1. Safety Notes

3D Cell Explorer-fluo

Thank you for purchasing the Nanolive 3D Cell Explorer-fluo. Before using your 3D Cell Explorer-fluo and the CoolLED illumination system, carefully read the safety notes to ensure safe handling and usage of the device at all times. Failure to do so may result in personal injury or damage to other items.

1. Safety Notes for the 3D Cell Explorer-fluo

   - Verify that the computer is plugged and grounded earthed with its own power supply during usage with your 3D Cell Explorer-fluo.
   - Switch the 3D Cell Explorer-fluo OFF before connecting it to a power supply.
   - Disconnect and lockout the power supplies of the 3D Cell Explorer-fluo 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-fluo only with the provided power supplies.

3. No special laser safety required when operating the 3D Cell Explorer-fluo (Class I laser).

   For measurements, the 3D Cell Explorer-fluo 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. 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.

![Safety Symbols]
1. **Safety Notes**

3D Cell Explorer-fluo

4. **Contaminations**

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-fluo. Please refer to our Maintenance and Care notes for proper cleaning instructions.

5. **Cleaning**

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-fluo or other measures.

6. **The 3D Cell Explorer-fluo conforms to the requirements of the Safety Standards as follows:**

- EN/IEC 61010-1:2010 Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory use.
- EN62471:2008 Photo-biological Safety of Lamps and Lamp Systems/Guidance on manufacturing requirements relating to non-laser optical radiation safety. **No risk**
1. Safety Notes

CoolLED illumination system

7. Safety Notes for the CoolLED illumination system

• UV light may be emitted from this product depending on the configuration selected. Avoid eye and skin exposure. Never look directly into the light output beam from the Light Source or accessories. The emissions could damage the cornea and retina of the eye if the light is observed directly.
• Always ensure that the Light Source is securely attached to the microscope with its light guide, prior to turning on the power. This will minimize the risk of injury and damage.
• If for any reason the Light Source is to be operated when not attached to a microscope, all personnel should wear eye shielding and clothing to protect the exposed skin.
• Disconnecting the main supply is achieved by unplugging the power cord from the power supply block or the Light Source. Only plug in the power cable, once the Light Source is attached to the microscope.
• There are no serviceable parts within the Light Source. Removing any of the screws and covers will result in the safety of the Light Source being impaired. The DC power supply unit should be inspected periodically throughout the lifetime of the system.
• To clean the exterior of the Light Source, use a slightly dampened cloth with a simple water/detergent solution only. Avoid the optical surfaces and lenses. Cleaning of optics should only be carried out using the provided optical swabs with grade isopropanol or ethanol. Please note that the DC power supply unit should be isolated prior to cleaning.

8. Compliance:

The light source conforms to the requirements of the Safety Standards as follows:

• EN/IEC 61010-1:2010 Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory use.
2. **Maintenance & Care**

3D Cell Explorer-*fluo*

**Maintenance and Care for the 3D Cell Explorer-*fluo***

1. Carefully, open the shipment box with the top face up to avoid damaging the 3D Cell Explorer-*fluo*.
2. Handle the 3D Cell Explorer-*fluo* 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. Keep the instrument out of direct sunlight, high temperature or humidity, dusty and easy shaking environments. Make sure the table surface is flat, horizontal and firm.
5. All elements (e.g. microscope objective, mirrors, lenses) 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 section “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.
2. Maintenance & Care

CoolLED illumination system

Maintenance & Care for the CoolLED illumination system

1. Carefully unpack the components from the shipping cartons.
2. The CoolLED illumination system requires little or no maintenance throughout its life. There are no field serviceable parts so there is no need to remove the covers.
3. Cleaning of the external surfaces can be carried out with a mild soap and water solution used to lightly dampen a lint-free cloth. Ensure that no liquid is allowed to enter the product through vents and panel edges. Avoid optical surfaces.
4. Cleaning of optical surfaces maybe necessary if debris or finger prints accidentally come into contact with the excitation filters during installation. In the first instance remove any loose debris with an air duster (aerosol or rubber blower).
5. Finger prints or other liquid type contaminants should be removed using standard lens cleaning procedures. Do not flood the optical surfaces with fluid as liquid could enter the product and cause damage.

Care of the light guide

⚠️ The light guide is a fine, high quality optical instrument. The useful life can be prolonged by following these simple guidelines.

