User’s Manual for the 3D Cell Explorer
Thank you for purchasing the Nanolive 3D Cell Explorer.

You are now ready to start exploring living cells. Before using your 3D Cell Explorer, carefully read the safety notes to ensure safe handling and usage of the device.

Safety Notes

1. Verify that the computer is plugged and grounded earthed with its own power supply during usage with your 3D Cell Explorer.
   • Switch the 3D Cell Explorer OFF before connecting it to a power supply.
   • Disconnect and lockout the power supply before completing any maintenance work tasks or making adjustments.
   • Do not use electrical equipment in wet conditions or damp locations
   • Do not clean tools with flammable or toxic solvents

2. Supply the 3D Cell Explorer only with the provided power supply.

3. No special laser safety required (Class I laser).
   For measurements, the 3D Cell Explorer uses a Class I laser. A Class I laser is harmless for your eyes under any circumstances and thus does not require laser safety goggles when properly operated.

4. By no means should the user open the covers and thus have access to the laser path within the microscope frame without a clear authorization from Nanolive. Operating the microscope in non-appropriate conditions may then be a risk. It is thus forbidden for a non-authorized user to open the covers and manipulate the laser.

5. The user has the responsibility to avoid disastrous contaminations by biological samples. Nevertheless, contamination might occur when working with biological samples. Please ensure appropriate cleaning of the 3D Cell Explorer. Please refer to our Maintenance and Care notes for proper cleaning instructions.

6. Please note that alcohol is highly flammable, do keep it away from fire or potential sources of electrical sparks, and use it in a well-ventilated room when using it for cleaning the 3D Cell Explorer or other measures.
Maintenance and Care

1. Carefully, open the box with the top face up to avoid damaging the 3D Cell Explorer.
2. Handle the 3D Cell Explorer from the side or the bottom plate; unplug all cables before moving it.
3. When moving the microscope, carefully carry it without lifting it from the camera and avoid any shocks.
4. Do keep the instrument out of direct sunlight, high temperature or humidity, dusty and easy shaking environment. Make sure the table surface is flat, horizontal and firm.
5. All elements (e.g. microscope objective, mirrors, lenses, etc.) have been specially and carefully adjusted; please do not dismount, reposition, or modify them. In such case the warranty becomes automatically void and rework shall be charged to the customer.
6. Do not block the rotating arm or prevent it to rotate freely.
7. Do not disassemble any parts of the microscope, as this affects the function or reduces the performance of the microscope. Please be aware that the warranty expires if you remove the covers.
8. Keep the instrument clean, and do not contaminate the optical elements when wiping away the dust on the instrument. It is highly recommended to leave the red cap in place on the microscope objective when the microscope is not in use in order to prevent contaminations to come on the optical surfaces.
9. Contaminations on the objective and the mirrors, like fingerprints and oil smudges, could be gently wiped with the provided cleaning swab and a bit of alcohol (Ethanol or Isopropanol). More information on the cleaning procedure to be found in Cleaning Procedure for the optical elements.
10. Do not attempt to use organic solvents to clean the microscope. To clean it, use a lint-free, soft cloth slightly moistened with clear water.
11. During use, if the microscope is splashed by liquid, cut off the power at once, and wipe away the splash.
12. Place the instrument in a cool, dry position. When not using the microscope or carrying it, keep it covered with the provided dust cover. Furthermore, protect the microscope objective with the red vinyl cover.
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1. Introduction

The 3D Cell Explorer: instrument description and main features

- Class I laser source
- 3D image reconstruction in probing volume
- Operation modes: 3D snapshot, single measurement, time lapse measurement
- Full and automatic self-alignment of the microscope
- Non-invasive technology for probing living in-vitro cells
- Measuring cellular processes with real-time kinetics: enables multi-parameter analysis at single cell and sub-cellular scale

Typical fields of application / potential applications

The 3D Cell Explorer is a tool of discovery for cell researchers and biologists in a limitless number of fields, including:

- Cell division
- Cell morphology monitoring
- Visualize and monitor microorganism interaction and internalization
- Cell differentiation
- Cell-cell interaction
- Intracellular trafficking
- Cellular remodelling processes
- Cell death (apoptosis or necrosis)
- Drug monitoring (i.e. monitor cell response to treatments, in term of modification in the cell's morphology as well as if it is sensible or not to drugs)
- In vitro fertilization
- Observe the consequence on cell morphology upon exposure to different types of nanoparticles as well as test the behaviour of them and monitor their location
- Tissues imaging: histopathological studies, tissue morphological studies, etc.
- For further information please visit our web-page: http://nanolive.ch/applications.
1. Introduction

3D Cell Explorer Terminology

Figure 1. views of the 3D Cell Explorer
1. Introduction

3D Cell Explorer Terminology

Figure 2. terminology of key elements in the 3D Cell Explorer
## 2. Technical Specification Sheet (TSS)

### Hardware

<table>
<thead>
<tr>
<th>Specification</th>
<th>Cell Explorer</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimensions (width x depth x height in mm)</td>
<td>380 x 170 x 445</td>
<td></td>
</tr>
<tr>
<td>Weight (in kg)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Storage and transport (in packaging)</td>
<td>Use the provided dust cover when not in use</td>
<td></td>
</tr>
<tr>
<td>Operation</td>
<td>Permissible ambient temperature, and can be operated between 20°C and 30°C</td>
<td></td>
</tr>
<tr>
<td>Voltage ranges</td>
<td>100-240 VAC / 50-60 Hz / 1.0-0.5 A</td>
<td></td>
</tr>
<tr>
<td>Camera</td>
<td>CMOS, 165 fps, 1024 x 1024 pixels, USB 3</td>
<td></td>
</tr>
<tr>
<td>Microscope Objective (MO)</td>
<td>60x magnification, Air</td>
<td></td>
</tr>
<tr>
<td>Laser</td>
<td>Class I laser low power (λ=520 nm, sample exposure 0.2 mW/mm²), Class I. No eye protection needed</td>
<td></td>
</tr>
<tr>
<td>Field-of-view (FoV)</td>
<td>85 x 85 x 30 µm</td>
<td></td>
</tr>
<tr>
<td>Lateral optical resolution</td>
<td>200 nm</td>
<td></td>
</tr>
<tr>
<td>Axial optical resolution</td>
<td>400 nm</td>
<td></td>
</tr>
</tbody>
</table>

### Software

<table>
<thead>
<tr>
<th>Specification</th>
<th>Cell Explorer</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral sampling</td>
<td>183 nm</td>
<td>In native output</td>
</tr>
<tr>
<td>Axial sampling</td>
<td>482 nm</td>
<td>In native output</td>
</tr>
<tr>
<td>Tomography frame rate (acquisition)</td>
<td>0.5 fps</td>
<td>One full 3D reconstruction every 2 seconds</td>
</tr>
<tr>
<td>Self-adjusting time</td>
<td>&lt;90 seconds</td>
<td>Depending on optical thickness</td>
</tr>
</tbody>
</table>
3. **Computer Technical Specifications for the full version of STEVE**

The 3D Cell Explorer and its software STEVE need a computer with certain specifications that we have listed below. Each of the requirements are **absolutely necessary** for the 3D Cell Explorer to operate correctly.

*Please pay particular attention to the graphics card requirements. The full version of STEVE performs heavy calculations using a Nvidia GPU while the microscope is acquiring data. For this reason, please refer to the minimum required specifications of the Nvidia GPU below. In addition, you need at least one USB 3.0 port for camera image acquisition.*

