nVista User Manual

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Thank you for purchasing this Inscopix product.

It is important to have a thorough knowledge of the instructions presented in this manual to operate the machine. Inscopix reserves the right to make any changes without advanced notice. This manual contains description of products that may not have been purchased by the user.

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1 Introduction

1.1 Customer Support

We are dedicated to our customers and strive to provide excellent support of our products. Please log on to the Inscopix website to access our online repository of documents, videos and more at https://www.inscopix.com/support

For more information about personalized scientific support, please contact support@inscopix.com

To reach our sales team, please contact sales@inscopix.com

1.2 Warranty

Inscopix guarantees that the product is free from defects in material and workmanship. If you encounter any defects, please contact us at support@inscopix.com immediately and take all necessary precautions to avoid further damage. Addressing the defects or delivering a defect-free system will be done at the discretion of Inscopix. Products or parts that have been subject by user to unusual physical or electrical stress, abuse, misuse, accident, alteration, neglect, modification, use under improper protocol or operating conditions, failure to provide suitable storage, unauthorized repair or installation, or use of the product for purpose or in a manner other than that for which they were designed are not covered by the warranty.

No maintenance or repair work, except for the instances mentioned in the manual, may be performed on the microscopes. Only Inscopix manufacturing personnel may perform repair work on the microscopes. If your instrument happens to malfunction please contact at support@inscopix.com.

Please keep the serial number (located at the bottom of the DAQ box) for future reference.

1.2.1 iCare Expanded Warranty

Additional expanded warranty can be purchased for complete system repair or replacement. The service provides coverage on all functional parts of the product and labor associated with the repair of the product.

Please contact an Inscopix Sales Representative (sales@inscopix.com) for more information.

1.3 Safety Guidelines

IMPORTANT - The nVista system is a precision optical instrument. Please handle the system with care and read this section for safety warnings and instruction icons used in the manual. Disregarding these instructions may lead to injury or damage the system.

Caution

Warns user of a possible/potential danger. Improper use could damage the equipment.

Attention

Follow these instructions carefully.

Take note of the following guidelines for safe and proper function of the system.

- Do not disassemble the nVista system as it may damage or result in system malfunction. Improper handling may damage and destroy the system. If you encounter any problems with your system please contact support@inscopix.com.

- Do not allow the system to come in contact with any liquid (water, chemical agent, etc.) as it may cause a short circuit or result in system malfunction. When the product comes in contact with any liquid, unplug immediately.

- Long durations of continuous imaging can result in temporary photobleaching of fluorescent indicators in live tissue, such as GCaMP.

- Always protect the microscope with the lens cover when not in use. Make sure the system is unplugged before using the cover.

- Never insert any metallic objects into microscope or DAQ box frame, as this could result in equipment damage or personal injury.

1.4 New Commercial Package contents

Below are the contents of your nVista system package

1. Miniature microscope x 1
2. Data Acquisition Box x 1
3. USB Cable x 1
4. Gripper Assembly x 1
5. Adjustment Tool x 1
6. ProView™ Lens Probes x 5
7. ProView™ Implant Kit x 1
    a. Lens holding device
    b. Stereotax rod
    c. Screw
    d. Adjustment tool

Mouse application module:

1. Base plates x 5
2. Base plate covers x 5
3. Dummy microscope x 1

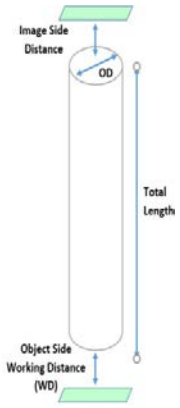
Rat application module:

1. Base plates x 5
2. Dummy microscope x 1
3. Protective cone x 1
4. Cable Sheath x 1
5. Cable sheath installation tool x 1

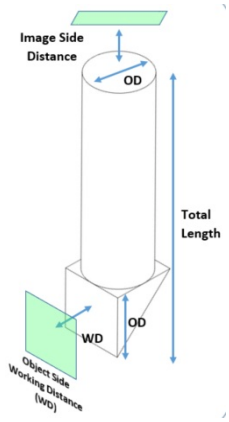
Keep the original factory box for future transportation and storage.

1.4.1 Lens Probe Variants

Your nVista Package includes five of the following lens probes and/or prism probes.



ProView™ Lens Probes	Part ID 1050-002202	Part ID 1050-002208	Part ID 1050-002211	Part ID 1050-002212	Part ID 1050-002209	Part ID 1050-002214
Diameter (mm)	1.0	0.6	0.5	0.5	0.5	1.0
Length (mm)	~4.0	~7.3	~6.1	~8.4	~4.0	~9.0
NA	0.5	0.5	0.5	0.5	0.5	0.5
Pitch	1/2	3/2	3/2	2	1	1



ProView™ Prism Probes	Part ID 1050-002203	Part ID 1050-002204	Part ID 1050-002213
Diameter (mm)	1.0	0.85	1.0
Length (mm)	~4.3	~3.3	~9.1
NA	0.36	0.36	0.36
Pitch	1/2	1/2	1

Numerical Aperture - NA

2 Technical Manual

2.1 Intended Use

The nVista imaging system is used for basic and preclinical neuroscience studies in rodents in:

- Science and research (colleges, universities) in biological fields
- Industrial applications (pharmacology/biotech)

The nVista Imaging System for rodents is a platform for visualizing large-scale neural circuit dynamics in freely behaving rodents. The complete system includes a miniature microscope, cables, Data Acquisition box (DAQ), nVista acquisition software (Mosaic) and hardware accessories, and will allow researchers to capture neural activity information at single cell resolution within specified cell types using epi-illumination. Today, nVista systems are generating transformative data sets in multiple brain regions of rodents such as:

- Cortex
- Hippocampus
- Hypothalamus
- Hindbrain

Key Applications of nVista:

1. Functional cell type mapping
2. Decoding neuronal ensembles

2.2 Getting Ready

- The nVista microscope is a precision instrument. Handle it with care and follow all warnings in the following sections.
- Do not keep the microscope under direct sunlight, in high temperature or humidity (For storage and operating conditions, refer to the section **Specifications**).

2.3 Care and Maintenance of the nVista system

Fig 2-1. nVista miniature microscopy system

- nVista Microscope
  - Always store your nVista microscope in the original box when not in use.
  - Handle the system only by the edges of turret to prevent damage to the objective lens. Any damage to the objective lens, including scratches and fingerprints, may degrade microscope images. Protect the objective lens with the provided lens cover when not in use.
  - Prevent hard impacts
  - ☞Keep the system away from liquids and adhesives. Clean the objective lens with 70% ethanol or pure water. (When using ethanol, always follow manufacturer instructions).

 Do not use too much liquid for cleaning, as it can enter the casing and damage the microscope.

- nVista set screws
    - nVista has two set screws: one placed at the microscope turret (focus set screw) and one used to assist in securing the microscope to the baseplate (baseplate set screw) when mounting for imaging.
    - Never overtighten the screws, as this may strip them and result in permanent damage to the system.
    - Always insert the provided hex key wrench completely into the screw.
    - ☞ Baseplate set screws should be tightened only until you begin to feel the resistance.
    - The focusing turret set screw should be tightened with a maximum of a ¼ to ½ turn after the screw makes initial contact with the turret.

- Lens probes
    - Handle the lens with care as chips or scratches may distort or obstruct the field of view inside the brain.
    - To remove any debris, acrylic deposits or condensation on the surface, very gently wipe the imaging surface clean with a lens paper and 70% alcohol.

- nVista DAQ Box
    - ☞ Always use original DAQ box and data cable pairing to ensure proper functioning and electrical safety of the equipment.
    - Clean the DAQ box with a soft, nonmetallic cloth and make sure it is always dry and free of contaminants.

- Cables
    - Avoid pulling on the cables and subjecting them to heavy strain. Refer to section **Connecting cables** for information on different cables used in the system.
    - When not in use, coil the microscope data cable gently.
    - To ensure data collection is not compromised, do not bend or twist the cables. Do not use force when connecting the cables.
    - ☞ Do not permit your animals to access the data cable. Suspend the cable above your set-up and provide some leeway so animals can move freely.

☞Regular inspection of the nVista system is recommended to maintain peak performance.

- Inspect the system for any dirt or dust; follow instructions in section **Care and Maintenance of nVista system** to keep system clean and dirt-free.
- Ensure that all cables are devoid of any wear and tear; damaged cables will cause electrical problems and may compromise data collection.

- Protect the system from liquids and ensure that it is always dry.

If you notice any defect in the product, contact support@inscopix.com

2.4 Operation : General optical principle and basic components of the nVista system

The nVista miniaturized microscopy system is a type of epifluorescence microscope which offers recording of neural circuit activity with high speed imaging of GCaMP calcium indicator fluorescence. The light source is a single wavelength LED which illuminates GCaMP labeled brain cells with 473 nm blue light.

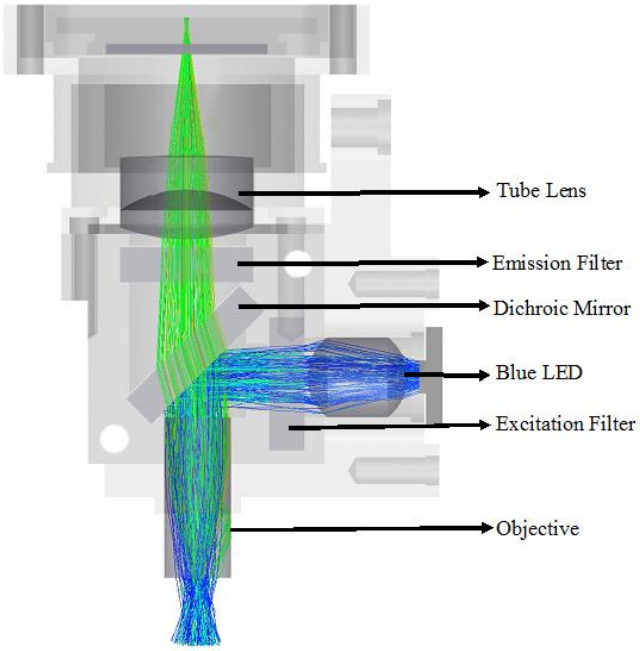


Fig 2-2. Schematic of internal light path in nVista microscope

The light output of the LED is passes through the excitation band-pass filter and is reflected by a **dichroic mirror** before being directed to the brain tissue by the **GRIN objective lens**. Green GCaMP fluorescence is collected by the objective lens, passes through the main dichroic element and emission bandpass filter, and is focused onto the **CMOS image sensor**. The **emission filter** blocks the wavelength of excitation light so that only the emission (green) light from the tissue is observed (Fig 2-2).

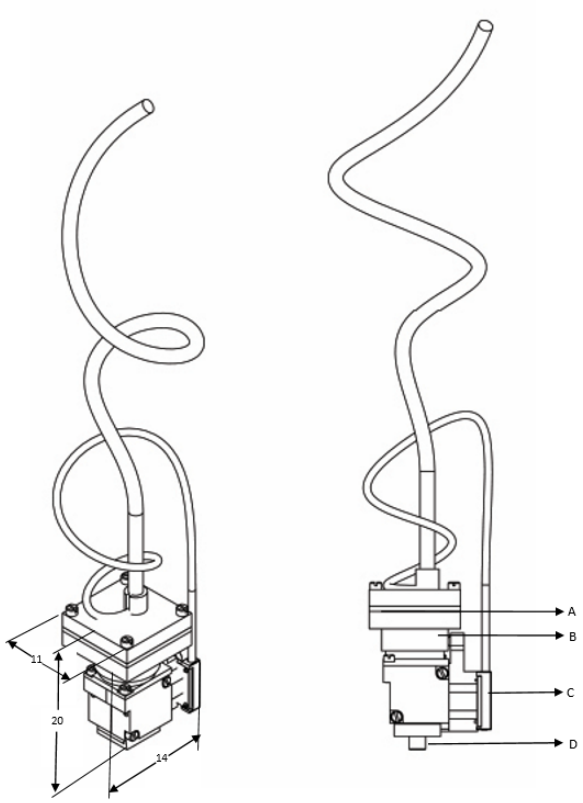
Focusing on a specimen plane using nVista is accomplished using a threaded turret at the top of the microscope. The turret allows for manual fine focusing by changing the distance between the image sensor and objective lens through a screw thread mechanism. Lowering or raising the

turret affects the focus plane of the tissue. For detailed information please visit **Introduction to Fluorescence Microscopy with nVista** page on our Knowledge base.

☞ Calcium indicators other than GCaMP can potentially be used with the nVista system, as long as the emission and excitation properties match the system specifications (Excitation: 475/10 (blue); Emission: 535/50 (green)), and signal to noise ratio is adequate for cell detection. For detailed information visit the **Guide on Calcium Imaging with Viruses and Indicators** page on our Knowledge Base.

2.5 System Overview

2.5.1 nVista miniature microscope system



Left - Outer Dimensions in mm

Right - Components of the nVista miniature microscope System
A - CMOS camera
B - Focusing mechanism (turret)
C - LED
D - Objective

Fig 2-3. Overview of nVista miniature microscope system

and its components

2.5.2 DAQ Box

The nVista system can communicate with an external hardware device using a TTL (**Transistor-transistor logic**) system. A TTL signal facilitates communication between nVista and the external device and makes it possible to coordinate and control multiple devices or instruments during an experiment. TTL signals are composed of an output voltage and current specification, an input voltage and current specification, and a signal rise/fall time specification. (Refer to DAQ Specifications for nVista and External Device Syncing Specifications).

2.5.3 Connecting nVista to external devices

The nVista DAQ box has two **GPIO** ports, a **Trig** port, and a **Sync** port that permit nVista hardware system to connect with external devices through coaxial cables with male BNC connectors (Fig 2-4).

Fig 2-4. nVista DAQ hardware showing BNC connectivity ports

2.5.4 General Purpose Input/Output (GPIO) ports 1-4

These input-output ports can be independently configured in the nVista software through the General Purpose Input/Output (GPIO) interface (Refer to **Interfacing with External Hardware** more information). All the ports might not be necessary for all experiments. These are passive "listeners" of external signal (input), or senders of a sync signal (output) that does not reflect the frame by frame timing of an nVista recording.

TRIG : This port allows an external device to trigger the start and end of an nVista recording.

SYNC : This port outputs the state of the nVista system during an nVista recording. This signal is used after recording to synchronize nVista movie data frame by frame to behavioral or other time-sensitive experimental data.

☞ The states of all the ports can be saved in an HDF5 formatted file along with the regularly imaged data. Make sure **GPIO Export** is checked under Preferences in the nVista Software to save the data from your ports as this is not a default setting.

2.5.5 How to trigger nVista

To enable triggering of nVista recordings via an input to the TRIG port, connect the output port of the external device to the Trig port of nVista with a BNC cable.

With nVista set to be controlled by the external device, configure the external device through its hardware and software interface to deliver the appropriate TTL pulse to nVista during experiment to start and stop a recording.

Use Device > Trigger from External Hardware to trigger a recording using an external TTL signal (see Interfacing with External Hardware for more information).

☞ Ensure that the external device is physically connected to the nVista Trig port before configuring the above, or nVista may begin to record as soon as you set "Trigger from external hardware".

2.5.6 How to use the Sync signal

The Sync signal serves as a record of whether the nVista image sensor was recording at any given time during a recording. You have the option of controlling what duty cycle the Sync signal is displayed at. Broadly speaking, **duty cycle** expresses the proportion of time that a device is operated as a ratio or percentage.

2.5.7 How to use the General Purpose Input-Output (GPIO) Ports

The additional two TTL-based I/O ports available on the nVista DAQ box are “passive”, as they cannot trigger nVista recordings. Their role is to provide additional ways to synchronize nVista with other devices being used simultaneously during experiments.

Refer to **Interfacing with External Hardware** for more information.