1. Avoid stretching the light guide, forming configurations, involving sharp angels or kinks, or contact with sharp or pointed objects. The internal light fibers are made of glass, a material that breaks under stress. Fiber breakage will result in diminished light output.
2. Any inadvertent cut or puncture to the outer wall will render the light guide unsafe for use and must be taken out of service immediately.
3. **Introduction**

**Instrument description and main features**

**Plug & Play systems**

- 2 imaging modalities:
  - Physical structural imaging (based on refractive index, RI)
  - Chemical fluorescence imaging
- 3 Fluorescence Channels: 2 configurations available:
  - DAPI (Excitation 392 nm / Emission 432 nm), FITC (Excitation 474 nm / Emission 515 nm), TRITC (Excitation 554 nm / Emission 595 nm)
  - FITC (Excitation 474 nm / Emission 515 nm), TRITC (Excitation 554 nm / Emission 595 nm), Cy5 (Excitation 635 nm / Emission 730 nm)
- 4 Fluorescence Channels:
  - DAPI (Excitation 392 nm / Emission 432 nm), FITC (Excitation 474 nm / Emission 515 nm), TRITC (Excitation 554 nm / Emission 595 nm), Cy5 (Excitation 635 nm / Emission 730 nm)

*Note: TRITC and Cy5 excitation filters are exchangeable on channel 3*

Please refer to the following link for details on the Filter configuration:
Likewise, refer to the following link for further data on the LED source:

- Operation modes:
  - 2D epifluorescence snap-shot (every second) & time-lapse (up to 96 hours)
  - 3D RI snap shot & time-lapse 3D RI & 2D epifluorescence snap-shot (every 3 seconds) & time-lapse (up to 96 hours)
- Co-localisation of Fluorescence (3 channels) and Digital Stains (7 channels at single acquisition)
- Time-lapse possibility up to 96 hours
3. Introduction

Typical Fields of Applications

The typical fields of applications for the 3D Cell Explorer-fluo

- Merging of fluorescence microscopy and 3D cell tomography
- Calibration of the refractive index map with markers (Chemical dyes, fluorescent antibodies, protein labeling with GFP, etc.)
- Combination of structural RI information with chemical information for specific protein/drug tracking, protein localization and mapping
- Long term (over a week) live cell imaging in 3D at high temporal resolution (1 image every 2 sec.) thanks to our technology that generates no phototoxicity
- Powerful partner for advanced image analysis strategies: high resolution and no phototoxicity allows to obtain more reliable cell measurements in subsequent image analysis
- Possibility to use 10 markers in parallel
3. Introduction

3D Cell Explorer-fluo terminology

- Rotating arm
- Scanning head
- Peripheral mirror
- Cell container interface
- XY knob for sample screening
- Hi-grade stage for sample screening
- Z knob for optical focusing
- Camera with USB 3.0 slot
- Injection Head
- USB 2.0 slot
- Power switch
- BNC cable plug port
- Power supply plug port
3. Introduction

CoolLED illumination system terminology

CoolLED illumination system – front view

CoolLED illumination system – rear view

CoolLED illumination system – side view 1

Liquid Light Guide (LLG)

CoolLED illumination system – Top/Side View

Excitation filters slots

Global Trigger

Not needed BNC plugs
# Technical Specification Sheet (TSS)

