Nikon C2Plus Confocal System Instructions

Updated October 2022

Instrument Set-up:

1. Turn all components of the Nikon C2Plus hardware "ON".
2. Open the Nikon Elements software using the **Nikon C2 Confocal** driver.
3. The software will automatically open the following Nikon C2 acquisition windows:

   C2Plus Pad
   Manual Microscope Pad
   C2Plus Scan Area
   LUT
   ND Acquisition
   XYZ Navigation

Focus on Specimen:

1. Select the objective for imaging and place specimen, coverslip up, on the stage.
2. Within the software, click on **Eyepiece-EPI or Eyepiece-DIA** to engage the laser interlock.
   The red "**Remove Interlock**" will be displayed, indicating that the laser light is currently blocked and will not be viewed through the ocular.
3. From the microscope, set the light path selector (located on the upper right) to **BINOC** for ocular viewing.
4. From the microscope, set the turret (located above the objectives) to the correct position:

   Position 1 – LSM – Confocal Laser Scanning, ocular will be blocked
   Position 2 – DAPI – Ocular view of Blue fluorescence
   Position 3 – FITC – Ocular view of Green fluorescence
   Position 4 – TX RED – Ocular view of Red fluorescence
   Position 5 – BF – Ocular view of Brightfield, Phase Contrast, or DIC
   Position 6 – EMPTY

5. When viewing fluorescence by eye (Eyepiece-EPI), select turret **Position 2, 3, or 4** and open the fluorescence light shutter using the foot pedal located below the microscope table.
6. The fluorescence intensity that can be seen through the oculars can be controlled using the Neutral Density filters located on the upper left of the microscope.
   ND4 = 25% Transmission (75% light blocked)
   ND6 = 16.7% Transmission (83.3% light blocked)
   ND16 = 6.25% Transmission (93.75% light blocked)
7. When viewing transmitted light by eye (Eyepiece-DIA), select turret **Position 5** and turn the halogen lamp "On" using the button on the lower left side of the microscope. The intensity of the halogen lamp can be controlled using the dial located below the on/off button.
8. For Brightfield imaging, use Condenser position "O" with any objective and set the polarizer position to 0-degrees. For DIC imaging, use Condenser position "N2", the 20x objective with the

20DIC prim, and polarizer at 90-degrees. For Phase Contrast, use the Condenser position "Ph2", the 40x objective, and the polarizer at 0-degrees.

9. The microscope focus can be controlled through either microscope focus knobs (left and right) or the focus knob located on the Prior Z-drive controller. To use the microscope focus knobs, set the Prior Z-drive controller to "Disengaged". To use the Prior Z-drive focus knob, set the controller to "Engaged". For either method, the focus knob may be set to coarse, medium or fine using the "Speed" button on the Prior Z-drive controller.
10. Once the specimen area and focus have been optimized, return to confocal laser mode:

    Set the turret to **Position 1**.

    Set the light path select to "**F**".

    Confirm that the "F" port is engaged by looking for the orange light at the top, front of the C2 confocal scan unit.

    Within the software, click on the **Nikon C2** tab and then click on the red **"Remove Interlock"** to disengage the laser lock.

    The "Remove Interlock" will turn from red to grey, indicating that the ocular port is now completely blocked in order to prevent laser light from being view by eye. If the "Remove Interlock" is still displayed in red, ensure that the "F" port is engaged by looking for the orange light at the top, front of the C2 confocal scan unit.

XY Image Optimization:

1. Select the correct **Objective Magnification** and set the **Pinhole** to 1.0 AU.
2. Select the correct Optical Configuration (OC) for fluorescence imaging. The OC's are located on the top menu bar:

    405-488-561
    405-488-561-TD
    488-561-640
    405-only
    640-only
    Reflection-only
3. Select correct combination of PMT Detector Channels to be used for your specimen.
4. Recommended Default Settings for Scan Parameters include:

Dwell Time:	2.4 μsec/pixel		
Pixel #:	1024x1024 pixels		
Averaging:	1		
Channel Mode:	CH Series		
Pinhole:	1.0 AU		
Blue Gain:	100	Blue Laser:	10%
Green Gain:	100	Green Laser:	10%
Red Gain:	100	Red Laser:	10%
Far Red Gain:	100	Far Red Laser:	10%
TD Gain:	50		

Note: In the Nikon C2 Software, image brightness will depend on the PMT Gain, Laser %, and Dwell Time. For a brighter image:

**Increase PMT* Gain *setting.*

This will affect the brightness of only the single detection channel. Gain *settings will not affect photobleaching.*

**Increase Laser %.*

In sequential mode, this will affect the brightness of only the single detection channel and may increase photobleaching of the same detection channel.