2.5.8 System Specifications

nVista 2.0	
Modality	Single-channel epifluorescence
Operating Conditions/Environment	Indoor use only Temperature: +10°C to +40°C Humidity: 85% RH max. (no condensation)
Transport/storage conditions	Temperature: -20°C to +60°C Humidity: 90% RH max. (no condensation)
External dimensions and weight (main body)	External dimensions: 11 mm x 14 mm x 20 mm Weight: ~ 2 g
Wavelength	Excitation: 475/10 nm (blue) Collection: 535/50 nm (green)
LED	2.0 mW/mm^2 at 100%. (Note: The LED power output is linear from 0-1.2mW; the software LED percentage is a linear scale from 0% to 100%.) Spectral peak : ~470 nm
Integrated HD Camera	Maximum FOV: 1440 pixels x 1080 pixels; 900 µm x 650 µm f Temporal Resolution: 25 fps at full FOV (dependent on computer system)
DAQ Specifications	Uses TTL signals (0-5 V) syncing and triggering of outputs through BNC ports to external hardware

DAQ	
USB Power Input	Max 5 V / 500 mA
GPIO Input (digit)	0-5 Low: < 1.5 V High: > 3.5 V
Trig Input Range	0-5 Low:<1.5 V High:>3.5 V
Dimensions Weight	15.7 x 6.82 x 2.85 281 g
USB Connector Type	USB 3.0 Micro-B
GPIOs Connector Type	BNC Connector

3 System Startup

☞ Please read the **Safety Guidelines** carefully before operating the microscope. Unpack all the components and verify completeness. Keep the original package to store the system and in the event you need to send it back to the manufacturer for servicing.

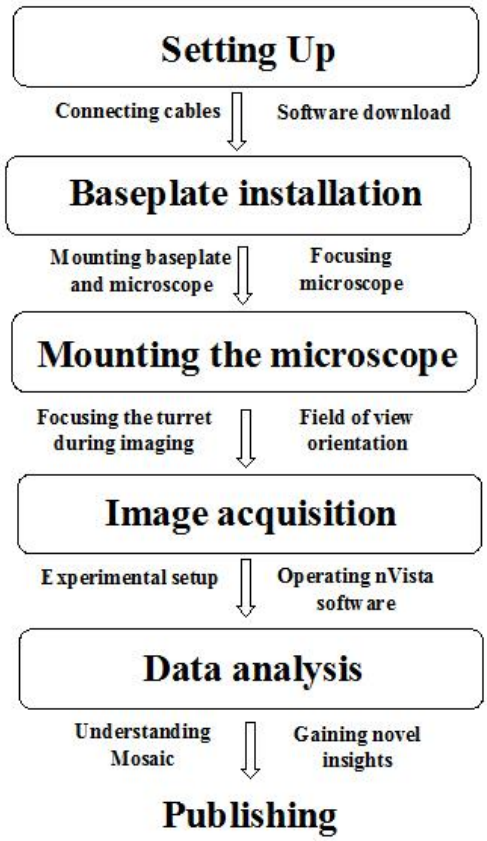


Fig 3-1. nVista Workflow

3.1 Connecting Cables

The nVista system is equipped with 2 cables:

- ➢ The microscope data cable, which connects the microscope to the DAQ box.
- ➢ The USB cable, which connects the DAQ box to the computer.

 Failure to assemble the system with correct cable connections could damage the equipment and interfere with proper system function.

☞Always secure the DAQ box to your experimental setup and ensure that cables are not put under stress. Make sure the cables are secured and do not hinder animals from engaging in desired behavior.

3.2 Mounting the baseplate and microscope during baseplate installation

Refer to the nVista Knowledge Base for your animal model for instructions on viral injection and probe insertion surgical procedures. For more streamlined lens probe insertion, use our **ProView™ Implant Kit** which comes in all configurations as your lens probe(s). Visit the **ProView™ Implant Kit** page on our Knowledge base for specs, assembly, and use.

☞ **ProView™ Lens Tip:** As the cuff takes up 1.23 mm length of each lens, make sure to consider this when calculating the desired depth in the brain. Visit the **ProView™ Implant Kit** page on our Knowledge base for detailed instructions.

Install the nVista microscope with the baseplate above implanted probe imaging face. The baseplate acts as a docking station for the nVista microscope and allows for easy attachment and detachment of the microscope for multiple imaging sessions. Visit the **Baseplate Install** page on our Knowledge base for the complete procedure.

☞ After baseplate installation, adhesive/acrylic shrinkage may change the distance between the objective and the probe imaging face, thus changing the location of the focusing plane within the tissue. It is important to take this into account to ensure access to the same imaging plane.

☞ Ensure that the baseplate is correctly oriented before mounting (see image below for reference).

☞When mounting the microscope on the baseplate, the baseplate will be locked tightly to the microscope by gently advancing the set screw inwards using a hex key (until it comes up against the microscope wall). Only ¼ to ½ turn of the set screw should be needed to secure and lock the baseplate to the microscope (Fig 3-2). Since baseplates and lenses are delicate and permanent adhesives are used for installation, their reuse is not recommended.

 Stop when you feel resistance to prevent damage to microscope housing.

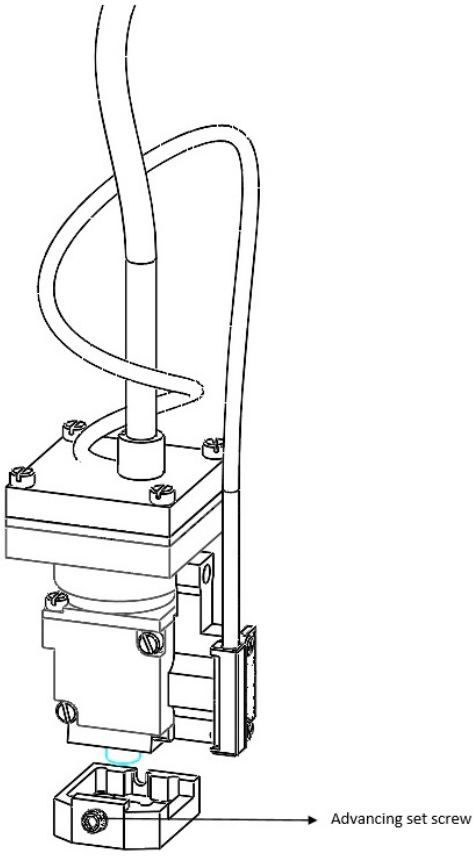


Fig 3-2. Locking the baseplate with the microscope

☞Always ensure that the nVista microscope objective lens and the probe imaging face are parallel.

Recommendation: Before you start using the nVista microscope, practice working with the dummy microscope to prevent potential damage to your nVista system.

3.2.1 Finding the focus during baseplate installation

The nVista microscope has a focus range of 100 µm - 300 µm below the face of the objective lens. The nominal focus is ~200 µm with ± 100 µm in focus adjustment. (When the microscope is on the baseplate, the objective protrudes 700 µm through the baseplate). The plane of focus depends on the proper position of these components:

1. **Baseplate** - An air gap of about ~500 µm between the two lenses (microscope objective and the lens probe) ensures optimal focus. Please refer to nVista Knowledge Base for your animal model for a detailed protocol on baseplate installation.

2. **Focusing Turret** - The working range (vertical) of the turret is 2 mm and functions as a fine focusing mechanism. During baseplate installation always position the turret at mid-height which will allow for finer focusing/allow greater focusing range, when imaging your brain region of interest. Do not move the turret from this position at this point.

   ☞There may be some adhesive/acrylic shrinkage after baseplate installation which will change the distance between the objective and the probe imaging face, thus changing the location of the focusing plane within the tissue. It is important to take this into account to ensure access to the same imaging plane.

3. **Lens Probes** - Straight lens probes have a maximum working distance (WD) of ~300 µm. Most lens probes are designed with an approx 500 µm ideal distance between the nVista objective lens and the lens probe, with the focusing turret at the middle of its focusing range (the overall system is optimized for this gap distance). As the gap increases, the WD shrinks. In certain situations, you may wish to alter this gap if the lens probe was not implanted in the ideal location relative to the labeled cells (Fig 3-3).

   ☞ Gaps smaller than 400 µm are unlikely to provide useful field of view due to light scattering and gaps larger than 650 µm would bring the WD inside the lens probe body.

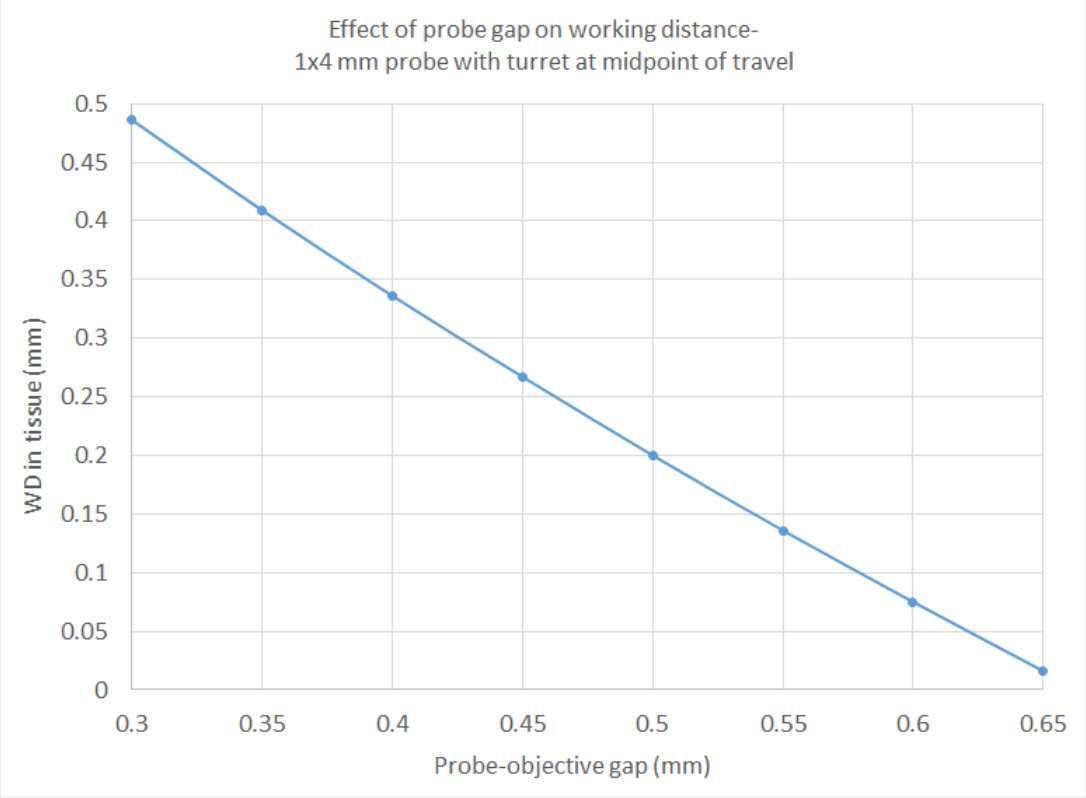


Fig. 3-3. Effect of changing air gap on the working distance: Graph illustrates effects of changing the air gap between nVista objective and a 1 mm diameter, 4 mm long straight lens probe on the WD in the tissue. For a probe-objective gap of 500 µm, the WD in the tissue is 200 µm. As the gap increases, the WD shrinks, and vice versa. Gaps smaller than 400 µm will cause tissue scattering of light and gaps larger than 650 µm will bring the WD inside the lens probe body (This effect applies to all lens probes).

3.3 Mounting and focusing the microscope during imaging sessions

Visit **Microscope Mounting** page on our Knowledge base for detailed description and video tutorial of baseplate installation and microscope mounting.

3.3.1 Focusing the microscope during imaging sessions

☞These steps should help you to refine your focus:

- Put on lens cover to prevent fingerprints. Hold microscope by the base and keep LED wires out of the way.
- Before changing the turret position, make sure you loosen the set screw with a hex key. Failure to do this will wear down the microscope housing and make it difficult to secure the turret in the future.
- Remove the lens cover and turn the top turret slowly to check the field of view (FOV) on the nVista software, if you are adjusting focus for an animal preparation. Make sure that the image gets sharper with this adjustment.
- It is normal for the FOV to rotate with the turning of the tower. Tighten set screw (¼ to ½ turn clockwise) once you have reached the desired position. **DO NOT OVERTIGHTEN**. Failure to do this will create a groove in the microscope housing and make it difficult to secure the turret in the future.
- When the focusing turret is in the middle of its 2 mm vertical range (2 turns from top or bottom), the working distance of the objective lens is ~200 µm below its imaging surface with a ± 100 µm focusing range. On turning the turret clockwise the FOV goes deeper into the tissue.

☞When rotating the turret, guide the LED cable to curl around the base of the thicker microscope cable.

☞It is recommended to adjust the focus by turning the turret in an anesthetized animal or after removing the microscope from the headcap if animal is awake.

3.4 Unmounting the microscope

Please follow these steps to unmount the microscope from the baseplate:

- ➢ Make sure the adhesive has completely cured and the baseplate is affixed to the skull after baseplate installation and before unmounting the (dummy/nVista) microscope from the baseplate.
- ➢ Loosen the set screw located on the side of the microscope.
- ➢ Gently grip the microscope by the upper tower, and pull straight upwards. Application of gentle downward pressure on the baseplate might make it easier to unmount the microscope.
- ➢ Make sure to use the baseplate cover after every imaging session which will help to keep the imaging surface clean.

3.5 Field of view orientation through the microscope and lens probes

The nVista microscope rotates the image of the recording brain regions depending on the orientation of the focus turret. The orientation of the image can be determined by observing the LED wires as they emerge from the cable. Additionally, the lens probe implanted in your animal also determines the orientation of your field of view. For more information on image transformation through lens probes and turret position visit **Guide on finding the field of view orientation when imaging with nVista** page on our Knowledge base.

Straight probes : Straight probes with odd numbers of pitches introduce a 180 degree rotation; straight probes with even number of pitches do not alter the image (Refer to the **Probe Variants** section for more information).

Prism probes : Prism probes introduce a vertical flip or mirroring of the image and 180° rotations with each added ½ pitch (Refer back to the **Probe Variants** page for more information).

☞ Straight lens probes themselves have no magnification, provided the gap between probe and microscope objective is set at 500 µm. Prism probes have a magnification of about 0.8 (they are demagnifying), which gives them a slightly expanded view relative to straight probes of equal diameter.

☞It is important to return to the same focus for longitudinal data collection to ensure the same cellular population is being studied over time as the orientation of image sensor changes with change in focus.

3.6 Image Acquisition and control

The nVista microscope allows real-time image acquisition for studying large groups of neurons at single-cell resolution. The data acquisition software is included in your package and supports control and acquisition of calcium imaging data and control and logging of optogenetic modulation. Follow these steps for efficient operation of your nVista system:

- **Preparing your computer** - Ensure that your computer is capable of obtaining and storing data with the nVista Acquisition software.
- **Connecting the system to the computer** - nVista sends data to DAQ box and the acquisition software acquires data from DAQ box and displays it on computer. Refer to section **Connecting the cables** for instructions.
- **Install software** - Install nVista data acquisition software and Mosaic processing and visualization software.
- **Settings** - Refer to **Mosaic software** manual for workflow tutorials.

4 Software Manual

4.1 Getting Started with nVista Software

4.1.1 System Requirements

The high-definition (HD) data acquired by the nVista system can require a significant amount of hard disk space during usage. As a guideline for hard disk budgeting, acquiring 30 minutes of data at the frame rate of 20 Hz and at full image resolution would require approximately 110 GB of hard disk space.

- Pentium i7 processor
- 8 GB RAM
- 200 MB free Hard Disk space (install only; more space is required for recording)
- 64-bit Windows 7 Professional and Windows 10
- USB 3.0 ports
- USB Hub (recommended device available here)

☞V1 Hardware will use USB2.0 protocol.