## 3D Cell Explorer-fluo & CoolLED illumination system

<table>
<thead>
<tr>
<th>Specification 3D Cell Explorer-fluo / Holotomography</th>
<th>Specifications CoolLED illumination system / Epifluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dimensions (width x depth x height in mm)</strong></td>
<td></td>
</tr>
<tr>
<td>380 x 170 x 445</td>
<td>77 x 186 x 162</td>
</tr>
<tr>
<td><strong>Weight (in kg)</strong></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td><strong>Environmental Operating Conditions</strong></td>
<td></td>
</tr>
<tr>
<td>Permissible ambient temperature, and can be</td>
<td>5-35°C</td>
</tr>
<tr>
<td>operated between 20° C and 30° C</td>
<td></td>
</tr>
<tr>
<td><strong>Voltage ranges</strong></td>
<td></td>
</tr>
<tr>
<td>100-240 VAC / 50-60 Hz / 1.0-0.5 A</td>
<td>110-240 VAC / 50-60 Hz / 2 A</td>
</tr>
<tr>
<td><strong>Camera</strong></td>
<td></td>
</tr>
<tr>
<td>USB3.0 CMOS Sony IMX174 sensor</td>
<td></td>
</tr>
<tr>
<td>Quantum Efficiency (typical)</td>
<td>70 % (at 545 nm)</td>
</tr>
<tr>
<td>Dark Noise (typical)</td>
<td>6,6 e⁻</td>
</tr>
<tr>
<td>Dynamic Range (typical)</td>
<td>73,7 dB</td>
</tr>
<tr>
<td><strong>Microscope Objective (MO)</strong></td>
<td></td>
</tr>
<tr>
<td>Dry objective / 60x magnification / NA 0.8</td>
<td></td>
</tr>
<tr>
<td><strong>Illumination Source</strong></td>
<td></td>
</tr>
<tr>
<td>Class I laser low power (λ=520 nm, sample exposure</td>
<td>High speed switchable &lt;100 µs</td>
</tr>
<tr>
<td>0.2 mW/mm²) (Class I. No eye protection needed)</td>
<td>Lifetime &gt;20'000 hours each channel</td>
</tr>
<tr>
<td><strong>Field-of-View (FoV)</strong></td>
<td></td>
</tr>
<tr>
<td>90 x 90 x 30 µm³</td>
<td>90 x 90 µm</td>
</tr>
<tr>
<td><strong>Lateral resolution</strong></td>
<td></td>
</tr>
<tr>
<td>200 nm</td>
<td>400 nm</td>
</tr>
<tr>
<td><strong>Axial resolution</strong></td>
<td></td>
</tr>
<tr>
<td>400 nm</td>
<td>-</td>
</tr>
<tr>
<td><strong>Tomography frame rate (acquisition)</strong></td>
<td></td>
</tr>
<tr>
<td>0.5 fps</td>
<td>3 fps each channel</td>
</tr>
<tr>
<td><strong>Self-adjusting time</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 90 seconds (Depending on sample mounting medium</td>
<td></td>
</tr>
<tr>
<td>thickness)</td>
<td></td>
</tr>
<tr>
<td><strong>Channels</strong></td>
<td></td>
</tr>
<tr>
<td>Up to 7 simultaneously</td>
<td>3: DAPI (blue), FitC (green), TritC (orange)</td>
</tr>
<tr>
<td></td>
<td>3: FitC (green), TritC (orange), Cy5 (red)</td>
</tr>
<tr>
<td></td>
<td>4: DAPI (blue), FitC (green), TritC (orange) and Cy5 (red)</td>
</tr>
<tr>
<td></td>
<td>(Note: TritC and Cy5 excitation filters are exchangeable on</td>
</tr>
<tr>
<td></td>
<td>channel 3)</td>
</tr>
</tbody>
</table>
5. Computer Technical Specifications

3D Cell Explorer-fluo

The 3D Cell Explorer-fluo is provided without a computer. Therefore, before you acquire a computer, please be aware of the minimum requirements listed below which are absolutely necessary for correct operation of the instrument.*

- **CPU:** 1.5 GHz or faster multi-core CPU
- **CPU Memory:** minimum 8 GB DDR3 RAM
- **Hard disk:** 450 MB free space for STEVE and 1 TB for acquisition data
- **GPU:**
  - NVIDIA GeForce GTX 780 or later NVIDIA GTX or Quadro GPU of the Kepler or newer architectures
  - minimum 4 GB dedicated
- **USB ports:** 3 ports, minimum one USB 3.0 port
- **Operating System:** 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 Operating System*

**In addition:** Internet access for STEVE installation and for the newest updates.

*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.

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 above. In addition, you need at least one USB 3.0 port for camera image acquisition.
6. **Start-up**

**Components**

The 3D Cell Explorer-\textit{fluo} package contains the following:

1. the 3D Cell Explorer\textit{-fluo}
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. WEEE certificate
8. protective cap for the MO and connection to the microscope
9. protective cap for the LLG
10. 1 BNC cable.
6. **Start-up**

**Components**

The CoolLED Illumination System packaging contains the following:

1. LED Light Source
2. DC Power Supply
3. IEC Power Cable
4. 1 USB 2.0 cable
5. Liquid light guide (LLG) protected with red vinyl caps (use the red vinyl caps to protect both end of the LLG when not in use)
6. 3 holders equipped with excitation filters.
6. **Start-up**

Installation and setup

Important Information: It is critical to follow the installation and setup steps as described in the following pages to ensure the safety standards of the CoolLED illumination system. Please refer to chapter 1: Safety Notes.