**Increase Dwell Time.*

This will make all channels brighter but may increase photobleaching.

5. To Reuse scan parameters from a previous experiment:
   Open the previous data set
   Right click on the image
   Select **Reuse Camera Settings**
6. The image field of view may be optimized through the **C2Plus Scan Area** window.
   For a full field of view, ensure that in the C2Plus Scan Area, **Zoom** = 1.
7. Click **Scan** and optimize the image.
8. Click **Capture** to record the image.

Saving Options:

1) Data must be saved directly to a **USB**, remote hard drive or CD/DVD.
   DO NOT SAVE ANY DATA directly to the Computer Hard Drive or Desktop.
2) Select **File / Save As** to save the raw data the *.nd2 raw data file format.
3) Select **File / Save/Export to TIFF Files** to save the image(s) in a *.tif file format.
   For a TIF image that can opened on most computers, select:
   Standard TIFF
   RGB Image for Each Channel, in channel color, scale to 8-bit. This will generate an individual 24-bit color image for each detection channel for each image in the series.
   All Channels Merged to RGB Overlay, scale to 8-bit. This will generate a 24-bit color image of the overlay image for each image in the series.
4) Select "**X**" from the keyboard to generate a Snapshot of any image. This will permit you to save exactly what you see as a 24-bit color TIF image.
   After pressing the "**x**" on the keyboard, select **File / Save As** to save the snapshot in a 24-bit color *.tif file format.

ND Acquisition: XY – Time
XY – Z Depth

XY-Time:

1) Select the **ND Acquisition** window.
2) Select **Time**.
3) Select **Phase #1.**
4) Enter **Interval**. Interval = time from the start of image 1 to the start of image 2

5) Enter **Duration** or **Loop** (but not both).
   Duration = total time for completion of Phase 1
   Loop = number of times Phase 1 is repeated
6) For a simple XY-T series, be sure that the additional ND Acquisition options (XY Position, Z Series and Lambda Series) are not selected.
7) To see a graph of Intensity versus Time during the image acquisition, select the **Perform Time Measurement** option. If no ROI is selected, the average intensity for the entire image will be plotted over time.
8) Select **Run Now**.
9) To stop the scan early and **SAVE DATA**, select **Finish**.
   To stop the scan early and discard the data, select **Abort**.

XY-Z:

1) Be sure that the Prior Z-drive controller is set to "**Engaged**".
2) Select the **ND Acquisition** window.
3) Select **Z**.
4) Select the first icon on the left to **Define By Top Bottom.**
5) Begin scanning and set **Top** and **Bottom** boundaries of the Z-depth range.
   Set **Bottom** boundary by turning the focus knob towards you and locating the lower boundary. Click **Bottom**.
   Set **Top** boundary by turning the focus knob away from you and locating the upper boundary. Click **Top**.
   Note: Top and Bottom boundaries may also be located by using the up/down arrow buttons in the **XYZ Navigation** window.

Note: Top = highest stage focus position
Bottom = lowest stage focus position

6) Enter **Step** interval or number of **Steps**.
   To set the Z-step interval to Nyquist, click on the number (**Suggested Step Size**) that is displayed between the **Step** and # of **Steps** options.
7) Select **Run Now**.
8) To stop the scan early and **SAVE DATA**, select **Finish**.
   To stop the scan early and discard the data, select **Abort**.

To crop in XY:

Select **Image / Crop**.
Optimize the size and location of the red box on the image.

To crop in Z:

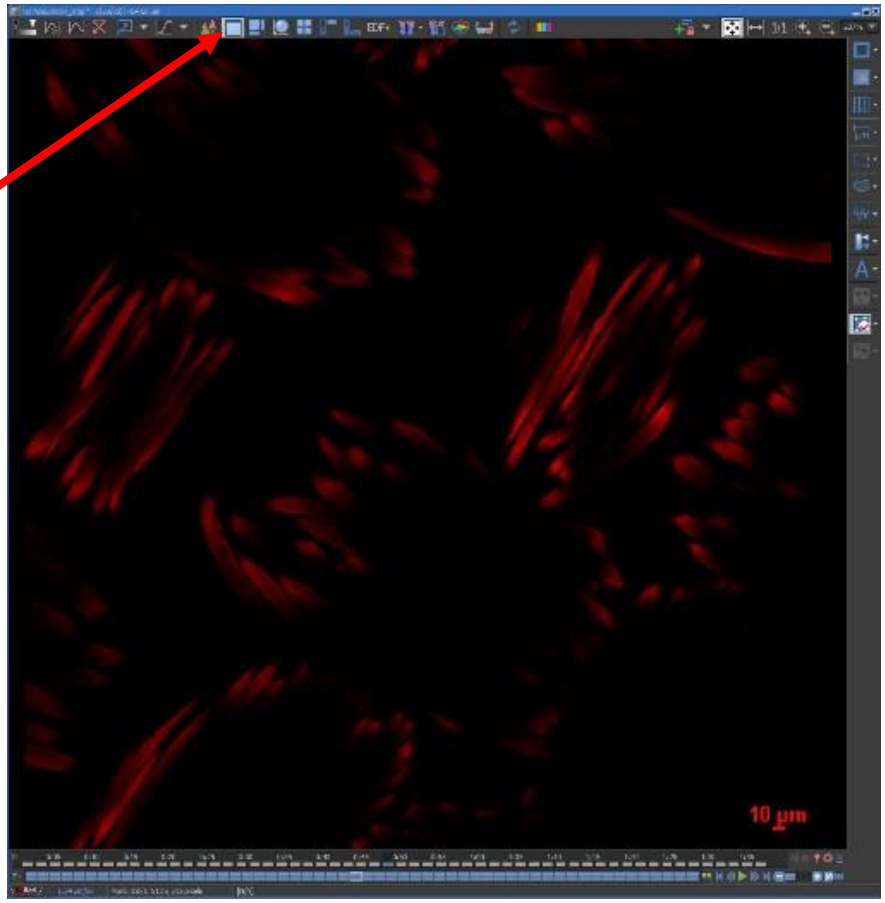
At the bottom of the XYZ series, press the **Shift** key, **left click** the mouse and **drag** to the right in order to select the desired range of images.
The selected buttons will turn from a blue color to a green color.
Right click on the selected buttons to "Keep Selected Frames" or "Delete Selected Frames".

3-Dimensional Image Display Options

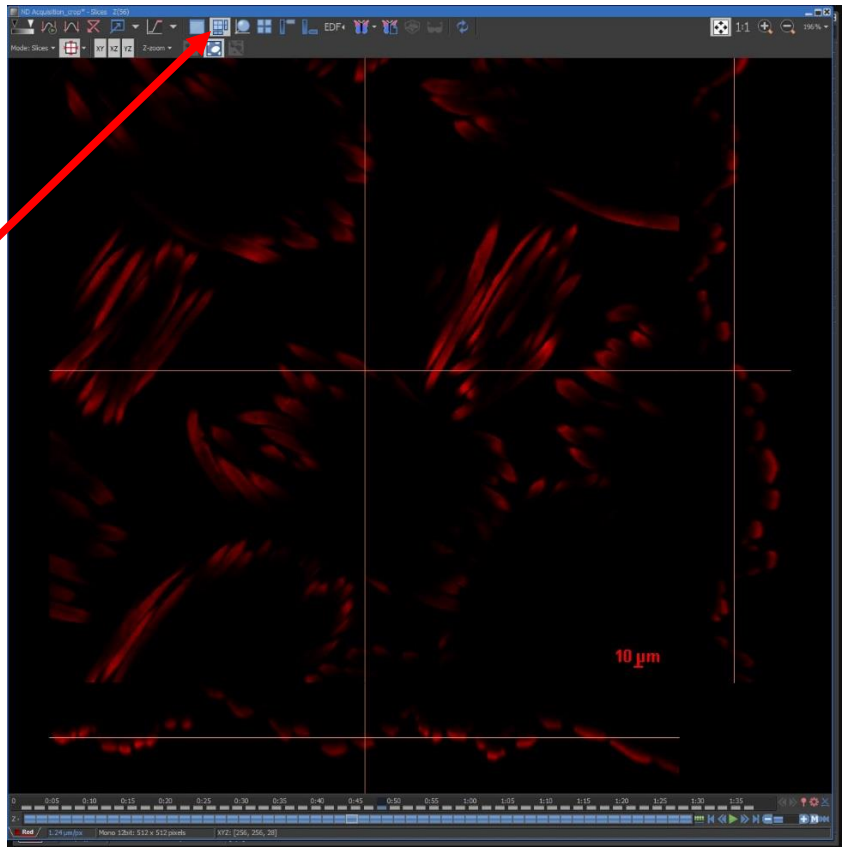
1) **Main View** = Single XY image through Z-series
2) **Slices View** = Orthogonal (Cross-Sectional) View
3) **Volume View** = MIP or Depth Shaded (Topographic)
4) **Tiled View** = Matrix of all images in XYZ series
5) **Maximum Intensity Projection** = MIP image

The 3D Image Display icons are located at the top of the XYZ image series.