4.1.2 Installation

This section describes how to install nVista on your computer. First check that your computer meets the recommended System Requirements.

The installation file for **nVista Acquisition Software (Version 2.0)** is available on the Inscopix website.

During installation, Python 2.7 and several Python modules (64-bit versions) will be installed to support the nVista System. The installation steps are as follows:

1. Download the software installation package from Inscopix website.
2. Run the software installation package. Python 2.7 will be installed at this time.

Run Inscopix nVista: use the desktop shortcut or select Start > Programs > Inscopix > Inscopix nVista.

☞ Unless otherwise noted, software update distributions can be installed without manually uninstalling the current software version. Please contact Inscopix technical support at support@inscopix.com if any issues arise during the installation process.

☞ From version 2.0.4, the installer includes an option to send anonymous installation statistics to Inscopix.

5 Data organization and acquisition modes

5.1 Project and Project Directory Relationship

Data in the form of single images ('snapshots') and image series ('recordings'), log files, and data acquisition settings are stored in *Projects* within a *Project Directory*. Each *Project* can represent an experiment, an experimental subject, or different experimental paradigm. One *Project* can be opened at a time in the nVista software. A *Project Directory* is a system folder that contains *Projects* created by the nVista software. This is shown in Fig 5-1.

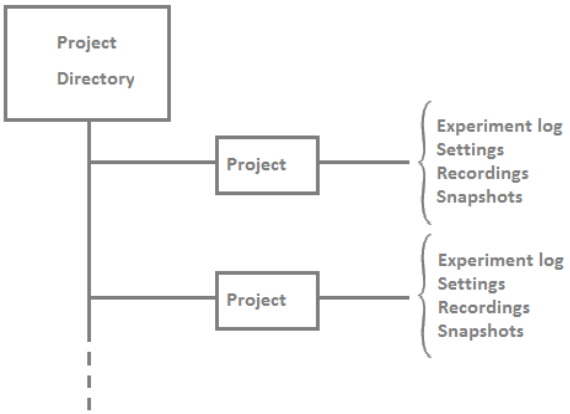


Fig 5-1. Schematic illustrating the relationship between *Projects* and the *Project Directory*

After launching the nVista software, select the *Project* to use in the session. In the opening dialog box select a *Project* from the available *Projects* in the active *Project Directory* (green boxed area). To create a new *Project*, enter a *New Project* name and click **Create** (blue boxed area) to add the Project to the list. To change the active *Project Directory*, click **Browse** (red arrow) and navigate to the desired location on the computer.

By default, the *Project Directory* is C:\Users\<username>\inscopix. Click **Open Project** after selecting the *Project* as shown in Fig 5-2.

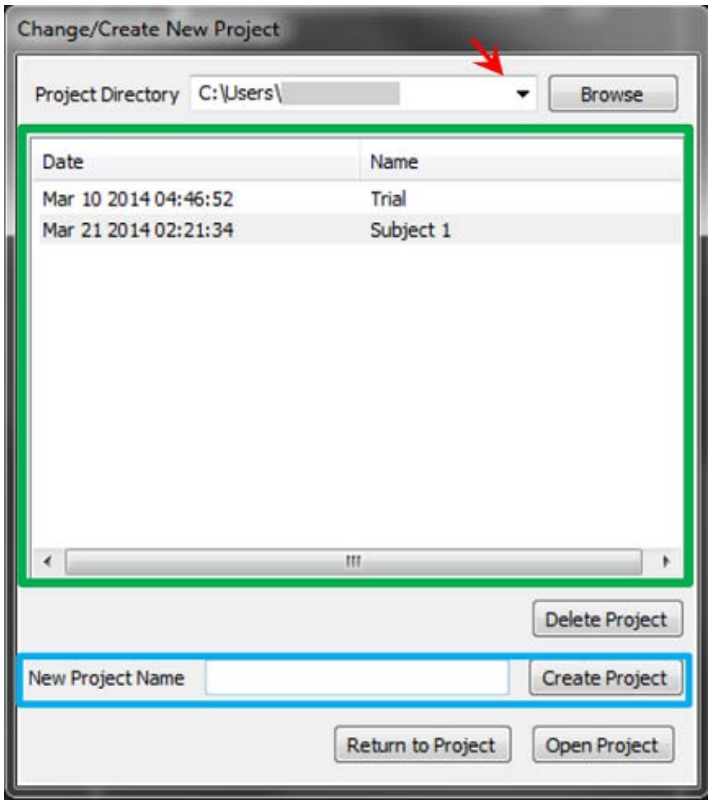


Fig 5-2. Using the Select/Create Project dialog box to select the Project

For best performance, it is recommended to keep the Project Directory on an internal hard drive instead of a USB-connected remote hard drive.

5.2 Storage of project settings

Project acquisition settings are stored in a master settings file for the Project ('settings.prj'). The master settings file can include multiple user-determined settings profiles. A given saved settings profile can be reloaded in the software at a later time in order to resume acquiring data using those settings, accessible through the Edit menu.

See Best practices for Conducting Imaging Sessions section for tips on how to adjust the project settings.

6 Data management

It is recommended to set up and maintain a separate Project for each experimental subject (see Fig 5-1). Different settings profiles can be saved for each imaging session as noted in Storage of project settings. This settings management feature will facilitate quick retrieval of settings profiles among various imaging sessions. The image sensor contained within the camera module records data at 12-bit depth (0–4095) and has a maximum image resolution of 1440 × 1080 pixels.

Snapshot and recording data can be saved by the software directly to one of three formats: compressed **RAW** format, uncompressed **TIF** format, or hierarchical data format **HDF5** (see *Data Acquisition Modes: Snapshots and Recordings*). RAW files must be decompressed with the Inscopix Decompressor software (see *Using the Image Decompressor*) into TIF or HDF5 files for data analysis. Uncompressed TIF files are 16-bit grayscale, multi-page TIF files which can be viewed in any software that can open multi-page TIF files. HDF5 files can be opened in Mosaic and Mosaic Matlab API.

6.1 File naming convention

Imaging data (see *Data Acquisition Modes: Snapshots and Recordings*) are automatically saved by the nVista software using either the default file name system or a custom file name system. The current file name pattern is available by selecting **File > Custom File Names**. In the default file name pattern, the following format is applied for automatic timestamping:

- snapshot_yyyymmdd_hhmmss
- recording_yyyymmdd_hhmmss

For example, a snapshot acquired on February 15, 2016, at 03:34:18 PM could be named “snapshot_20160215_153418.tif”. Alternatively, different text markers and/or timestamps in a custom file name pattern can be arranged by selecting **File > Custom File Names**. This is shown in Fig 6-1.

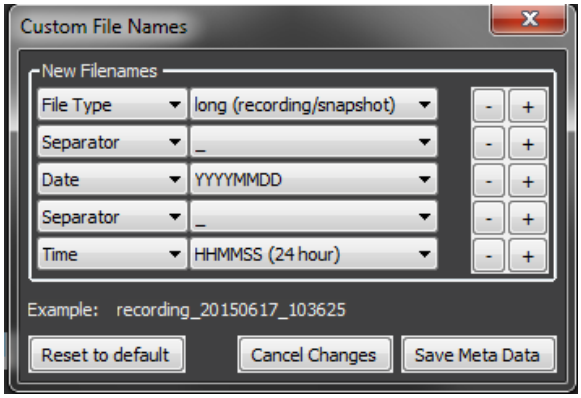


Fig 6-1. Setting up a custom file naming pattern

6.2 Data Acquisition Modes: Snapshots and Recordings

Data is acquired on-demand either by pressing the Record or Snapshot button (see Fig 6-2) or by external hardware triggering (if enabled). A specific schedule can be set by configuring a recording schedule.

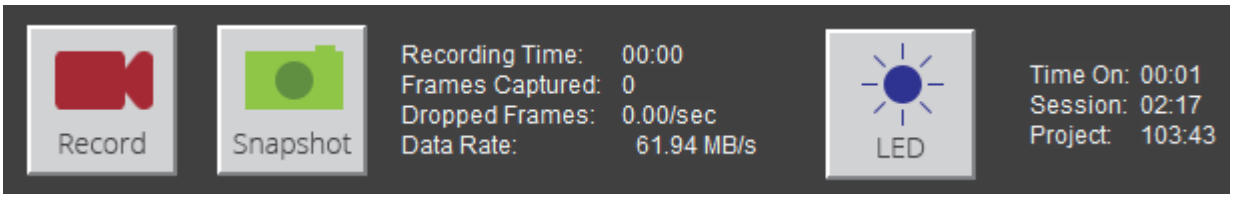


Fig 6-2. Data acquisition can be initiated on-demand from the main display (arrows shown)

There are two data acquisition modes: single image ('snapshot') or continuous series of images ('recording'). Each acquisition mode generates data in two or more distinct file types:

- One or more RAW (compressed), TIF (uncompressed) or HDF5 files
- One XML document.
- One settings.prj document (only if recording in .RAW format)

The XML document can be opened by any text editor and contains information about the following default data acquisition parameters, with more user-configurable information available in Preferences:

- Size and location of the captured field of view (in units of pixels)
- Exposure time (in units of ms)
- Frame rate (in units of fps)
- Sensor gain (unitless)
- Light source enabled-on-status and power level (in percentage 0-100%)
- Number of captured frames, excluding dropped frames (unitless)
- Total count and indices of dropped frames (in absolute frame count)
- Duration of the recording (in the format <min:sec>)
- RAW, TIF, or HDF5 file name(s) associated with the recording

Specification of the content for XML files is set through the *Edit menu*.

Each single image acquisition, or 'snapshot', captures a single frame of image data. The single image acquisition mode allows for collection of static data as well as reference images. nVista 2.0 allows for snapshot data to be saved in three formats:

Format	Pros	Cons
(RAW)	• Smaller file size	• Additional decompression step lengthens analysis workflow
(TIF)	• Immediate data visualization	• Larger file size
(HDF5)	• Efficient file structure for data analysis	• Larger file size

6.3 Capturing recordings

Each image series recording generates one or more files which contain many successive frames of image data. nVista 2.0 allows for recording data to be saved in three formats:

Format	Pros	Cons
(RAW)	• Smaller file size	• Additional decompression step lengthens analysis workflow • Maximum individual file size ~ 4 GB
(TIF)	• Immediate data visualization	• Largest file size • Maximum individual file size ~ 4 GB • Maximum speed of data acquisition (i.e. fps) may be limited by computer specifications • Inefficient file structure for data analysis
(HDF5)	• Efficient file structure for data analysis • Unlimited (OS-dependent) individual file size	• Largest file size • Maximum acquisition fps may be limited by computer specifications

Recordings saved as RAW and TIF files will be greater than a certain total data size (approximately 3.9 GB) are automatically split into multiple file segments. Each individual data

file shares the same file name stem (see *File naming convention*) and receives a different, sequential end tag. For example:

- First file segment: recording_yyyymmdd_hhmmss.raw
- Second file segment: recording_yyyymmdd_hhmmss-001.raw
- Third file segment: recording_yyyymmdd_hhmmss-002.raw

Data files saved with a custom file name pattern will be similarly tagged with a different, sequential end tag if multiple data files are necessary. There is no data loss between subsequent file segments since the software executes continuous data acquisition without interruption.

☞TIF and HDF5 data formats not available with V1 Hardware.

7 Display Interface

7.1 Opening the Display

After connecting the microscope as described, open the nVista software. You can either create a new Project, or select an existing Project from the current Project Directory. Available Projects are shown in the menu on the left, see Fig 7-1.

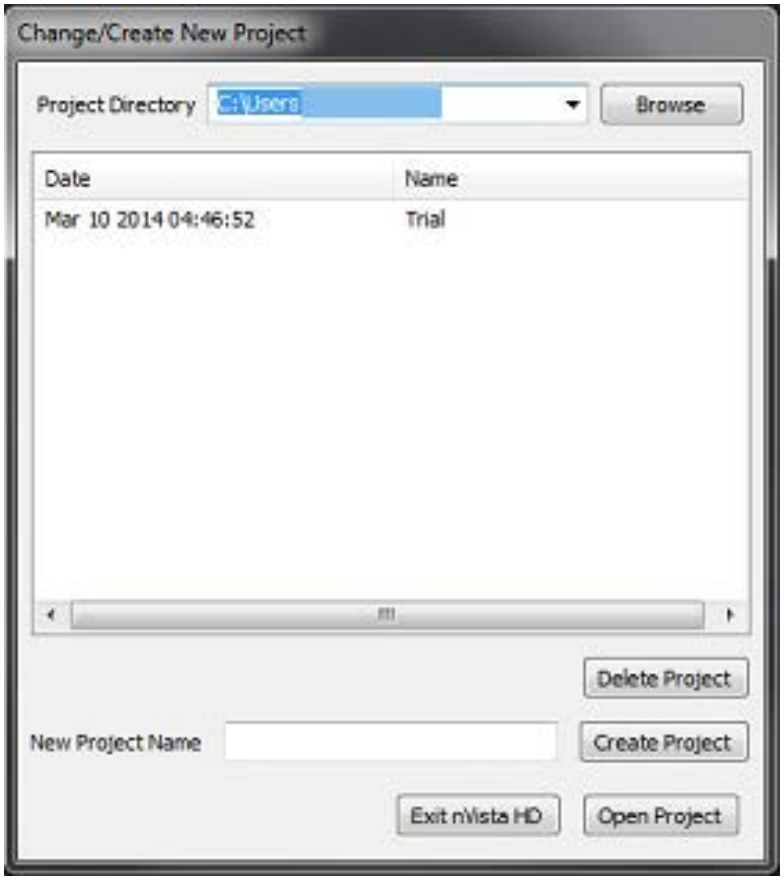


Fig 7-1. Using the Select/Create Project dialogue box to select a Project

Click **OK** after selecting the Project (see *Project and Project Directory Relationship*). The nVista software display will be activated and the name of the Project will be displayed in the upper left corner of the display.

The microscope can be in the connected or disconnected state. With the microscope plugged in to the DAQ box, and the DAQ box connected to the USB3 device, click **CONNECT** in the middle of the nVista software screen (or select **Device > Connect**) to open communication between

the devices. When a microscope is connected to the software, the **Status field** displays Status: Connected.

If you see Status: Disconnected, this indicates that the microscope has not connected properly with the software. Ensure that the DAQ box is properly plugged in, that the computer's USB3.0 driver has been updated to the latest version (check your computer's website), and that all nVista system and USB firmware has been correctly installed. Please refer to the troubleshooting section of this manual for more information.

The software supports connection to a single device and only one active instance of the nVista software is supported. Attaching multiple nVista systems simultaneously to the computer may prevent the software from working.

☐ Do not attach multiple nVista systems simultaneously to the computer; doing so may prevent the software from working.

7.2 Display Screen

Data acquisition is initiated from the main display. Controls for image sensor settings, light source settings, and recording and snapshot acquisition are available in menu pull-downs and the moveable windows on the right of the nVista software.

There are three microscope control buttons in the main display window, shown in Fig 7-2:

- Record - begin or end an image series recording
- Snapshot - capture a single image
- LED On/Off - toggle on or off the LED light source.

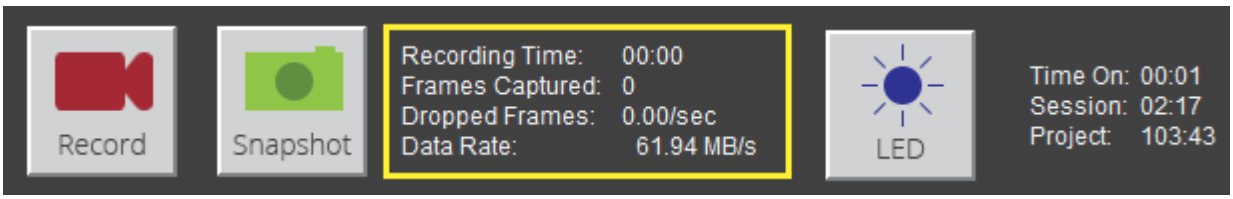


Fig 7-2. The Record button begins or ends a recording; the Snapshot button collects a single image; the LED button toggles the light source on and off

While a recording is streaming to the hard disk, information about the recording status (number of frames acquired and recording duration), is displayed and periodically updated in the **Status field** to the right of the microscope control buttons (yellow boxed area in Fig 7-2). The file name

is displayed after the recording has ended. The settings controls in the subwindows are disabled during recording.