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPU</td>
<td>1.5 GHz or faster multi-core CPU</td>
</tr>
<tr>
<td>CPU Memory:</td>
<td>minimum 8 GB DDR3 RAM</td>
</tr>
<tr>
<td>Hard disk:</td>
<td>450 MB free space for STEVE and 1 TB for acquisition data</td>
</tr>
<tr>
<td>GPU**:</td>
<td>NVIDIA GeForce GTX 780 or later NVIDIA GTX or Quadro GPU of the Kepler or newer architectures</td>
</tr>
<tr>
<td>GPU RAM:</td>
<td>minimum 4 GB dedicated</td>
</tr>
<tr>
<td>USB ports:</td>
<td>2 ports, minimum one USB 3.0 port</td>
</tr>
<tr>
<td>Operating System:</td>
<td>64-bit versions of Windows Vista, 7, 8 or 10 (Updated drivers: you need to have the latest Nvidia Graphics Card drivers, USB 3.0 drivers and fully updated Windows)</td>
</tr>
<tr>
<td>In addition:</td>
<td>Internet access for STEVE installation and for the newest updates.</td>
</tr>
</tbody>
</table>

*We are happy to provide you with a list of computers and laptops that we have tested and validated. Please contact us.

**If you are unsure about the minimal requirements, please contact us for an updated list of tested GPU cards.
4. **Start-up**

**Components**

The 3D Cell Explorer package contains the following:

1. the 3D Cell Explorer
2. 1 USB 2.0 cable
3. 1 USB 3.0 cable
4. 1 power supply
5. 1 power cable
6. 1 cleaning set
7. Reusable dust cover
8. WEEE certificate
9. protective cap for the MO

Figure 3. Contents of the 3D Cell Explorer packaging
4. **Start-up**

**Installation and setup**

1. Install the 3D Cell Explorer on a stable, flat, horizontal and firm workbench.
   - Lift the 3D Cell Explorer carefully out of the box by holding it at the indicated spots in the image below.
   - Never lift the 3D Cell Explorer by holding the camera.

![Holding spots for the 3D Cell Explorer](image)

**WARNING:** Nanolive does not bear the damage produced by wrong handling of the 3D Cell Explorer while it is removed out of its box.

In case of damages, Nanolive reserves the rights to examine the 3D Cell Explorer and verify how the damage occurred. All warranty claims will be cancelled in case of mishandling the 3D Cell Explorer by ignoring the instructions.

The warranty does not apply even if the 3D Cell Explorer has been subject to accidental damage.
4. **Start-up**  
**Installation and setup**

2. Carefully remove the protective accessories  
   - Properly dispose of the original packaging, or keep it for storage or return of the instrument to the manufacturer.  
   - Remove the disposable plastic foil and red vinyl protective cap (Figure 5.).

![Figure 5. Disposable plastic foil and red vinyl cap.](image)

You are ready to install the 3D Cell Explorer. Please check the following page and follow steps 1-5.
4. **Start-up**

Installation and setup

1. Connect your 3D Cell Explorer to your Computer.
2. Make sure the power switch is off.
3. Connect the power cable to the power supply. Then, plug the power supply into the power port.

4. Plug the USB 3.0 cable into the USB 3.0 port on the side of the 3D Cell Explorer, tidly screw the locks and connect it to the USB 3.0 slot on your computer.

5. Plug the USB 2.0 cable into the USB 2.0 port on the back of the 3D Cell Explorer and connect it to the USB slot on your computer.

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Figure 6. Connecting plugs of the 3D Cell Explorer
4. **Start-up**

**Installation and setup**

**Download and install STEVE**
- Switch on your PC and make sure it is connected to a power source.
- Updated drivers: you need to have the latest Nvidia Graphics Card drivers, USB 3.0 drivers and fully updated Windows.
- Go to www.nanolive.ch/register, fill out the form to register the 3D Cell Explorer and download the software.
- When requested, insert the serial number (you can find your serial number on the bottom of your 3D Cell Explorer, the delivery note or on the back of the quick start guide).
- Download and install STEVE.

⚠️ **Please note that you must run STEVE with Nvidia graphics processor**
- right click on the Steve exe file
- then click on "run with graphics processor"
- select "Nvidia high-performance"

- Start STEVE while still connected to the internet.
- The user’s manual and important information are downloaded during installation.
- Switch on your 3D Cell Explorer and make sure it is connected to a power source.