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

**WARNING:** Nanolive does not bear the damage produced by wrong handling of the 3D Cell Explorer-\textit{fluo} while it is removed out of its box. In case of damages, Nanolive reserves the rights to examine the 3D Cell Explorer-\textit{fluo} and verify how the damage occurred. All warranty claims will be cancelled in case of mishandling the 3D Cell Explorer-\textit{fluo} by ignoring the instructions. The warranty does not apply even if the 3D Cell Explorer-\textit{fluo} has been subject to accidental damage.
6. **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.

⚠️ For safety reasons, please proceed to install the CoolLED illumination system first.
6. **Start-up**

*Installation and setup*

3. Install the CoolLED Illumination System on a stable, flat, horizontal and firm workbench
   - Lift the CoolLED Illumination System carefully out of the box together with its accessories.
6. **Start-up**

Installation and setup

4. **Install the excitation filters on the CoolLED Illumination System**
   - Place the little drawers containing the excitation filters in the dedicated slots.
   - Mind the order and the direction.
6. **Start-up**

**Installation and setup**

5. Install the LLG on the CoolLED Illumination System
   - Remove the red cap from the LLG from one end (Figure 1)
   - Insert the LLG as far as it can go into the LED Light Source as shown below (Figure 2).
   - Use an **Allen key n°1.5** to secure the LLG in place. Once the screw is in mechanical contact with the LLG header, gently apply an extra force to make sure the LLG is maintained in place. There is a risk of damaging the LLG header and reducing the coupled power from the LED Light Source to the sample if too much force is applied by the screw.
6. **Start-up**

Installation and setup

6. The CoolLED illumination system with the inserted LLG should look like the image below.

Place the light source close to the 3D Cell Explorer-*fluo* in order to avoid any strain on the LLG or too short radius turns.

![Image of the CoolLED illumination system](image)

**Figure 3**

Ensure that the Light Source sits upright on a flat surface and keep a clearance of 200mm on each side to ensure adequate airflow for the cooling system.

![Diagram showing clearance space](image)

**Figure 4**
6. **Start-up**

Installation and setup

7. **Connect the LLG to the 3D Cell Explorer-fluo**
   - Remove the red cap from the LLG from the other end (Figure 5)

   ![Figura 5: Protective cap y LLG header](image1)

   **Figure 5**

   - Insert the LLG as far as it can go into the injection head on the 3D Cell Explorer-fluo as shown below (Figure 6).

   ![Figura 6: Injection Head y LLG en el cabezal de inyección](image2)

   **Figure 6**
6. **Start-up**

Installation and setup

- Use an **Allen key n°1.5** to secure the LLG in place.
- Bring the 3 screws first in mechanical contact without effort one after the other. Gently apply some force to keep the LLG in place one after the other. Please be aware that any damage made to the LLG might lead to irreversible loss of coupled power brought to the sample (Figure 7).
6. **Start-up**

**Installation and setup**

8. **Handling note on the LLG (Figure 8)**
   - The LLG is carrying the light from the CoolLed illumination system to the 3D Cell Explorer-fluo. It is fragile and should be handled with care.
   - Avoid scratches and damages to the LLG ends (especially the silica surfaces) when connecting them to the CoolLed illumination system and the 3D Cell Explorer-fluo.

   ![Figure 8](image)

   - Do not stretch, squeeze or bend the LLG excessively.

**Important Note**

**Short-term bending radius** \( R_{\text{short}} > 40 \text{ mm} \) / 
**Long-term bending radius** \( R_{\text{permanent}} > 75 \text{ mm} \) (Figure 9)

To avoid permanent damage, it is highly recommended to leave a clear and safe path for the LLG from the CoolLed illumination system to the microscope without any strain (Figure 10).
6. Start-up
Installation and setup

9. Diverse connections of your CoolLed illumination system
   • Plug the power connector from the DC power supply to the CoolLED illumination system as shown below. Ensure that the DC power supply
     is the one supplied with the product. Using a non-CoolLED power supply may damage the illumination system and will invalidate the
     warranty. At this stage do not connect the main power lead to the DC power supply (Figure 11).
   • Plug the CoolLED illumination system USB 2.0 cable into the USB 2.0 port on the top of the CoolLED illumination system and connect it to
     one of the USB slots on your computer (Figure 11).
   • Then plug the BNC cable to the rear panel of the CoolLED illumination system and to the rear panel of the 3D Cell Explorer-fluo (Figure 12).