To view the intensity level for an individual pixel within the active image area, hover the mouse cursor over the desired pixel within the field of view. The intensity level and the pixel's (x,y) coordinates are shown in the bottom of the display (white boxed area in Fig 7-3).

If any errors are detected during operation, an Error Icon will appear by the LED statistics  . Clicking on the Error Icon will bring up an error menu containing the error log text, which can be exported for software troubleshooting. For troubleshooting help, please refer to the Troubleshooting section of this manual. To report a bug, please email support@inscopix.com.

7.3 Customizing the Display User Interface

To provide maximal experimental flexibility and functionality during image acquisition, the main software tools and capabilities are packaged into discrete windows, which can be displayed or hidden (Fig 7-3). Each window constitutes a central function and allows the user to have easy and convenient control of the hardware and imaging settings for the task at hand.

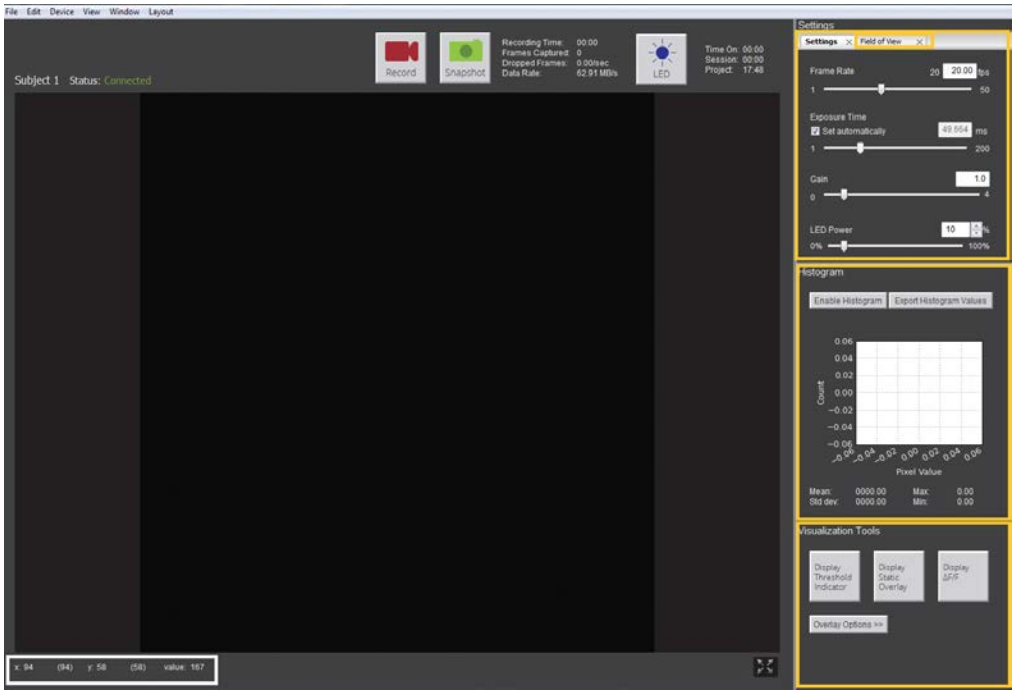


Fig 7-3. The yellow boxed regions in the software workspace highlight the Settings window, with the Field of View window tabbed behind it; the Histogram window; and the Visualization Tools window

The software workspace can be customized with any combination of windows by opening them from the Window, or Layout menus and physically moving them to your desired physical location within the software workspace. A given window will modify its dimensions to fit the workspace based on where you place the window within the workspace.

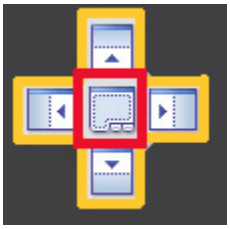


Fig 7-4. Location icons for docking and tabbing windows. The yellow boxed icons will dock a floating window in the location indicated. The central red boxed icon will dock a floating window in the middle of the current window in a tabbed format.

- **Undocking**: windows can be undocked from the main workspace by clicking the top of the window and dragging. The undocked window will float in place once you release the mouse button. If you hold the left mouse button down while dragging the window, location icons will appear in the workspace at the four outer edges as well as in the center of the nearest workspace area.
- **Docking**: Drag the window to the desired workspace location and position your mouse beneath the location icon pointing to the region you wish to dock into: top, bottom, right or left (Fig 7-4, yellow boxes). The corresponding workspace region will be overlaid in blue . When you release the mouse button, the window will dock in that location.
- **Tabbing Windows**: In order to tab a Window behind another Window, first grab the Window to undock it and then click and hold the left mouse button while dragging the Window. Tab the Window directly behind another existing Window by positioning your mouse in the center location icon of the existing Window's workspace window (Fig 7-4, red box).

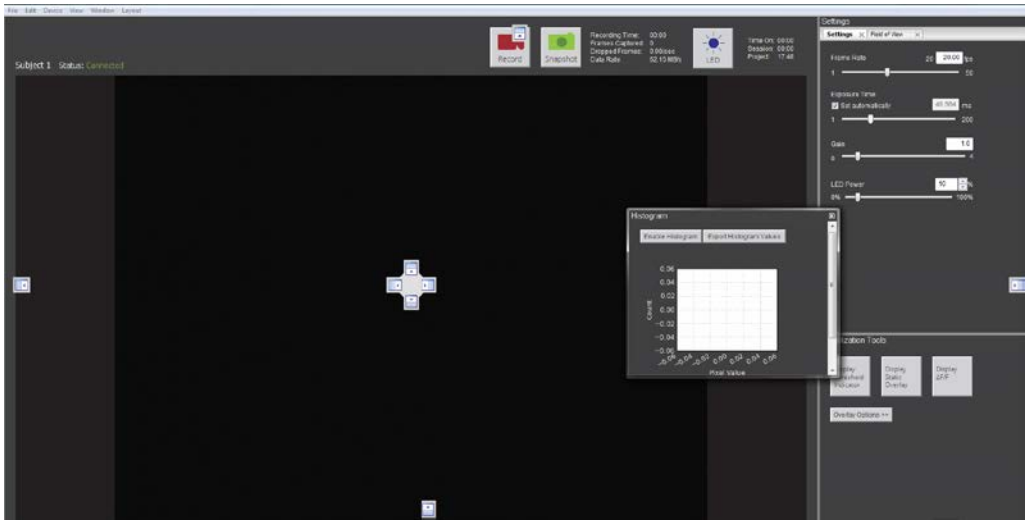


Fig 7-5. Dragging the undocked Histogram Window causes Location Icons to appear in the workspace. These provide you the option of docking the window in any location by moving the Window over the corresponding location icon.

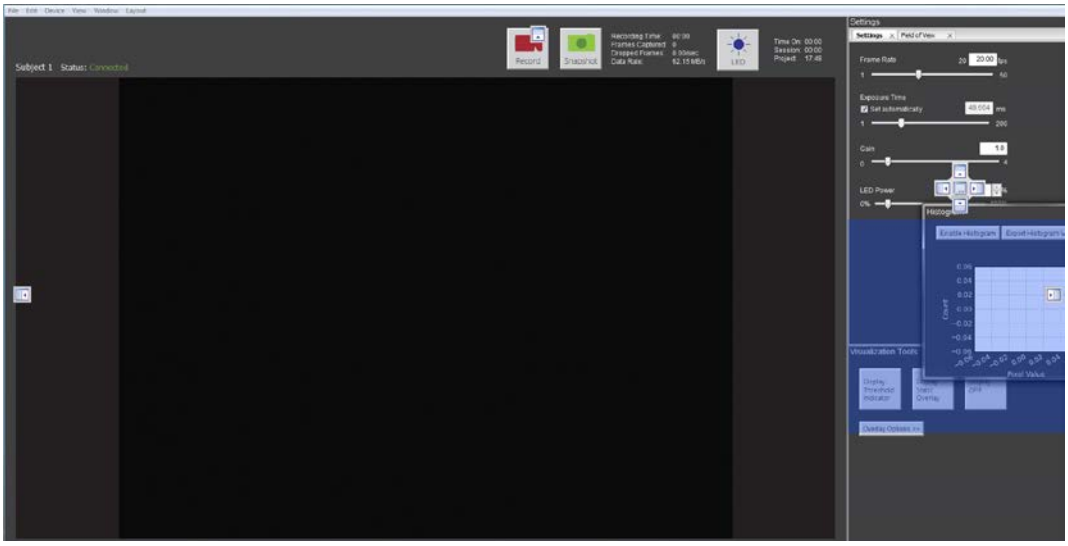


Fig 7-6. A blue shaded region appears where the Histogram Window will be redocked in the workspace when it is dragged over the relevant location icon.

7.4 Display Menus

There are six menus in the menu bar of the main display:

1. File menu

2. Edit menu
3. Device menu
4. View menu
5. Window menu
6. Layout menu

File menu

File > About
Use this window to find information about the current hardware serial numbers and software version.

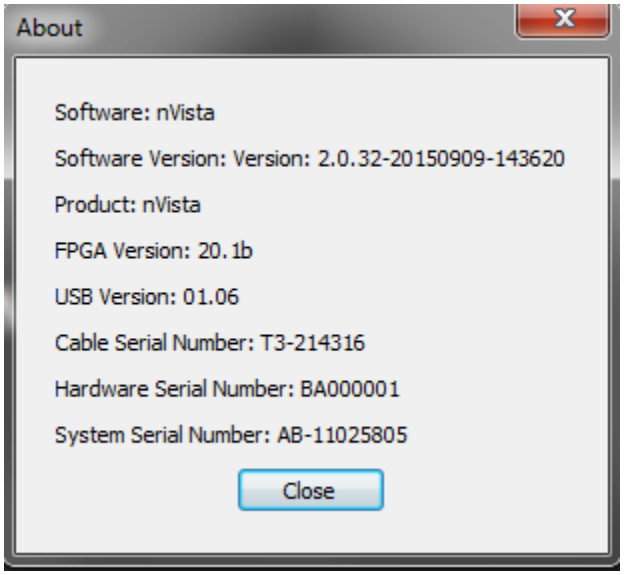


Fig 7-7. Displaying information about the software and hardware

☞**NOTE:** When contacting support@inscopix.com regarding any software or hardware issues, it is often very useful to attach a screenshot of the “About” window.

File > Help
Use this window to find information about the website, support email and nVista user manual.

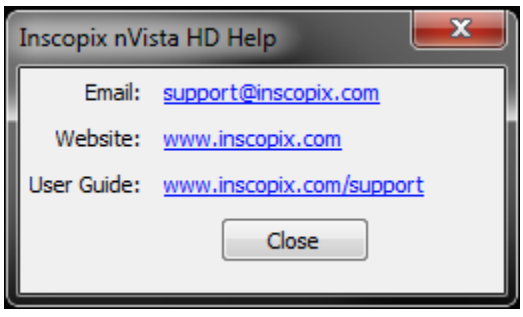


Fig 7-8. Displaying help and support contact information

File > Change/Create New Project

Use this window to change the active Project. Enter a name for a new Project and click **Create** or choose an existing Project listed in the Recent Projects window, and then click **OK**.

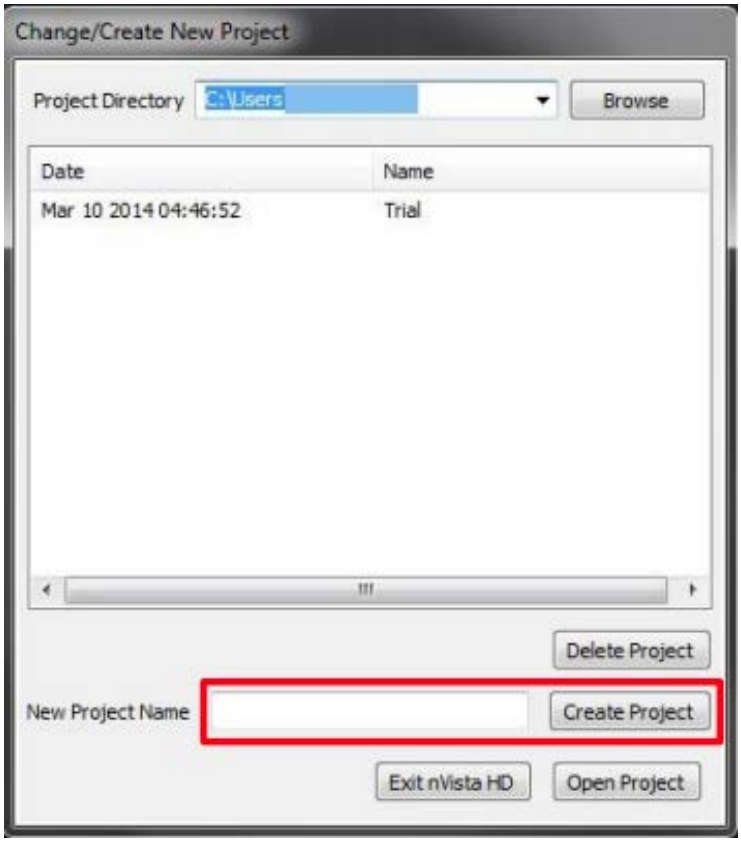


Fig 7-9. Change/Create New Project

To access a Project in a different Project Directory, first change the Project Directory using the Browse button, then select from the available Projects or create a new Project.

File > Edit Project Name

Use this window to change your current Project name.

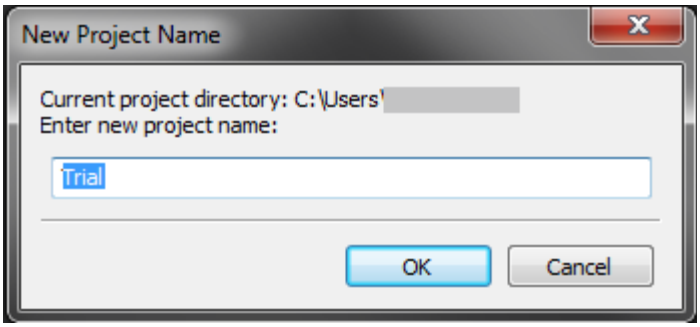


Fig 7-10. Editing your current Project name

File > Custom Meta-Data

Add custom meta-data to your Project files by checking the box and typing in the desired information.

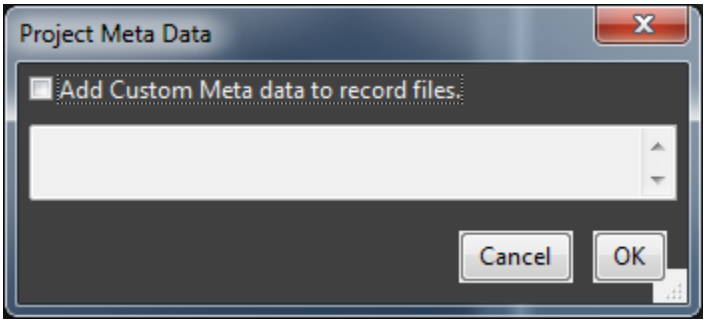


Fig 7-11. Specifying project meta-data

File > Custom Filenames

To create a custom file naming pattern, select **File > Custom File Names** and configure the desired naming pattern. Enter additional fields by pressing - or +.