⚠️ **Before to start exploring your cells, check our Sample Preparation Protocol on how to properly prepare your samples.**
4. **Start-up**

Correct handling of the 3D Cell Explorer

The following section's purpose is to explain how to avoid any damages to the 3D Cell Explorer during moving, carrying, transportation and repacking.

**Important note**

If you want to move, carry, transport or repack the 3D Cell Explorer, please make sure to:

1. **Unplug** all cables: (i) power, (ii) USB2.0, (iii) USB3.0
2. **Secure** sample stage in top position
3. **Respect** the holding precautions

**NB:** Should you repack the 3D Cell Explorer, please use the original packaging.

**Securing the sample stage**

Before moving the 3D Cell Explorer, please ensure that the sample stage is blocked in top position with the z-screw to avoid damaging the Microscope Objective as shown in the picture below:

- High-risk of damages during transport
- Secure top position for transport with z-screw in top position
4. **Start-up**
Correct handling of the 3D Cell Explorer

**Instructions on how to protect the Microscope Objective (MO) with the red vinyl protective cap**

Place the protective cap on the MO through the sample stage aperture

Push it until it leans on the knurled part of the MO
4. **Start-up**

Correct handling of the 3D Cell Explorer

**Instructions on how to carry the 3D Cell Explorer correctly**

Once all **cables** are **unplugged** and the **sample stage** is **secured** and in top position, hold/carry your device as shown below.

- Secure hand positions at the places provided for lifting, carrying, and holding
- Do not lift the device by its sample stage or by the rotating arm
5. Sample Preparation

Sample Preparation Manual:

The 3D Cell Explorer allows measuring the inside of a living cell offering the researcher the possibility to acquire high resolution 3D images of a cell within seconds:

• cells fixed on glass coverslips or FluoroDish™
• cells grown on FluoroDish™

Thanks to extremely low light exposure and compatibility with cell culture accessories, the 3D Cell Explorer is suitable for long-term live cell imaging. We have designed a Sample Preparation Manual to guide researchers through the sample preparation methods for observation. Please download here our Sample Preparation Manual.

Rules of Thumb for Sample Preparation & Imaging:

The Rules of Thumb document presents 10 important key points related to your sample preparation and live cell imaging. Please download here our Rules of Thumb.
6. Cleaning Procedure for the optical elements

First, inspect the optical elements to determine the location of the contaminants. This allows you to anticipate the cleaning (typically by a swiping movement) so that the contaminant is removed from the surface of the optical element as soon as possible (avoid dragging it around).

Optical surfaces are sensitive to scratches and must thus be cleaned carefully with the appropriate equipment. We advise to use only new clean swabs (Texwipe™ Microdenier or Alpha series) soaked with ethanol or isopropanol to clean the optics.

Take a fresh and clean swab soaked in ethanol or isopropanol, and without pressure swipe the contaminant away from the center of the optical element. Use a fresh side of the swab after each contact with the optical surface in order to avoid re-deposition of removed contaminants.

Please clean first the most exposed optical surfaces, with thus higher probability of contamination:

- The MO is quite exposed to contamination and it may thus be required to clean it regularly. (Figure 7)
- The scanning mirror of the rotating arm is not much exposed, and its cleaning should not be required too often. (Figure 8)
- Finally, the central mirror is not exposed and its cleaning should be exceptional. (Figure 9)

High concentrations of contaminants may require repeated cleaning.

Figure 7. Cleaning procedure for microscope objective.
6. Cleaning Procedure for the optical elements

Figure 8. Cleaning procedure for scanning mirror.

Figure 9. Cleaning procedure for central mirror.
7. Software STEVE and how to operate the 3D Cell Explorer

Short introduction of STEVE

STEVE is Nanolive’s software for exploring the data acquired using the 3D Cell Explorer. For each recorded frame, the 3D Cell Explorer measures the refractive index (RI) of your cell in three dimensions. Use STEVE to digitally stain your data and interactively explore your cells and its subcomponents.