![Figure 11](image1)

![Figure 12](image2)

**Important Note**

Orientation of the power plug: Plug the power cable with the flat surface facing inward (Figure 13)
6. **Start-up**

**Installation and setup**

- With the CoolLED illumination system now attached to the microscope it is safe to connect the power cable. Connect the power cable supplied to a convenient socket, plug in the IEC connector into the DC power supply and switch the power on at the socket.
6. **Start-up**

**Installation and setup**

10. **Diverse connections of your 3D Cell Explorer-fluo (Figure 14)**

   - Make sure the power switch of your 3D Cell Explorer-fluo is off.
   - Connect the Nanolive power cable to the Nanolive power supply. Then, plug the Nanolive power supply into the power port.
   - Plug the Nanolive USB 3.0 cable into the USB 3.0 port on the side of the 3D Cell Explorer (the camera), tightly screw the locks and connect it to the USB 3.0 slot on your computer.
   - Plug the Nanolive USB 2.0 cable into the USB 2.0 port on the back of the 3D Cell Explorer-fluo and connect it to one of the USB slots on your computer.

![Figure 14](image)

Figure 14
6. Start-up
Installation and setup

11. 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-fluo and download the software.
   • When requested, insert the serial number (you can find your serial number on the bottom of your 3D Cell Explorer-fluo, 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-fluo and make sure it is connected to a power source.
   • Connect the power supply of the CoolLED source to the grid.

⚠ Before to start exploring your cells, check our Sample Preparation Manual on how to properly prepare your samples.
7. Handling

Correct handling of the 3D Cell Explorer-fluo

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

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

1. Unplug all cables from both devices (3D Cell Explorer-fluo and CoolLED illumination system): (a) power, (b) USB2.0, (c) USB3.0 (d) BNC and (e) light guide.
2. Secure the sample stage of the 3D Cell Explorer-fluo in top position.
3. Protect the Microscope Objective with its dedicated red vinyl cap.
4. Protect the injection head with its dedicated black vinyl cap
5. Respect the holding precautions of the 3D Cell Explorer-fluo.
6. Protect both ends of the LLG with the red vinyl cap.
7. Avoid stretching the LLG, forming configurations, involving sharp angels or kinks, or contact with sharp or pointed objects.
8. Transport the 3D Cell Explorer-fluo and the CoolLED illumination system in their original packaging.
7. Handling
Correct handling of the 3D Cell Explorer-fluo

Instructions on how to secure the sample stage
Before moving the 3D Cell Explorer-fluo, please ensure that the sample stage is blocked in top position by using 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
7. **Handling**

Correct handling of the 3D Cell Explorer-**fluo**

**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
7. Handling

Correct handling of the 3D Cell Explorer-fluo

Instructions on how to protect the LLG header and how to properly take care of the LLG

- Use the protective cap for both ends when the LLG is not connected to the devices (Figure 15 & 16)
- The light guide is a fine, high quality optical instrument. The useful life can be prolonged by following these simple guidelines
  - Avoid stretching the light guide, forming configurations, involving sharp angels or kinks, or contact with sharp or pointed objects. Light guide breakage will result in diminished light output (Figure 16).
  - Any inadvertent cut or puncture to the outer wall will render the light guide unsafe for use and must be taken out of service immediately.

- Avoid scratch or damage to the LLG ends (especially the silica surfaces) when connecting them to the CoolLed illumination system and the 3D Cell Explorer-fluo (Figure 17).
- Clean ends with optical tissue and grade alcohol
7. **Handling**

Correct handling of the 3D Cell Explorer-*fluo*

**Instructions on how to carry the 3D Cell Explorer-*fluo* 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.
8. Sample Preparation

**Sample Preparation Manual:**

The 3D Cell Explorer-*fluo* 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-*fluo* 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.

**Note:** If you are using fluorescent markers to label your cells, please follow the known concepts of Sample Preparation for Fluorescence Microscopy!
9. Cleaning Procedure for 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 18)
• The scanning mirror of the rotating arm is not much exposed, and its cleaning should not be required too often. (Figure 19)
• Finally, the central mirror is not exposed and its cleaning should be exceptional. (Figure 20)

High concentrations of contaminants may require repeated cleaning.