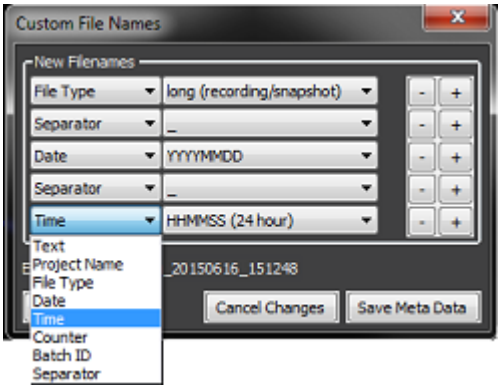


Fig 7-12. Setting up a custom file name pattern for snapshots and recordings

File > Quit

Exit the program.

Edit menu

Edit > Undo/Redo

Undo or redo the last setting change.

Edit > Save/Load Settings
Save and recover past session settings.

Edit > Change Preferences

Customize your preferred settings. There are several tabs in this window:

- **General**
  1. **Export Log Automatically** allows the user to toggle automatic log exports to file when program closes.
  2. **Prompt to send error** log prompts the user for bug submission notification when errors occur.
  3. **Use IO 1/2 as output** define the functionality of the I/O ports (checked=output; unchecked=input).
  4. **Export GPIO data file when recording** allows the user to toggle saving of GPIO data to a separate file when recording. This feature is only available if the frame rate is larger than 17 frames per second.
  5. **External record trigger polarity** allows the user to control external record trigger polarity. For example, when set to “Active Low”, the hardware records while it sees a 0 V signal on the external trigger port and is idle while it sees a 5 V signal. (**Note**: the external trigger is tied internally to 5 V if no external device is attached to the port.)
  6. **External signal duty cycle** controls duty cycle of sync signal output port.

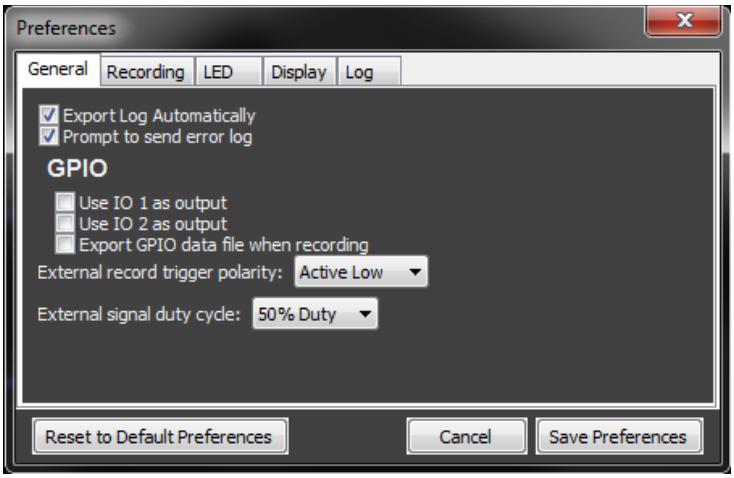


Fig 7-13. General Preferences dialog box

☞**NOTE:** GPIO functionality is not available with nVista V1 hardware.

- **Recording**
  1. **Save Format** controls which file format will be used to save data.
  2. **Max filesize before rollover** sets the maximum individual file size before creating a new file for data storage.
  3. **Downsample saved images** enables saving directly to 2X, 3X or 4X spatially **downsample**d or binned files.
     - **Standard (recommended)**: downsampling is performed via decimation (i.e. with anti-aliasing).
     - **Binning**: downsampling is performed via binning or averaging.
  4. **Minimum recording diskspace time** allows the user to specify the minimum available hard disk space threshold before a warning is generated, in terms of recording duration.
  5. **Reset time traces each recording** toggles time trace reset for each new recording.

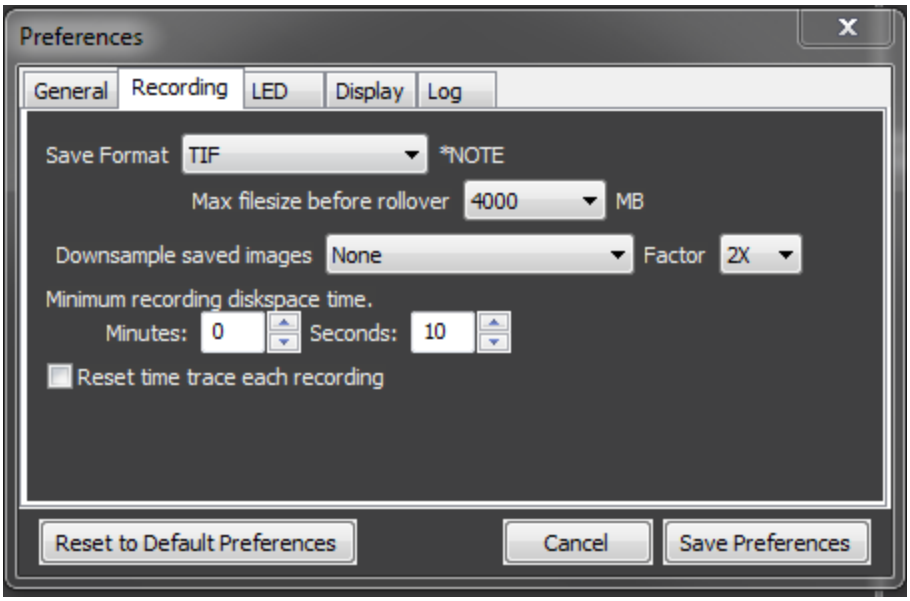


Fig 7-14. Recording Preferences

NOTE: TIF and HDF5 formats are available only with V2 hardware connected to a USB3.0 port.

- **LED**
  1. **On when recording** automatically turns on LED while recording and turns off LED when not recording.
  2. **On for snapshot** automatically turns on the LED while taking snapshots.
  3. **Off if not recording** for automatically turns off the LED after the specified period of time while not recording.
  4. **Delay** for sets a delay between when a recording is started and the LED is turned on (automatic LED turn-on must be enabled).

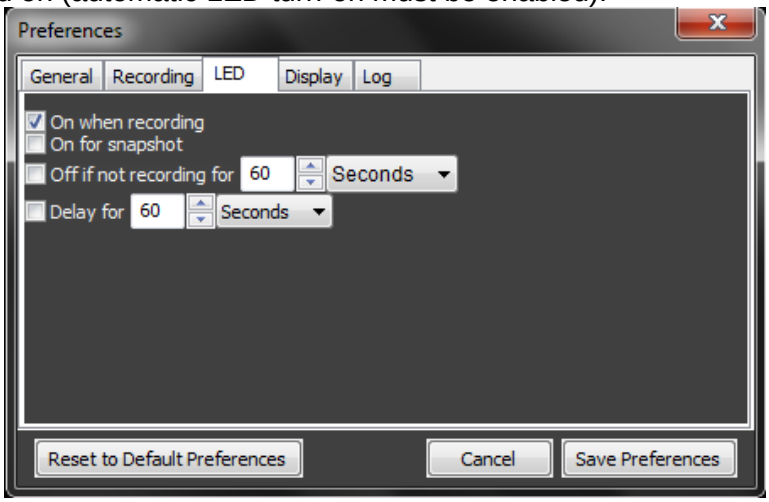


Fig 7-15: LED Preferences

- **Display**

1. **Display Range** sets the display range to map to the lookup table for display contrast for Delta f over f calculations.
2. **Average interval** sets the time over which the image average is calculated for Delta f over f calculations.
3. **Lookup Table** sets the color map selection for display.
4. **Saturation** sets the pixel color for Display Threshold calculations; when in Display threshold mode, the saturated pixels will be displayed in this color.
5. **Saturation level** sets the threshold value for display of saturated pixels for Display Threshold calculations.

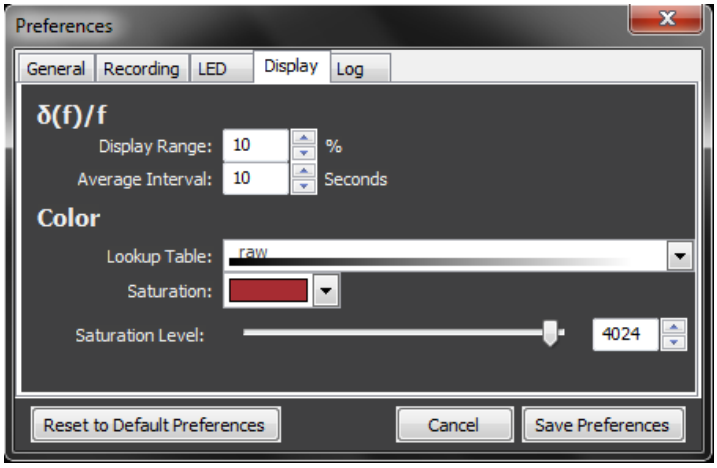


Fig 7-16: Display Preferences

- **Log**
  1. The user can specify what additional information is stored in the log file and in the XML files.

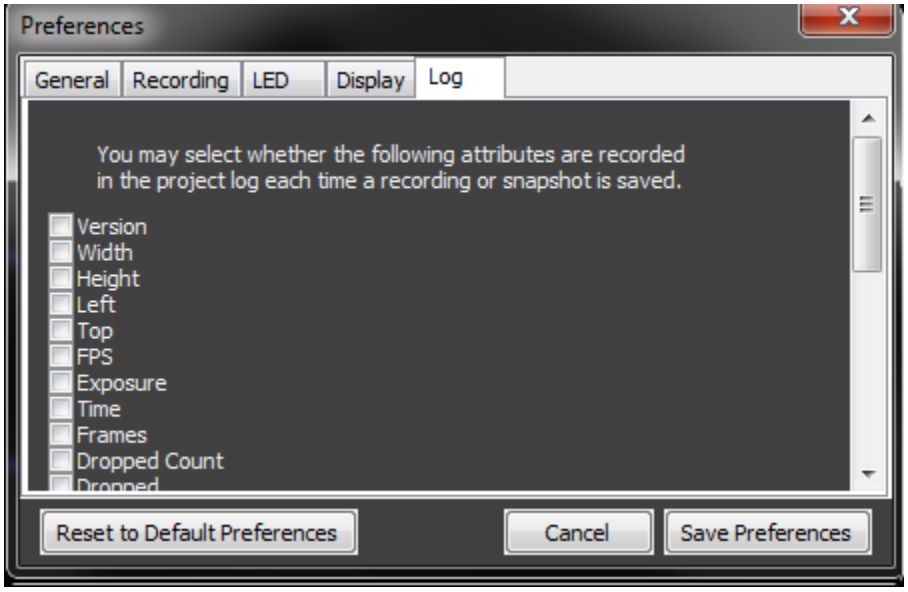


Fig 7-17: Log window preferences

Device menu

Device > Connect & **Device > Disconnect**

Depending on the current connection state, either Connect or Disconnect will be available in the Device menu. Selecting Connect will connect the nVista software to an attached microscope, if possible. You will see the Status: Connected status.

Selecting Disconnect will terminate the connection between the nVista software and the microscope. You will see the Status: Disconnected status.

Device > Turn On LED & **Device > Turn Off LED**

Turn off and on the microscope LED; also accessible by the LED button 

on the main menu.

Device > Start Recording

Begin or end a Recording; also accessible by the Record button 

on the main menu.

Device > Take Snapshot

Take a Snapshot; also accessible by the Snapshot button 

on the main menu.

Device > Trigger from External Hardware

Use this to trigger a recording using an external TTL signal (see *Interfacing with External Hardware* for more information).

Device > Hardware Bandwidth Test

Run this test to confirm whether or not your computer system can handle the data stream according to your selected settings. After the test is complete, the maximum frame rates and data speeds available on your computer system is displayed for each image size; view them by clicking through on the upper right window (red box).

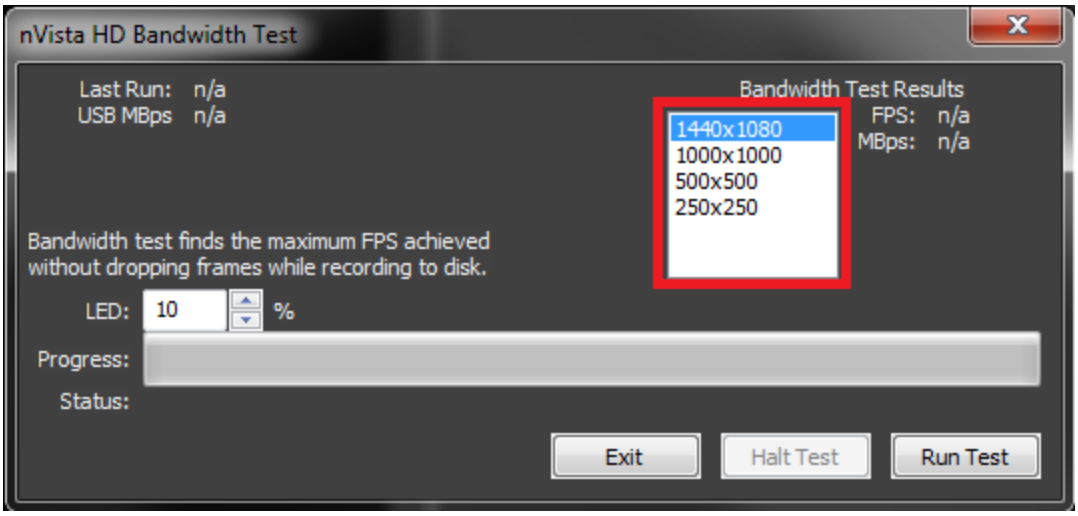


Fig 7-18. Bandwidth test

This warning will appear when the bandwidth of your computer system has not yet been tested.

Fig 7-19. Bandwidth test warning

Device > Firmware Update

Use the Firmware Update dialog box to update the hardware firmware. Firmware updates will be available on the Inscopix website.

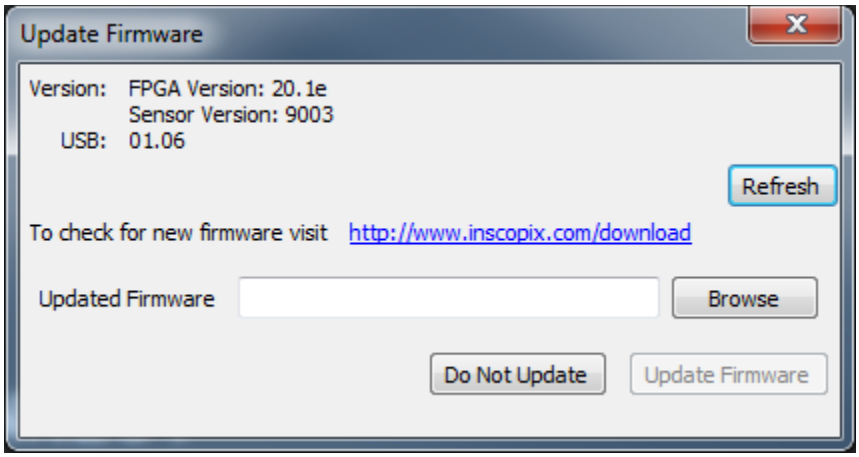


Fig 7-20. Firmware update window

 Warning: When upgrading firmware, be sure to upgrade the USB firmware first if required, before upgrading the FPGA firmware. Failure to do so may render the V2 hardware permanently inoperable. Do not close this window until firmware update is complete.

View Menu

View > Zoom In
Zoom in the field of view. This is also available at the bottom right corner of the main screen when mousing over the area as shown in Fig 7-21.

View > Zoom Out
Zoom out of the field of view. This is also available at the bottom right corner of the main screen when mousing over the area as shown in Fig 7-21.

View > Zoom 1:1
Adjust the zoom to achieve a one to one x-y ratio. This is also available at the bottom right corner of the main screen when mousing over the area as shown in Fig 7-21.

View > Zoom to Fit
Adjust the zoom to fit the field of view in the screen. This is also available at the bottom right corner of the main screen when mousing over the area as shown in Fig 7-21.