Software features

Simple and advanced microscope control
- Single Shot acquisition
- Configurable time-lapse acquisition
- Object selection using auxiliary bright field mode
- Full self-adjustment

Intuitive user interface
- Quantitative staining based on physical markers (RI)
- Easy management of your digital stains (creation, edit, enable/disable, delete, save)
- Playback options for time-lapse data

Comprehensive visualization options
- 2 visualization modes: Control mode and Viewer mode
- 2D slice per slice viewer (refractive index and stained data combined)
- 3D experience/scientific viewers

Multiple data export options
- Classical Formats: raw, tiff, obj, png/jpeg
- Compressed proprietary formats: vol/volx
- Screenshot capture for 2D and 3D viewers
- Video capture of the 3D viewer (.avi)

Data annotation system
- Semi-automatic update of the software
- GPU- accelerated 3D processing
7. Software STEVE and how to operate the 3D Cell Explorer

Please also refer to our video on our homepage “Introduction and getting started video for STEVE”.

Getting started with STEVE

STEVE is Nanolive’s software for exploring the data acquired using the 3D Cell Explorer. For each recorded frame, the 3D Cell Explorer measures the Refractive Index of your cell in three dimensions. Use STEVE to digitally stain your data and interactively explore your cell and its subcomponents.

2D VIEW
The 2D view shows an X-Y slice of the sample’s Refractive Index overlaid with the user-defined stains. In this view you can:
- Move to a different slice ( or via Slices slider) • Zoom in or out ( ).
- : Measure (or stop measuring) a user-defined path length. Click this button and click on the image to draw a path • : Show the Refractive Index and the Index Gradient in the Panel Viewer of a selected voxel in the 2D view • : Take a screenshot of the 2D view • / : Switch between Standard mode and Viewer mode.

PANEL VIEWER
The panel viewer represents a 2D space of Refractive Index (horizontal axis) and Index Gradient (vertical axis). Stains are shown as rectangles covering a region in the staining space. Regions on the right have a high Refractive Index, regions on the top have a high Index Gradient.
In this view you can:
- Change a stain’s position ( on the stain’s center) or shape ( on the stain’s edge) • Change a stain’s transparency ( on the stain’s center/edge or via the Opacity and Edge Softness sliders).

3D VIEW
The 3D view shows the stained data in three dimensions. In this view you can rotate ( ) • Zoom ( ) • Move (SHIFT + ) the 3D data.
- : Switch between Voxel View and Surface View • : Detach the 3D view from STEVE’s main window • : Take a screenshot of the 3D view • : Start or stop a live video recording of the 3D view.

BUTTONS AREA
: Visualize your sample in white light. Move the microscope’s knobs to search and focus your cell of interest • : Single shot acquisition: acquire a single 3D frame • : Time-lapse acquisition: acquire multiple 3D frames in a range of time • : Load a measurement file (.vol) • : Save a 3D or 4D measurement file (.vol). To save a 4D file, please indicate the desired range on the time line using markers and • : Open the Export dialog.

SLIDERS AREA
Overlay: Fuzzy switch between stained data and unaltered Refractive Index. Slices: Selects the X-Y slice from the 3D data stack. Opacity: Chooses the opacity for the currently selected stain. Edge Softness: Use this to make a stain’s edges soft and avoid solarized images in 2D.

STAIN COLORING AREA (PANEL)
Load Panel: Load a previously saved panel (.xml format) • Save Panel: Save the current panel (stains) • Rescale: Modify the viewing range of the panel viewer to fit the current time frame.

STAIN PICKER
: New stain - choose a name (not required) and a color for the stain. Next, begin painting your region of interest in the 2D view ( ). A rectangular stain is computed based on the painted part of the image and is shown in the panel viewer.
- / : Hide or show a stain • : Delete a stain • : Show the stain properties and allow background definition.