Figure 18
9. Cleaning Procedure for optical elements

Figure 19

Figure 20

Figure 19

Figure 20
10. **Software STEVE**

**Important Note: Fluorescent filter configuration**

⚠️ **Fluorescence filter configuration**

Before you start your acquisitions, set up STEVE with the correct fluorescence filter configuration as shown below. The 3D Cell Explorer-*fluo* is delivered with the options FITC (green), TRITC (orange) and DAPI (blue) or Cy5 (red). The configuration may be changed from the Microscope —> Options menu. (Figure 21 & 22)

![Microscope Options](image1)

![Fluo channels configuration window](image2)
10. Software STEVE

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 (🔍). Move the view when zoomed in (SHIFT + 🎧).

🚀: 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 2DView. 📸: 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 ( מבין 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
ıldığı: 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 ()view.

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.
10. **Software STEVE**  
**Interface Fluorescent**

**2D View**  
The 2D view shows a X-Y slice of the sample’s Refractive Index overlaid with user-defined digital stains and fluorochromes resulting in a multi-channel image.

**Panel Viewer**  
The Panel Viewer represents a 2D space of Refractive Index and Index Gradient. Multi-channel images are not represented here.

**3D View**  
The 3D view shows the stained data in three dimensions.
10. Software STEVE

Preparation for a measurement

**Note!**

In order to start your measurements, verify that all the cables are properly plugged in, the 3D Cell Explorer-\textit{fluo} and the CoolLED illumination system are turned on and connected to your computer and STEVE is running.

Operate and control the 3D Cell Explorer-\textit{fluo} and the CoolLED illumination system 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)
- Localization of organelles at different cell depths
- Co-localization of fluorescence (3 channels) & Digital Stains (7 channels at single acquisition)
- 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-\textit{fluo}, 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**

Take the sample previously prepared (for details on how to prepare the sample, please refer to the “Sample Preparation Protocol”) and place it on the 3D Cell Explorer-\textit{fluo} stage.

Open STEVE and click on the white light button. On the left panel of STEVE you can visualize your cells in the white light mode. Use the XY and Z knobs of the 3D Cell Explorer-\textit{fluo} to search for the cell of interest and focus on it.

Now you can choose to either do a single-frame image acquisition or a time-lapse acquisition with your desired fluorescent channel choice.
10. Software STEVE

Selecting the mounting medium’s refractive index

How to select the mounting medium’s refractive index

Before either a single-shot or a time-lapse acquisition is started, a dialog will appear in STEVE (Figure 23) prompting you to select the mounting medium refractive index (e.g. DMEM, Figure 24).

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 “New” and entering the new mounting medium refractive index and its name (Figure 25). 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 is 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.
10. Software STEVE

Single-frame image acquisition

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 (see section "How to select the mounting medium's refractive index" previously explained) and the acquisition will start once a refraction index is selected (Figure 26).

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 26 Acquisition type dialogue window
Multi-channel image

A multi-channel image combines a series of monochrome images into one image. The multi-channel image generally shows a sample that has been stained with numerous different fluorochromes. The multi-channel image is a combination of the single fluorescence images. You can have the individual fluorescence images displayed separately (showing, e.g., the plasma membrane or mitochondria) or also as a superimposition of all of the fluorescence images (overview) (Figure 27).

Figure 27 Single fluorescence images and overview fluorescence image
10. Software STEVE

Acquiring fluorescence images

How to create multi-channel images

To perform a single shot, click on the 3D button or on the Single Shot from the Microscope menu. A selection dialog will appear to choose the acquisition type which can be individually set up (Figure 28). Once a refractive index and the fluorescence channels are selected, a new dialogue will appear to adjust the fluorescence channel parameters (Figure 29). Adjust the fluorescence parameters as per your needs. Then click on “ok”. Your acquisition will start.

Figure 28 Acquisition type dialogue window

Figure 29 Dialogue window fluorescence parameters
10. **Software STEVE**

Acquiring fluorescence images

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**TIP**

To be able to acquire good fluorescence images, follow our guidelines hereafter.

1. At the time the dialogue appears you are able to adjust the fluorescence channel parameters, in order to acquire the best image possible (Figure 30).