View > Display Full Screen
Reset to full screen view. This is also available at the bottom right corner of the main screen when mousing over the area as shown in Fig 7-21.

View > Reset Field of View
Reset to the default screen view. This is also available at the bottom right corner of the main screen when mousing over the area as shown in Fig 7-21.

Fig 7-21. Zoom in, zoom out, zoom 1:1, and zoom to fit icons in main window

Window menu

The window menu allows the user to access any combination of windows used during calibration or recording.

Window > Visualization Tools
This window controls the visualization mode of the data during streaming or recording. The modes do not affect the recorded data and are used for visualization purposes only. The mode settings can be adjusted through **Change preferences** in the *Edit menu*.

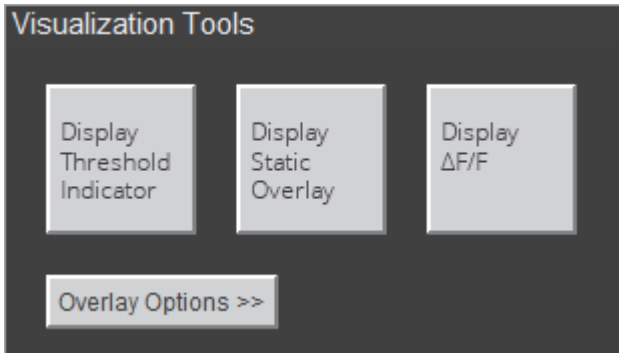


Fig 7-22. Visualization window

- Threshold Indicator provides a visual indicator of pixel saturation. The color indicative of saturation, and the display color table, can be edited through **Edit > Change Preferences > Display > Color**. This is shown in Fig 7-23.
- Static Overlay enables simultaneous co-display of the live imaging stream along with a static image. This tool adjusts the nVista system so that the FOV from a previous imaging session can be recaptured. A file browser window will appear for the selection of the desired static image file, which should be saved in 16-bit grayscale TIF or HDF5 format. This is shown in Fig 7-24.
  - Overlay Options provides control over the settings for loading the static image, adjusting the opacity of the static image, and re-positioning the static image relative to the live image stream as desired.
- Display ΔF/F enables the display of real-time ΔF/F data within the nVista software. Each pixel is redisplayed as its percent change from its own average pixel value calculated over a specified moving time window. The default time window for the average pixel calculation is 10 seconds; to change the time value,select **Edit > Change Preferences > Display > Average Interval**. This is shown in Fig 7-25.

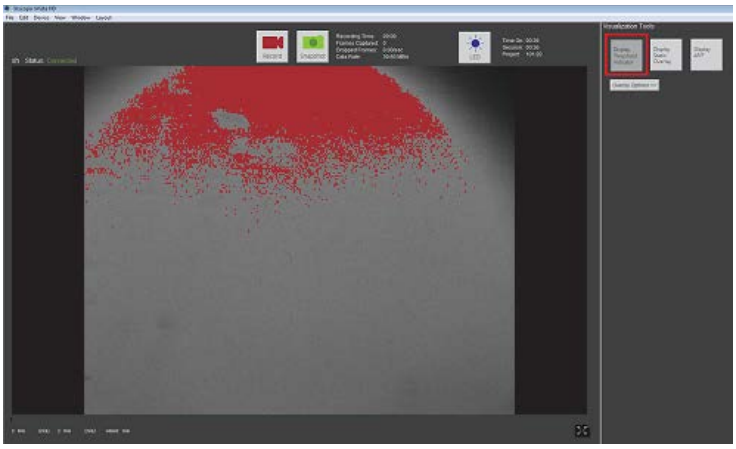


Fig 7-23. Threshold indicator, with saturated pixels displayed

Fig 7-24. Static overlay, displayed on top of the field of view

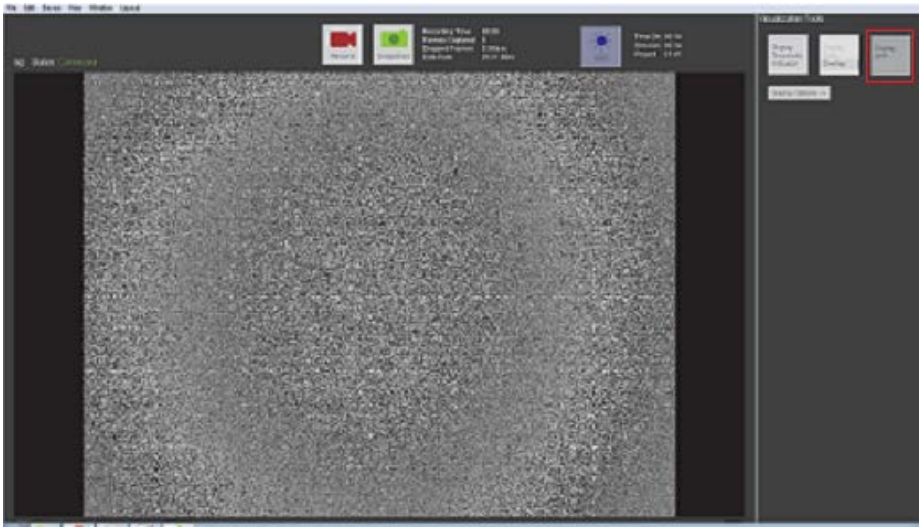


Fig 7-25. ΔF/F

Window > Field of View

This window controls the field of view (FOV) for recordings and/or snapshots. The field of view can be specified manually by dragging the FOV window boundaries or specified in pixel units. It can also be moved and/or reset as necessary. This panel is shown in Fig 7-26.

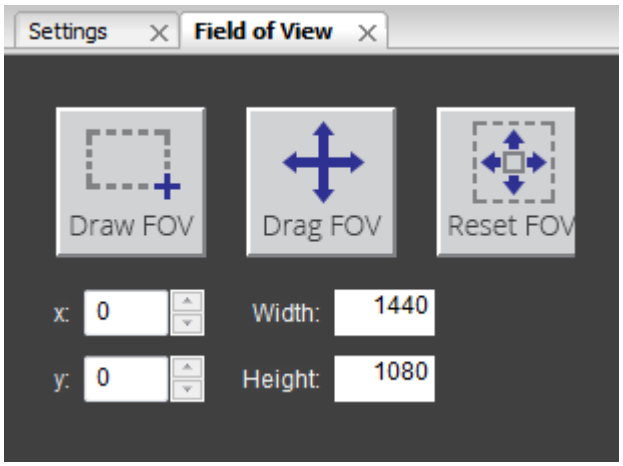


Fig 7-26. Field of view window

- **Draw FOV** allows the user to manually specify a field of view window using the computer mouse.
- **Drag FOV** allows the user to manually move the field of view from one location to another using the computer mouse.
- **Reset FOV** resets the field of view to the full resolution view, 1440 by 1080 and will clear any other drawn field of view.

Window > GPIO

Either of the two I/O ports on the DAQ box can be configured as an input or output port; set your preference using **Edit > Preferences > GPIO**. This Window can be used to test your hardware configuration to ensure that the nVista system is communicating properly with your external device. The colored dots show the current state of the GPIO port (green = HI, grey = LO). This panel is shown in Fig 7-27.

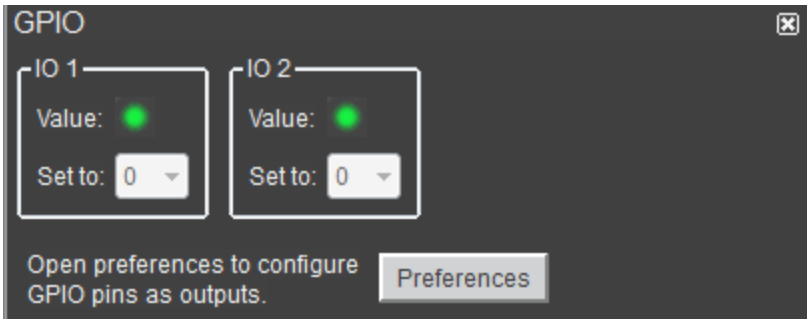


Fig 7-27. GPIO window

☞ **NOTE:** GPIO functionality is not available with nVista V1 hardware. GPIO ports both have pull-up resistors and will appear HI (green) by default (even when set as inputs in the software) unless a signal is sent to the port.

Window > Histogram

This window displays a histogram of pixel intensity values. The histogram is used to determine whether signal levels are sufficient for imaging. For tips on how to achieve an ideal histogram, see Best practices for Conducting Imaging Sessions. The histogram absolute max and min values as well as the mean and the standard deviation are displayed at the bottom of the histogram. The histogram x axis is fixed to a range of 0 to 4500. The histogram y axis updates dynamically. This panel is shown in Fig 7-28.

- **Enable histogram** enables and disables the histogram display in real time during calibration or recording.
- **Export histogram values** saves the histogram values in a text file.

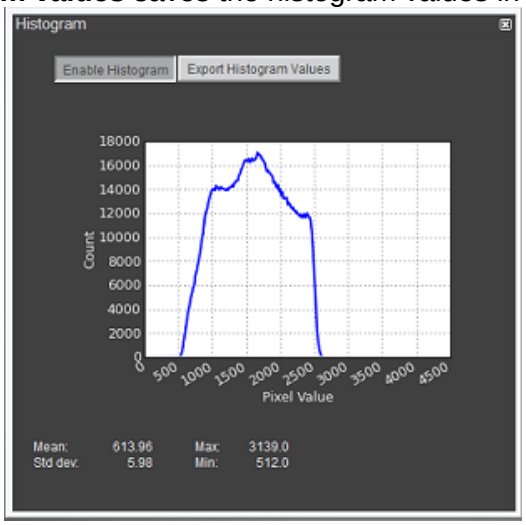


Fig 7-28. Histogram window

Window > Log

The Log tab contains a record of log files for the current session's acquired snapshots and streamed recordings and user notes. Log files containing information about all session recordings are automatically generated and listed in the Log tab record. One can add comments and notes to the log record by typing in the comment box and hitting **Enter**. All log comments are time-stamped. To export the log record for later review, first check whether you want all log details or only the user defined comments. Then, click on Export and enter the desired file location in the Export Log dialog box. This panel is shown in the image on the left in Fig 7-29. The log can be set to automatically export through **Preferences > General > Export Log Automatically**. The user can also specify what additional information is stored in the log file through **Preferences > Log** as shown in the image on the right in Fig 7-29.

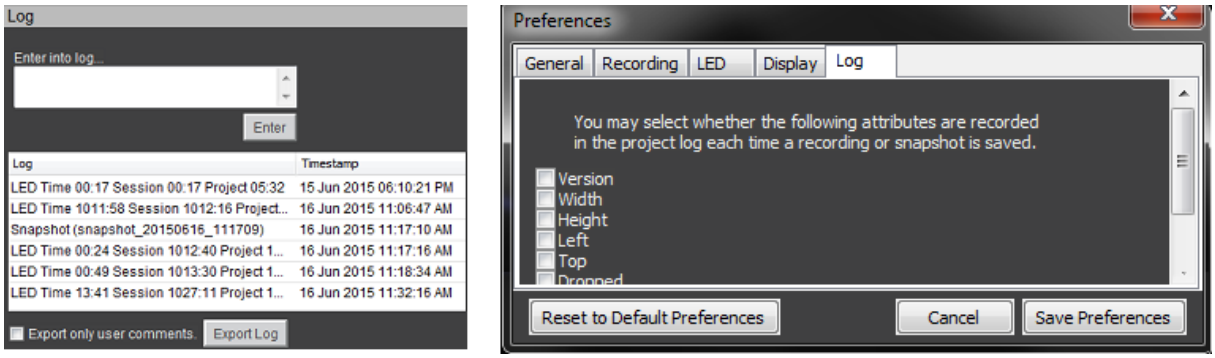


Fig 7-29: Log window (left) and log window preferences (right)

Window > Recording Schedule

Control your experiment with a custom recording schedule. In each step, schedule a specific task (e.g. Record, Wait, Toggle GPIO) from the pull down menu, then choose the action or timing. To add a subsequent step or delete a step, click on the '+' or '-' button at the end of each recording step. Steps can be rearranged by holding down the left mouse button and dragging the step to the desired position. The last step on the scheduler controls the number of ties the recording scheduler sequence is replayed or allows you to run the scheduler until halted.

To run the scheduler, click **Run Schedule** and the progress bar at the bottom will display the scheduler progress. Recording schedules can be saved as .sch files and reloaded using "Load Schedule'.

An XML file is generated for each recording schedule run, which lists all recordings associated with a given recording schedule. This window is shown in Fig 7-30.

Fig 7-30. Recording schedule window

For more information on how to use this window refer to *Recording Schedule*.

Window > Time Traces

This window controls visualization of average ROI pixel intensity over time in real time; multiple ROI displays can be displayed at once on the main screen. This panel is shown in Fig 7-31.

- **Add ROI** adds a Region of Interest (to the image acquisition screen).
- **Load ROI** displays a past Region of Interest.
- **Save ROI**: Save the current Region of Interest.
- **Manipulate ROI** allows the user to move, adjust shape and color, or delete ROI.

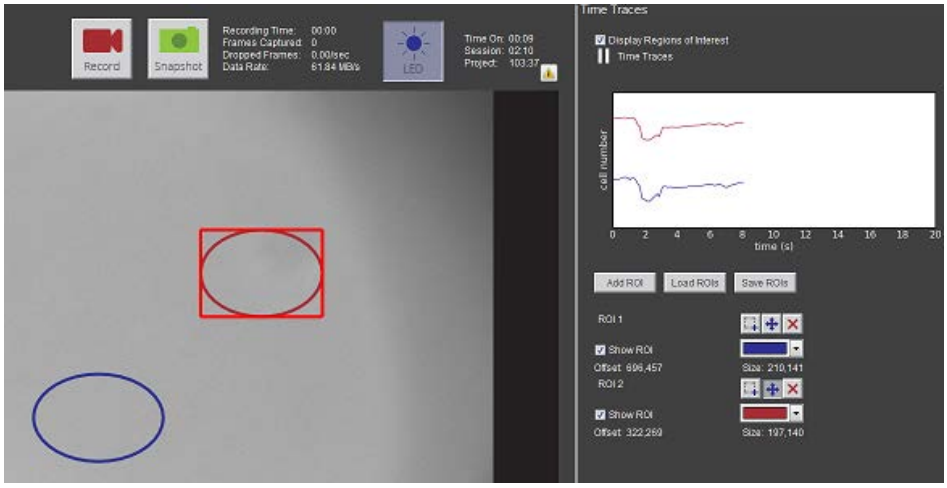


Fig 7-31. Time traces window, with two ROIs on the main window

Window > Settings

This window sets the basic image acquisition settings here. The default settings are: frame rate (20 fps), exposure time (50 milliseconds), gain (1) and LED power (10%). This panel is shown in Fig 7-32.

- Frame rate controls the frame rate at which images are collected during movie recording. The maximum frame rate possible for your system will depend on your hardware setup and Field of View. The computer hardware limited frame rate is displayed to the left of the input text box for reference. If the **Export GPIO data file when recording** setting under **Edit > Change Preferences** is checked, the frame rate cannot be lowered below 17 fps. In order to set the frame rate to lower values, uncheck this setting.

- Exposure time sets the exposure time as high as possible without saturating the image to achieve the highest quality images. If **Set automatically** is unchecked, the exposure time can be manually set to lower values.
- Gain adjusts the analog gain on the image sensor. Refer to **Best Practices for conducting imaging sessions** for selecting the settings for data acquisition.
- LED Power adjusts the power of the LED light incident on the tissue. LED power of 100% is equivalent to ~2.0 mW. The LED will shut off for values below ~5%.