3D CONTROL AREA
: Change the background color for the 3D view. Hide Axes & Cube: Show/hide the coordinate axes and the outline cube in the 3D view.
Show Caption: Show/hide the labels in the 3D view.
Cropping sphere: Cut away part of the 3D view. Move the plane through the sphere to crop the 3D data. The plane can be moved (SHIFT + ) or rotated ( ).

TRANSPORT CONTROL
Use this to explore your data in time. The transport display shows the frame number and acquisition time of the current frame.
- : Jump to the beginning of a data set • : Set marker A to the current cursor position • : Play/pause playback • : Set marker B to the current cursor position • : Jump to the end of a data set • : Set playback speed. (Note: Markers A and B select a time range for saving/exporting purposes.)

TIME LINE AREA
Displays the current time frame ( ) and the selected range ( ). Click on the top part of the area to jump to the specified frame. Drag on the tick region to set markers A and B.
7. **Software STEVE and how to operate the 3D Cell Explorer**

![Diagram of STEVE software](image)

- **2D View**: The 2D view shows a X-Y slice of the sample’s Refractive Index overlaid with the user-defined stains.

- **Panel Viewer**: The Panel Viewer represents a 2D space of Refractive Index and Refractive Index Gradient.

- **3D View**: The 3D view shows the stained data in three dimensions.

**Explanation of Icons**

- **Sliders Area**
- **Buttons Area**
- **Transport Control**
- **Stain Coloring Area**
- **Stain Picker**
- **Time Line Area**
- **3D Control Area**

Figure 10.
7. **Software STEVE**

**Preparation for a measurement**

In order to start your measurements, verify that all the cables are properly plugged in, the 3D Cell Explorer is turned on and connected to your computer and STEVE is running.

Operate and control the 3D Cell Explorer easily with STEVE if you wish to do the following:

- Fully automated microscope calibration
- Single-frame image acquisition (single 3D reconstruction)
- Time-lapse acquisition (multiple 3D reconstructions in a range of time)
- Sample screening using auxiliary bright field mode (white light)

**Data Storage**

An acquisition generates a lot of data. It is thus necessary to store it on the control PC hard drive. The location of this acquisition buffer may be changed from the Microscope → Options menu. By default, it points to a location on your system drive (C:). You may choose another location with more available space to avoid acquisitions being interrupted due to insufficient space.

**Fully automated microscope calibration**

With the 3D Cell Explorer, there is no need for manual calibration. After positioning your sample, the microscope will instantly self-adjust so you can be sure you are getting the best possible images of your cells. You can always check the calibration progress as it is displayed on STEVE. After calibration is completed the cell image is displayed on the left panel of STEVE (2D view).

**Step-by-step guide on how to prepare for a measurement**

1. Take the sample previously prepared (for details on how to prepare the sample, please refer to the “Sample Preparation Manual”) and place it on the 3D Cell Explorer stage.
2. Open STEVE and click on the white light button. On the left panel of STEVE you can visualize the white light mode. Use the XY and Z knobs of the 3D Cell Explorer to search for the cell of interest and focus on it.
3. Now you can choose to either do a single-frame image acquisition or a time-lapse acquisition.
7. Software STEVE

Selecting the mounting medium’s refractive index

How to select the immersion medium refractive index

Before either a single-shot or a time-lapse acquisition is started, a dialog will appear in STEVE (Figure 12) prompting you to choose the mounting medium refractive index (e.g. water; Figure 13).

You may select the mounting medium from the drop-down list of commonly used media. If yours is not listed you may add it to the list by clicking “Add new RI” and entering the new mounting medium refractive index and its name (Figure 14). In case you are using an aqueous mounting medium not found in the list for which you do not know the refractive index, it is safe to assume it close to water. If using oil mounting media however, it is important to specify a correct refractive index as it can be significantly larger than of aqueous solutions.
How to do a single-frame image acquisition

A single frame image acquisition is an instantaneous measurement that results in a unique image of the object.

To perform a single shot, click on the 3D button or on the Single Shot from the Microscope menu. The mounting medium refractive index selection dialog will appear and the acquisition will start once a refraction index is selected.