![Figure 30 Dialogue window fluorescence parameters](image)

- **Live-image of the selected channel**
- **Tab for setting-up each of the selected channels**
- **Tab for activating the white-light mode and perform bright-field of the sample**
- **Exposure setting of the camera in ms**
- **Gain setting of the camera in percentage: it is recommended not to go beyond 40% to acquire a high-quality image.**
- **Intensity setting of the LED source in percentage: it is recommended to operate at about 10% for the sample screening to limit the sample photobleaching**
10. **Software STEVE**

Acquiring fluorescence images

**TIP**

For the best image quality, keep the intensity as low as possible in order to avoid bleaching while you search for a suitable fluorescent cell. You will need to maximize the gain to be able to see something. At the same time, the exposure time should be typically below 200 ms, to have enough frame refreshing rate and screen comfortably the sample (Figure 31).

2. Now you are ready to focus on the fluorescent image (use the Z knob for that).

![Figure 31 Dialogue window fluorescence parameters](image-url)
10. **Software STEVE**

Acquiring fluorescence images

TIP

3. To acquire an image without background, adjust the intensity parameters. The intensity should stay low (<20-30) (again to minimize photobleaching). Now, reduce the gain until the background disappears. The maximum gain to be used can be up to 40%.

Increase the exposure time if you need to see more details of the image but try to keep it as low as possible (100 ms or lower = very good; 200 ms = good) (Figure 32).

![Figure 32 Dialogue window fluorescence parameters](image-url)
10. Software STEVE
Displaying multi-channel images

When you visualize a multi-channel image, the overlay parameter must be set to 0 (Figure 33). The fluorescence control area in the lower right part of your software window provides access to all of the fluorescent channels (Figure 34).

- You can have the individual fluorescence image displayed separately or as a complete overview of all the fluorescence images (refer to Figure 34); to do so use the navigation bars in the fluorescence control area.
- Move a color bar to make the channel appear or disappear. All of the color channels that are at the moment displayed on your monitor will be recognized through the color bars (0 % to 100% transparency) (Figure 34).
10. Software STEVE

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 (see section "How to select the mounting medium's refractive index" previously explained). Once a refractive index is selected, a new dialog will open to specify the speed and the duration of the acquisition (Figure 35 green frame).

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 35 time lapse setting window
10. Software STEVE
Fluorescence time-lapse acquisition

Acquiring fluorescence time-lapses
To perform a time lapse, click on the 4D button or on Time Lapse in the Microscope menu. A selection dialog will appear to specify the speed and the duration of the acquisition, which can be individually set up (Figure 36). Once the settings for the time lapse, the refractive index and the fluorescence are selected, a new dialogue will appear to adjust the fluorescence channel parameters (Figure 37). Adjust the fluorescence parameters as per your needs (Figure xy9). Then click on „ok“. Your acquisition will start.

⚠️ Please note: in case of maximal speed during a time-lapse acquisition, the fluorescence channels are disabled!

![Figure 36 Time lapse setting window](image)

![Figure 37 Dialogue window fluorescence parameters](image)
10. Software STEVE

Time Line Area

While the 3D Cell Explorer-\textit{fluo} acquires multiple 3D reconstructions to create a time-lapse, you will be able to follow the exact chosen fluorescent and refractive index parameters for each frame in the lower part of the STEVE interface (Figure 38).

Figure 38 Time Line Area
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.
10. **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.
10. Software STEVE

Data Saving

How to save your data

The data can be saved by clicking on the Save icon (Figure 39). The holograms are kept by default within the .vol file.

Figure 39 Saving Window
10. 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 40.

Figure 40 Export data setting

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).
10. Software STEVE

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 41 Export data setting
10. **Software STEVE**

**Software Updates**

**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.
11. 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](http://www.nvidia.com/Download/index.aspx). If the error persists, please contact our customer service including error detailed information displayed in the error popup.
12. Compliance

WEEE
All qualifying products that are subject to the WEEE Directive and supplied by Nanolive are compliant with the WEEE marking requirements. Such products are marked with the "crossed out wheelie bin" WEEE symbol and in accordance with European Standard EN 50419.

RoHS
Based on information obtained from our component suppliers, this statement certifies that ALL products manufactured and supplied by CoolLED Ltd are in compliance with Directive 2011/65/EU of the European Parliament and of the Council of 8 June 2011 on the restriction of the use of certain hazardous substances in electrical and electronic equipment (also known as "RoHS"). This declaration is correct to the best of CoolLED Ltd knowledge, information and belief at the date of its release.