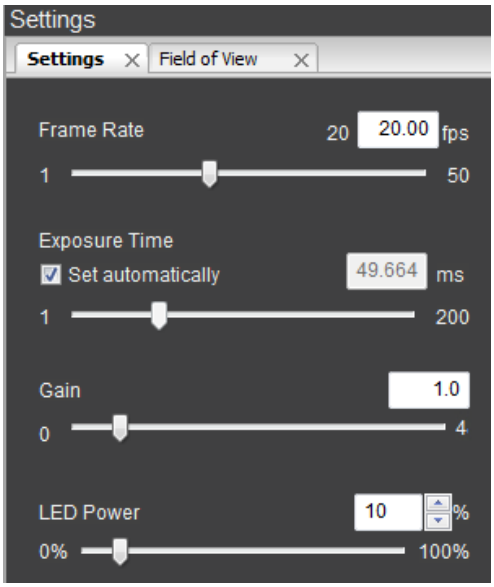


Fig 7-32. Settings window

☞ **NOTE:** For tips on how to adjust the settings window parameters for best imaging results, see Best practices for Conducting Imaging Sessions.

Layout menu

The native workspace layouts includes the main image acquisition window and some combination of Windows.

Layout > Default

The Default layout contains the Field of View, Settings, Histogram and Log windows. These are often windows used for quick calibration and quick recording.

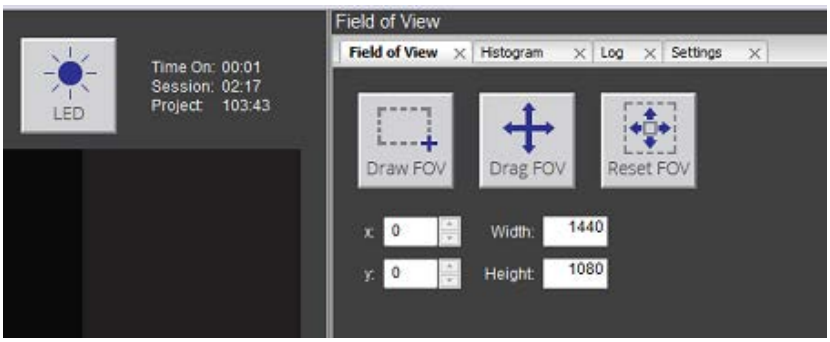


Fig 7-33. Default layout

Layout > Calibration

The Calibration layout contains the Settings, Field of View, Histogram and Visualization Tools windows. These are often windows used for calibration and specifying operating conditions.

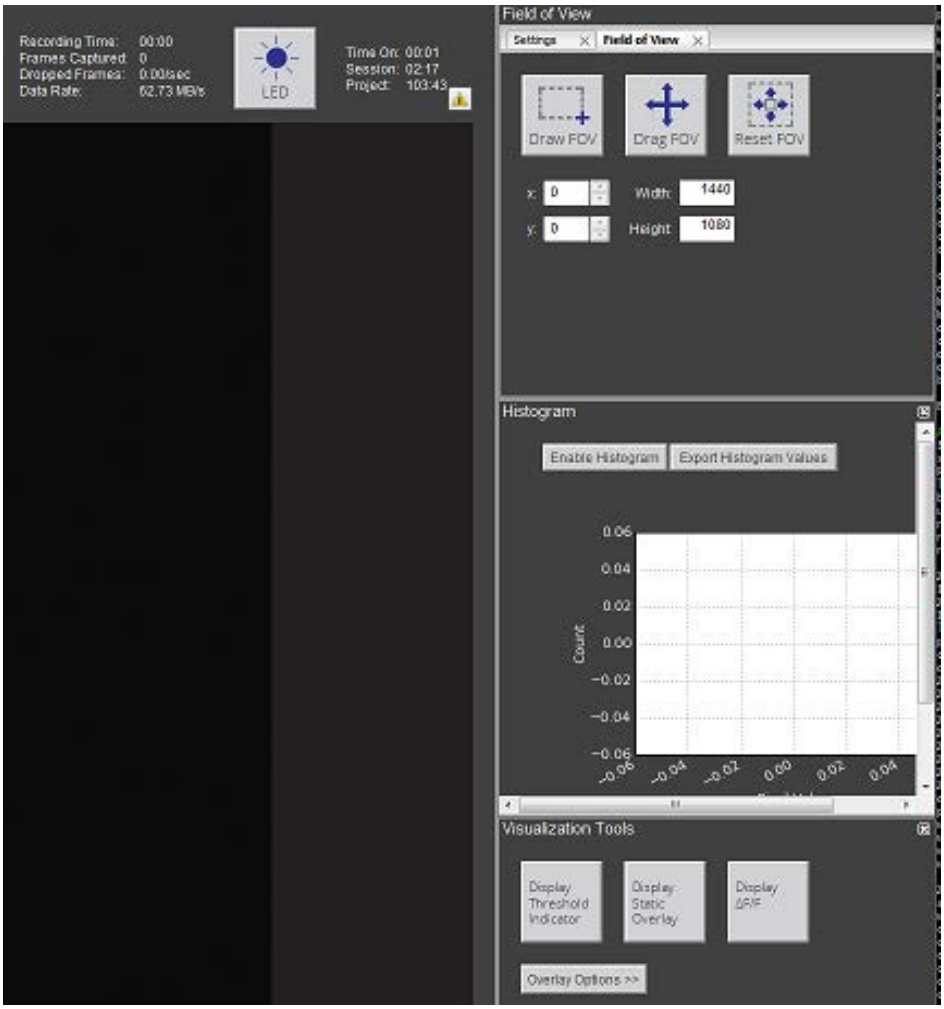


Fig 7-34. Calibration layout

Layout > Recording

The Recording layout contains the Log and Recording Schedule windows. These are the main windows necessary for recording manually or using a recording schedule.

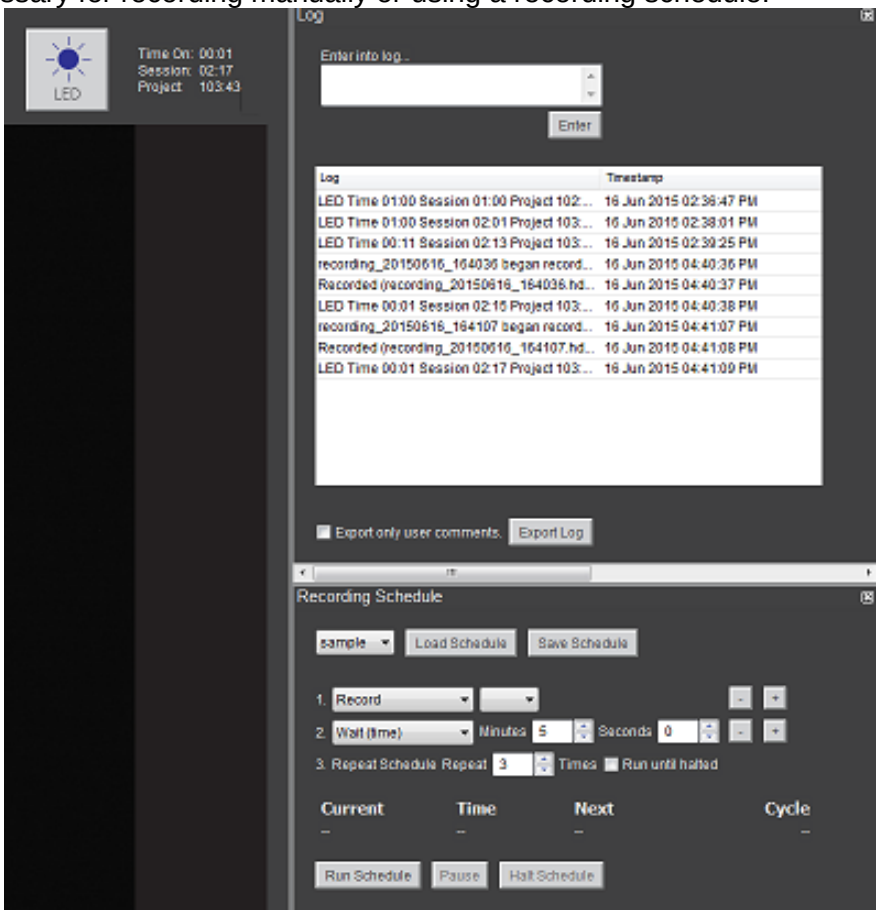


Fig 7-35. Recording layout

Layout > Save Layout

Save the current layout of windows.

Layout > Load Layout

Load a previously saved layout of windows.

8 Interfacing with External Hardware

Fig 8-1. BNC ports for synchronization (SYNC) and external triggering (TRIG) on the left and additional I/O ports on the right

8.1 SYNC and TRIG Ports

On the back of the DAQ box there are two BNC ports as shown in Fig 8-1: SYNC and TRIG. These ports enable the user to interface the nVista system with other external hardware using standard TTL signals (0-5 V).

While recording, the BNC port labelled SYNC on the DAQ box outputs a TTL HI pulse synchronized to the acquisition time of each frame. The SYNC port outputs a TTL LO signal while the software is not recording.

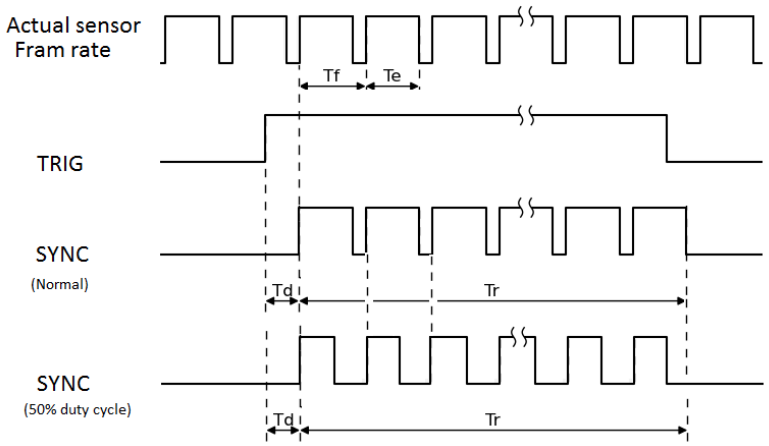


Fig 8-2. Timing of the TRIG and SYNC signals

- Tf = frame time (1/fps)
- Te = exposure time
- Td = delay between TRIG HI signal and start of recording (Td , Tf)
- Tr = total recording time

The SYNC port Duty Cycle can be controlled during recording by selecting “50% Duty” with the ‘External signal duty cycle’ setting (**Preference > General window > GPIO**). Selecting “Normal” will generate pulses as illustrated in Fig 8-2. The “Normal” option can have very short ‘LO’ duration; that is, Tf-Te can be so short that not all devices would be able to detect the rising edge of the next pulse. Hence, it is strongly recommended that the “50% Duty” option is used.

To enable external triggering of recordings via input to the “TRIG” port, select “Trigger from external hardware” under the *Device menu*. When enabled, a new recording will start when the TTL input goes HI and terminate when the TTL input goes LO. The polarity of this behaviour is configurable with the ‘external record trigger polarity’ setting (**Edit > Preferences > General > GPIO**). Automatic naming will proceed according to the convention noted in *File naming convention*.

8.2 Additional I/O Ports

Two additional TTL-based I/O ports are available on the V2 DAQ box as shown in Fig 8-1. The ports can be configured in **Preferences > General** to either be input or output ports.
As outputs, the GPIO can be toggled either manually through *GPIO window* or automatically through *reschedule*.

As inputs, the GPIO ports are sampled at 1 kHz and the states of all the ports are saved in an HDF5 formatted file along with the regularly recorded image data (if the GPIO export option is selected under preferences).

While using the recording scheduler, GPIO data is saved during periods that the system is not recording image data, in addition to being saved while the system is recording image data. The resulting data is stored in a single HDF5 file per recording.

☞ **NOTE:** When interfacing with external hardware, signals can only be saved to a file for frame rate values larger than 17 frames per second.

☞ **NOTE:** Additional I/O port functionality not available on V1 hardware.

9 Using the Image Decompressor

To begin, run Inscopix Image Decompressor by either using the desktop shortcut or selecting **Start > Programs > Inscopix > Inscopix Image Decompressor**. In the interface, available snapshots and recordings (RAW files with accompanying .xml and settings.prj files) for decompression are listed in the right selection window for the Project selected in the left selection window. Change the Project Directory by clicking **Browse** and navigating to the desired location on the computer. Select single or multiple snapshots or recordings or decompression and click **Decompress**.

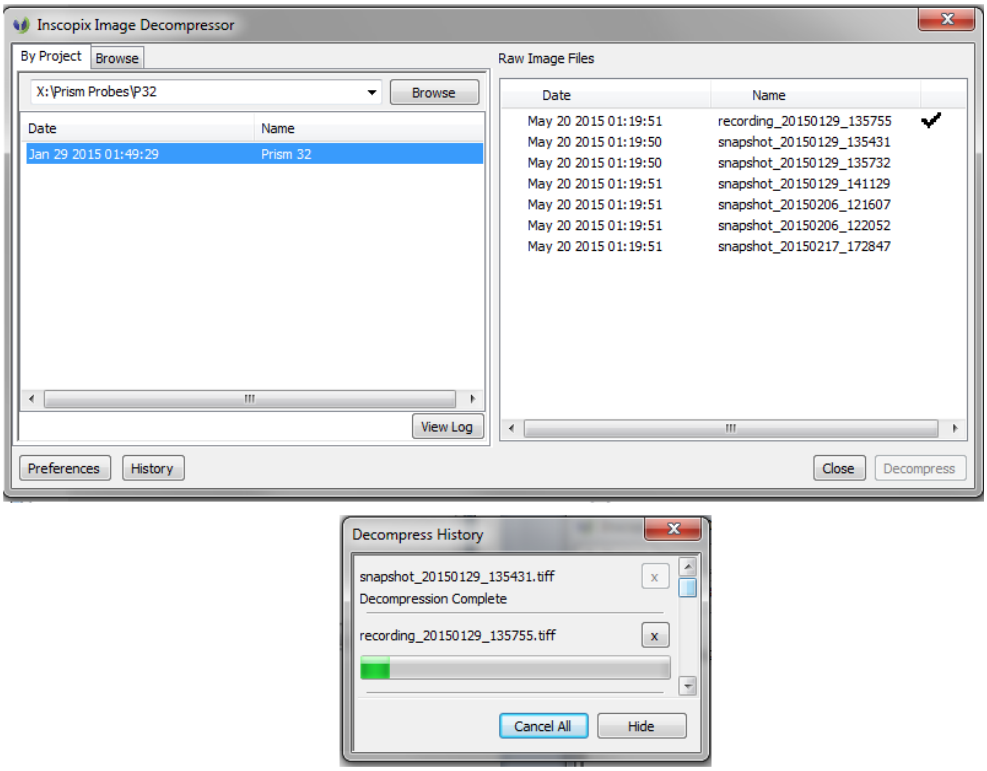


Fig 9-1. The Inscopix Image Decompressor interface (top) and decompression history window (bottom)

A status bar will appear to display the decompression progress. The RAW file(s) will not be automatically deleted from the Project after decompression has finished.

A recording with multiple associated RAW files will not be listed multiple times in the selection window. If the size of the decompressed TIF file exceeds approximately 3.9 GB, the recording will be decompressed to multiple TIF files with the same file name stem and different end tags (see *File naming convention*).

The Image Decompressor recognizes Project by the presence of a valid project settings file ('settings.prj') and lists Projects using the name stored in each Project settings file (see *Opening the Display* for information about renaming a Project).

Copy the settings file (settings.prj) along with all the RAW and XML file pairs when transferring data between hard drives. Note also that the RAW files for a recording will not be automatically deleted once decompression has finished.

☞ **NOTE:** .raw, .xml and settings.prj file are required to decompress any .raw recording.

10 Best practices for conducting imaging sessions

When conducting experiments with nVista, you should select your acquisition settings to optimize the Signal to Noise Ratio (SNR) of the calcium response, and avoid photo-bleaching to ensure ideal illumination conditions.