At the end of the acquisition, STEVE will enable to digitally stain the data and interactively explore the cell and its subcomponents. Further down, we explain the exact steps on how to achieve this. Please refer to “How to digitally stain cells with STEVE”.

Figure 15. Acquisition type dialogue window
7. Software STEVE and how to operate the 3D Cell Explorer

Time-lapse acquisitions

How to do a time-lapse acquisition

In a time-lapse acquisition, multiple 3D reconstructions in a range of time are attained.

To perform a time lapse, click on the 4D button or on Time Lapse in the Microscope menu. The mounting medium refractive index selection dialog will appear. Once a refractive index is selected, a new dialog will open to specify the speed and the duration of the acquisition (Figure 16).

The time lapse can be performed either at the maximum speed or by setting the time interval between two consecutive frames.

The duration of the time lapse acquisition can be determined either by stopping it manually (by clicking on the Stop Acquiring button) or by setting the ending time for it to stop automatically.

At the end of the acquisition, STEVE will enable to digitally stain the data and interactively explore the cell and its subcomponents. Further down, we explain the exact steps on how to achieve this. Please refer to “How to digitally stain cells with STEVE”.

Figure 16. time lapse setting window
7. **Software STEVE**

   Digitally staining

**How to digitally stain cells with STEVE**

1. Choose a meaningful slice on the 2D visualization panel (left side).
   - Dragging the mouse up to down (left click pushed) on the 2D visualization panel or by moving the Slices slider.

2. Pick a new stain.
   - Click on the + button.

3. Choose a name (optional).
   - Click on the black-gray line edit form.
   - Write down the name in the line text input.

4. Choose a color.
   - Click on your desired color.
   - Option: drag the mouse to have a more precise vision of the color that you could select.

5. Go to the 2D visualization panel and draw.
   - Click and/or drag the mouse on your desired region of interest. The pixels under your cursor are glowing in the panel view. When you release the mouse, we compute the stain from your selected pixels.
   - Option: You could zoom in on the 2D visualization panel by wheeling the mouse.

6. Now the stain is represented in the panel view and superimposed on the 2D visualization panel.
   - Option: you could change the weight of the colored image on the 2D visualization panel by moving the Overlay slider.
7. **Software STEVE**

Digitally staining

7. How to manipulate your stain:
   - Change the opacity (Opacity slider or drag up-down with the right click pushed).
   - Change the edge softness (Edge softness slider or drag up-down with the right click pushed on a stain edge).
   - Move a stain in the panel view (click on the stain and drag it on the desired position in the refractive index – index gradient space).
   - Change the shape of the stain (select an edge or a corner and drag it).

8. To add other stains repeat the above steps

9. Stain representation: opacity, edge softness, stain edge /rectangle, panel view axes

10. Stains options:
    - Enable/Disable the visualization of the stain
    - Delete the stain
    - Change the color (by double clicking the colored button or by clicking on the palette icon)

**Note!** For more in-depth information about digital staining, please refer to our best staining practices on our support online page.
7. Software STEVE

Data Saving

How to save your data

The data can be saved by clicking on the Save icon (Figure 16). The holograms are kept by default within the .vol file.

![Figure 16. Saving Window](image)

Figure 16. Saving Window
7. Software STEVE

Data Saving

STEVE allows to save data as a 3D or a 4D .vol file (readable only with STEVE).

Alternatively, it is possible to export the data in other file formats by opening the export dialog shown in the Figure 17.

The data can be exported by choosing the following parameters:

- Single acquisition or Time-lapse. For time-lapse, it is possible to export all frames or one every 2, 3, etc.
- the type of data: RI or fluorescence channel
- the format of the file: Raw, Tiff, Obj, Png/Jpeg (available depending on the above selections).