10.1 Selecting settings for data acquisition

For a typical experiment measuring Ca^{2+} neural activity, we advise adjusting the LED power, the frame rate and gain in the following order:

1. The LED power: The LED power impacts the excitation level of GCaMP indicators in your active cells. To ensure sufficient excitation, gradually increase the LED power level to a maximum of 90% (see Note below) in the nVista settings panel. You will determine the appropriate LEDpower for each animal based on the instructions below.

High LED exposure can result in bleaching of GCaMP molecules and decrease the fluorescent signal across the recording session. To avoid photo-bleaching, do not increase the LED power more than necessary to obtain sufficient SNR of the calcium response. Design your experiment to alternate between imaging and tissue recovery periods (this can be done using the recording scheduler or synchronizing external hardware with nVista).

☞ **NOTE:** nVista software versions 2.0.31 and newer are temperature compensated for LED power levels of ~0 - 90%. Increasing the power level beyond 90% may result in power fluctuations and affect the interpretability of your data.

2. The frame rate and exposure time: Set the 'frame rate' to 20 fps in the nVista Settings Panel.. Ensure that the exposure time is 'set automatically'.

The frame rate can be adjusted to increase/decrease the sensor exposure time and thus the number of photons detected per frame. The temporal resolution of the indicator should be taken into account when choosing the frame rate.

Lower frame rates (between 10 and 20 Hz) can be used to increase the sensor exposure. Frame rates below 20 Hz may result in a loss of information depending on the temporal characteristics of the fluorescent indicator used (e.g. for GCaMP6 slow, medium, or fast allow for a temporal resolution of 10, 15, and 20 Hz respectively, refer to Chen et al., 2013). Frame rates below 10 Hz may result in motion blur and/or image distortions caused by the combination of brain motion and nVista system's rolling shutter.

Frame rates above 20 Hz can be used but may require to adjust the LED settings to ensure sufficient signal intensity, since the sensor exposure will be decreased.

☞**NOTE:** When interfacing with an external hardware, signals can only be saved to a file for frame rate values larger than 17 frames per second.

3. The Gain: Set the 'gain' to 1 in the nVista settings panel.

Changing the gain will change the visual appearance of the image **and** alter the data you collect. Increasing the gain will increase the histogram range, but will not improve the GCaMP fluorescence emission or the number of photons collected by the sensor. While increasing gain can improve your signal range, that should be done with caution, as discussed below. However, we do recommend increasing gain when imaging only for visualization purposes (e.g. when you are visually checking an animal for fluorescence).

For optimal SNR, the best approach is to increase the LED power and exposure time as much as practical first (i.e., before motion artifacts are too prominent or biological limits for phototoxicity/bleaching are reached), before increasing the gain.

The effect of increasing gain is increasing the signal within the bit range. For example: at 8 bits, values range from 0 to 255. If your original signal range is only 3-127, it is reasonable to increase gain by a factor of 2, which will result in a signal range from 6-254. If you use too much gain, though, you will clip and lose data at values beyond the bit range. We recommend increasing gain after adjusting your LED power and exposure time because increasing gain first may result in poor SNR. For example, you could use 30ms exposure time, and gain of 1, or 10ms exposure and gain of 3 to achieve the same signal range – imaging with 30ms exposure time and a gain of 1 is preferable from an SNR standpoint.

4. Check image histogram: The image histogram indicates the distribution of the fluorescence intensity of the pixels.

For a typical experiment with high cell density and high activity level, e.g. hippocampus CA1 in the awake mouse:

Upon visual inspection, the right edge of the histogram curve should have a value between ~1500 (Acceptable) and ~3000 (Ideal), as shown in Fig 10-1 (below).

☞**NOTE:** Determine the value of the right edge of your histogram plot by visual inspection. Do not refer to the 'max' value reading displayed below the histogram.

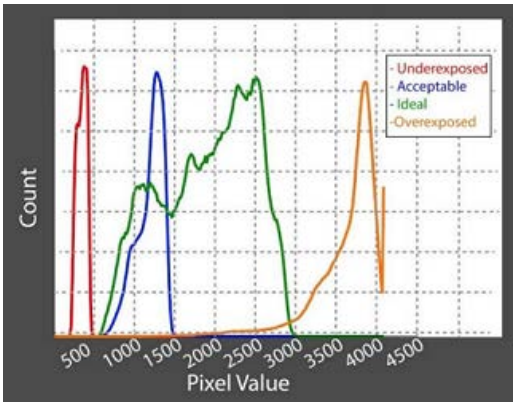


Fig 10-1. Overexposed (Orange), Underexposed (Red), Acceptable (Blue), and Ideal (Green) histogram plots.

If the right edge of histogram curve appears lower than ~1500 (see Fig 10-1, Underexposed), you may be collecting data with a low SNR. To correct for this:

1. Gradually increase the LED power level to a maximum of 90%.
2. If the histogram still appears Underexposed, lower the frame rate.
3. If your tissue is still Underexposed after performing Step 2, contact Inscopix Support (support@inscopix.com). Troubleshooting now may prevent lost time imaging a non-ideal prep.

If the right edge of histogram curve appears higher than ~4000 (see Fig 10-1, Overexposed), you may saturate your signal and increase your risk of photobleaching.
To correct for this:

1. Decrease the LED power.
2. If the histogram still appears Overexposed, uncheck “set automatically” for exposure time and reduce the exposure time.
3. If your tissue is still Overexposed, contact Inscopix Support (support@inscopix.com). Troubleshooting now may prevent lost time imaging a non-ideal prep.

☞ **NOTE:** In the case of applications with low cell density (e.g. some Cre-lines) and/or low activity level (e.g. non-active animal), the histogram will be shifted to the left, although the calcium signals may still be acceptable after extraction with Mosaic. Increasing the LED power to shift the histogram to the right can cause photobleaching and should be done with caution.

To determine the optimal illumination parameters and histogram shape, we recommend acquiring data with different sets of imaging parameters, processing these data in Mosaic to extract the cellular activities, and then comparing the SNR of the extracted signals in Mosaic to see which parameter set was optimal for your prep.

5. Acquire Data: Set up the recording scheduler or synchronize external hardware with nVista. Refer to the “Hardware Syncing” section in the Inscopix Knowledge Base Methods for guidelines on using nVista with external hardware.

In the Edit > Change Preferences Menu, choose your file format and downsampling/binning options in the Recording tab.

Downsampling by 2 at acquisition will decrease the data size and should not compromise the data analysis (because the spatial resolution of the camera is very high). The impact of further downsampling (x3 or x4) at acquisition on the data interpretability depends on the brain region, fluorescence levels, activity levels and desired analysis.

To ensure that further downsampling will not compromise the data interpretability, we recommend comparing the results of PCA/ICA on the same data set with different downsampling/binning options in Mosaic (with the Reduce or Bin Movie Apps).

☞ **NOTE:** Make sure to **acquire at least 20,000 frames** of movie data from the same field of view, in order to properly process these files in Mosaic. PCA/ICA results may not be accurate without enough data.

11 Troubleshooting Guide

If you are experiencing any problems listed in the table below, take remedial action as needed. If the problem persists and cannot be resolved by the described action(s), please contact Support (support@inscopix.com) for assistance.

Issue	Potential Cause	Action
Electrical system problems		
System will not connect, LED does not turn ON	Cables are not installed properly or is damaged.	Connect cables properly and ensure they are not damaged. Refer to section **Startup** for guide to connecting cables. If problem persists, contact

		Support.
Field of View Visualization Problems		
Field of view is black or obscured	Microscope Objective Lens Cover is not removed.	Make sure Microscope Objective Lens Cover is removed from the microscope.
	Debris or acrylic deposits on imaging surface of nVista objective lens or on the tip of the relay lens.	Carefully clean imaging surface of objective lens or top surface of the lens with lens paper and 70% ethanol. If problem persists, contact Support.
	Blood is obscuring the field of view.	If the region does not fully obstruct the field of view, imaging may not be affected. You may wish to wait another few days to see if blood deposits will be re-absorbed by the surrounding tissue. If problem persists, contact Support.
	Image is out of focus.	Adjust the turret height/ position; refer to section **Focusing the Microscope** for additional details. Change the microscope position above the relay lens, if image is out of focus during baseplate installation. If problem persists, contact Support.
Irregular dark patches, speckles or spots in field of view	Debris under or on the surface of the implanted lens.	Always inspect and clean lens before implanting.
	Blood deposits from broken	If the region does not fully

	blood vessels in the tissue.	obstruct the field of view, imaging may not be affected. You may wish to wait another few days to see if blood deposits will be re-absorbed by the surrounding tissue.
	Debris on or in the microscope objective.	If cleaning the microscope objective lens does not improve the field of view, contact Support.
The view is poor (insufficient contrast or poor resolution).	The field of view is not focus.	Adjust the turret position; refer to section **Focusing the Microscope** for additional details.
	Imaging parameters are not optimized.	Increase LED power, decrease frame rate, and then increase gain. Refer to **Imaging Best Practices Guide** for more information.
	Baseplate is not in right position.	Ensure that the baseplate is installed correctly. Refer to **Baseplate install** page on our Knowledge base for more information.
The focus is uneven or one side of the image is blurry.	Microscope is not properly seated in the baseplate.	Ensure that the microscope is properly seated inside the baseplate . Refer to the **Microscope mounting** page on our Knowledge base for mounting demonstration.
	Baseplate is not implanted parallel to the implanted lens probe.	The microscope objective must be parallel to the lens probe imaging surface and all edges should be in the same focal plane. Refer to **nVista baseplate install** page for mounting procedure.

Flickering nVista Acquisition Display.	Data cable has experienced wear or damage, and splits frames before they are fully acquired.	The data cable may be damaged. Contact Inscopix Support.
Unstable or shifting images despite proper microscope seating/ Motion and instability in nVista movies.	Baseplate is not attached stably to the animal skull; screws may not be stripped or not properly connected.	Practice baseplate installation on a slide before attempting an experiment on an animal. Ensure the head cap adhesive does not touch any skin or muscle tissue and screws are properly installed which will improve baseplate stability. .
	Lens and/or baseplate installation are not secure due to improper baseplate application.	Lens and baseplate must securely adhere and the skull must be free of debris and skin prior to lens and baseplate installation.
	The tissue is moving relative to the outline of the implanted lens in the field of view.	Some amount of translational motion can be corrected in data processing. If problem persists, contact Support.
Gradual darkening of movie (usually detected post-acquisition).	Photobleaching due to GCaMP overexposure that causes signal saturation.	Reduce LED power or the duration of the imaging session. Waiting for 24-48 hours before the next imaging session will allow reexpression of new GCaMP molecules in the imaged cells. Refer to **Imaging Best Practices Guide** for recommendations on optimizing your settings.
Images are inverted	Some lens probes used with our system optically invert images. Each half pitch of the probe inverts the image.	Refer to section **Orientation of images through lens probes** for more information.

The field of view is blurry at the perimeter/ Image quality is different with different lens probes	The blurriness at the perimeter of the field depends on the pitch length, but the field of view remains the same; as a result the useful field will shrink.	Increase in pitch length increases the optical aberration in the lens. Refer to section **Probe Variants** for information on pitch length.
Software/Connectivity problems		
nVista will not connect	nVista is not connected to computer	Confirm your nVista system DAQ box is connected to the computer USB port via the black USB cable. ● Check that the cable is firmly connected to the DAQ box. ● Check that the cable is firmly connected into a USB port.
	nVista is not receiving power (or consistent power) from USB port	Connect another USB-driven to the same USB port to confirm the port is functional.
	nVista data cable has physical damage	Inspect data cable for physical damage. Contact Inscopix Support.
	nVista is not getting power from computer (bad USB cable)	● Use this USB cable with another nVista system. If that is not successful, contact Support. ● Use a different USB cable to connect your nVista DAQ box to the computer. If that is successful, contact Support for help receiving a replacement cable.

nVista system disconnects during recording	nVista system disconnects and will not re-connect.	Refer to “nVista will not connect” troubleshooting steps.
	nVista system disconnects and is able to re-connect when prompted.	Refer to “nVista will not connect” troubleshooting steps.
nVista recordings have dropped frames	nVista software is out of date.	Ensure you are using the most current nVista software available. If problem persists, contact Support.
	nVista is not receiving power from USB port.	Refer to “nVista will not connect” troubleshooting steps.
	nVista data cable has physical damage.	Refer to “nVista will not connect” troubleshooting steps.
	nVista data cable does not show physical damage.	Refer to “nVista will not connect” troubleshooting steps.
nVista is not being triggered by my external device.	BNC port is not connected to external device.	Confirm external device is connected to the nVista TRIG port.
nVista SYNC port is not causing my external device to record/turn on	BNC port is not connected to external device	Confirm external device is connected to nVista SYNC port.

12 Property Rights

nVista ™ is a trademark owned or used by Inscopix, Inc.

The instruments, their components and the methods described in this manual are protected by the following patents:

- **US 9,161,694**
- **US 8,788,021**
- **US 9,195,043**

Other US and foreign patents.

13 Glossary

CMOS Image Sensor - CMOS (complementary metal-oxide semiconductor) image sensor in nVista is used for image acquisition and has 1440 x 1080 pixels. When light enters the camera through the lens, it strikes the image sensor and causes the photodetectors to accumulate an electric charge.

DAQ - Data acquisition (DAQ) is the process of measuring an electrical or physical phenomenon such as voltage, current or temperature with a computer.

Dichroic mirror - This is placed between the excitation and emission filters at 45° angle. It reflects the illumination towards the fluorophore-labeled tissue (here GCaMP) and transmits the emitted fluorescence towards the detector.

LED - A light-emitting diode (LED) is a solid-state light source that is used as an excitation source for fluorescence microscopy.

Emission filter - This filter is placed in the imaging path of a fluorescence microscope to transmit only the emitted wavelengths of the fluorophore (here GCaMP) and blocks the excitation wavelengths of the LED.

Excitation filter - This filter is placed in the illumination path of a fluorescence microscope to pass only the wavelengths of the light source (LED) required for excitation of the fluorophore (here GCaMP).

Field of view (FOV) - The observable area through the optical probe (the field of view changes orientation with every turn of the focusing turret).

Gain - Gain determines the signal intensity of the image. ~~Signal-to-noise ratio is unaffected by the gain setting.~~

GCaMP - Genetically encoded calcium indicator that is used to image and measure changes in Ca^{2+} concentrations associated with neural activity.

GRIN lens (gradient-index lens) - Cylindrical glass rod, composed of non-toxic silver ion exchange, in which the refractive index decreases quadratically with radius from the central axis.

Numerical aperture - A dimensionless number that indicates the light-collecting power of a microscope (0.5 - base scope ± straight probe; 0.35 - base scope ± prism probe).

Photobleaching - Photobleaching of a fluorophore causes irreversible loss of fluorescence and results from reaction of calcium indicators in their excited state with oxygen or other reactive intracellular species.

Pitch - The length of GRIN lens within which a single ray will propagate a complete sinusoidal cycle (the pitch length is proportional to the diameter of the lens).

Prism probe - Probes, shaped like right-angle prisms, used for simultaneous multilayer imaging of deep brain regions.

Spatial resolution - The shortest distance between two spatially separated points on a specimen that can be resolved by an imaging system as separate entities.

TTL signal - TTL signals are based on what's known as **Transistor-transistor logic**, a basic concept within digital circuit design where binary logic calculations are done by transistor elements (also called "logic gates") to store, process or transmit information. TTL signals are composed of an output voltage and current specification, an input voltage and current specification, and a signal rise/fall time specification.

Focus Turret - The revolving part of the microscope to which the image sensor is attached.

Working Distance - The distance from the end of the objective or probe lens to the cells in focus within the tissue. It ranges from 100 to 300 µm in the nVista system.