Figure 17. Export data setting
7. Software STEVE and how to operate the 3D Cell Explorer

Data Annotation

How to work with data annotation

For each measurement you can visualize and modify the measurement properties, called also data annotation. In the File Menu under “Properties” open the annotation dialog (Figure 41). All the visible properties, marked with “Eye icon” will be added to the current measurement after the button “Apply” is pushed. The invisible properties, marked with “Close Eye icon” will not be saved. The first property entries, such as: “X size”, “X resolution”, “Acquisition Time”, “Microscope”, etc. are not editable, you cannot modify them, but they will always be saved on the measurement. You can add new entries by clicking on the “Add Entry” button or you can delete the selected entry by clicking on “Delete Entry”. By deleting an entry, that entry will never be shown again on the annotation dialog, you will have deleted it permanently. Also, you can edit each entry by clicking on the “Edit icon” button on the right side of the entry.

Figure 18. Export data setting
How to update the software

Steve will automatically update when a new version is available.
The user will be alerted to close Steve and the Steve Maintenance Tool will start.
In the Steve Maintenance Tool choose “Update components” and click the “Next” button. Follow the messages provided by the update dialog.
8. Best Staining Practices

**Best Staining Practices:**

This document is a practical guideline about how to digitally stain your sample obtaining the best possible results. Please [download here](#) our Best Staining Practices.
9. Troubleshooting

Calibration
Calibration failed err.1.1
If applicable, reduce the quantity of liquid above your sample (-> Check our Sample Preparation Protocol in Section 5). Check that no opaque object can intersect the laser beam while the arm is rotating. If using a dish, check that you are imaging close to its center such that the laser beam cannot intersect the wall of the dish instead of the top.

Calibration failed err.1.4
Sample may be too absorbent. Try to move in a different area and re-do the acquisition. If the error persists, please get in touch with our customer service.

Acquisition
Acquisition failure err.2.1
If applicable, try to move to another position within your sample. If the error persists try to clean the microscope objective and check that you comply with the sample preparation protocol (-> Check our Sample Preparation Protocol).

Connection
Connection failure err.3.1
If STEVE cannot establish communication with the 3D Cell Explorer, please verify that the backside USB 2.0 is correctly plugged in to the 3D Cell Explorer and to your computer, that the microscope has electrical power and that it is switched on. If the error persists, unplug all cables, reboot your computer and plug the cables back (check Start-up - Section 4).

Connection failure err.3.2
If STEVE cannot establish communication with the 3D Cell Explorer, please verify that the USB 3.0 cable coming from the camera is correctly screwed in and that it is properly connected to your computer’s USB 3.0 port.
11. Troubleshooting

Connection failure err.3.3
STEVE cannot establish communication with the 3D Cell Explorer. The port to which the USB 3.0 cable (coming from the camera) was connected was recognized as USB 2.0. This cable must be connected to a USB 3.0 port. If you have correctly connected the USB 2.0 and USB 3.0 cables and the error persists, please verify that your USB 3.0 adapter drivers are properly installed or contact your system administrator.

Connection failure err.3.4
STEVE cannot establish communication with the 3D Cell Explorer and the microscope cannot initialize itself. Please make sure the rotating arm is not obstructed in any way. Try to switch it off and on again. If the error persists observe whether the arm rotates when you switch the microscope on. If this is not the case, please contact our customer service.

Connection failure err.3.6
The microscope needs maintenance. Please contact your distributor if applicable or our customer support.

Connection failure err.3.7
The connection to the fluorescence module failed. Please make sure that the fluorescence module is powered and that the USB cable from the module is connected to a USB port on your computer.

Connection failure err.3.8
Microscope firmware update error. Your microscope likely needs maintenance. Please contact our customer support.

Processing

Processing error err.5.1
The STEVE processing tool chain encountered a fatal error. Please make sure your computer meets the STEVE hardware requirements. In particular, make sure your graphical card is from the NVIDIA brand with at least 2GB of dedicated GPU memory. Please upgrade your graphical drivers using the NVIDIA website at www.nvidia.com/Download/index.aspx. If the error persists, please contact our customer service including error detailed information displayed in the error popup.