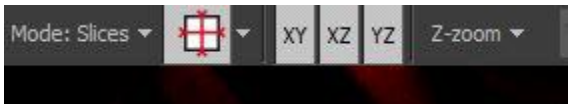
Main View



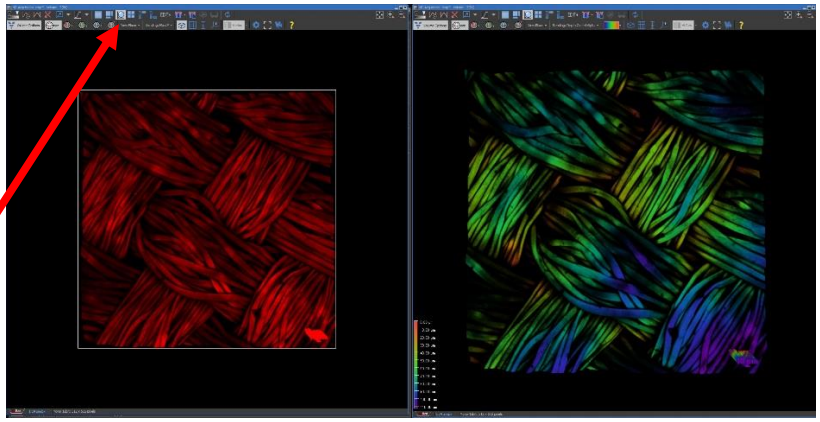
Slices View
Also called an Orthogonal or Cross-Sectional View



Slices View Options



Volume Views
Max Intensity Projection
Depth Shaded View



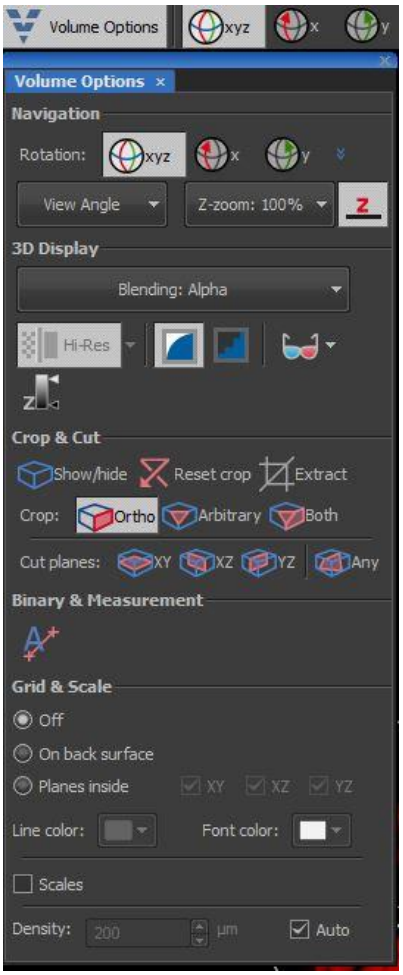
Volume Options

Rotation Angle
- XYZ
- X-only
- Y-only

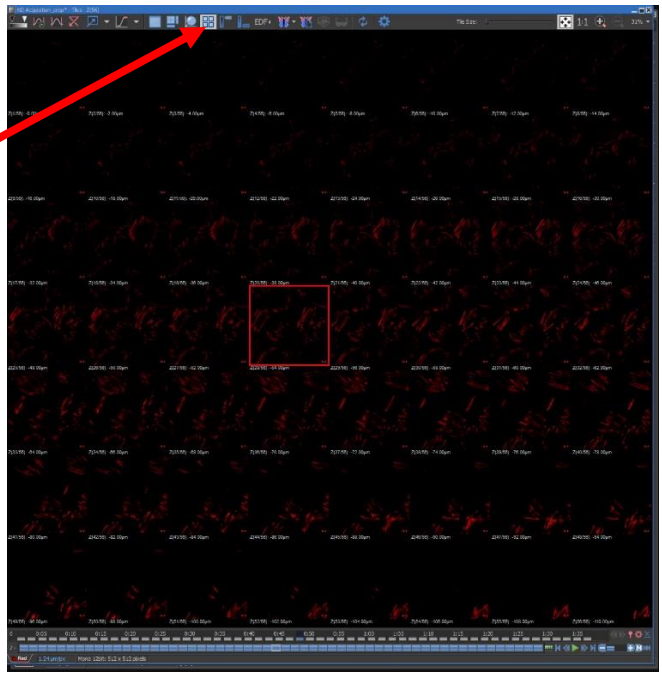
Viewing Angle
- XY
- XZ
- YZ

Z-Zoom
- Expand Z thickness

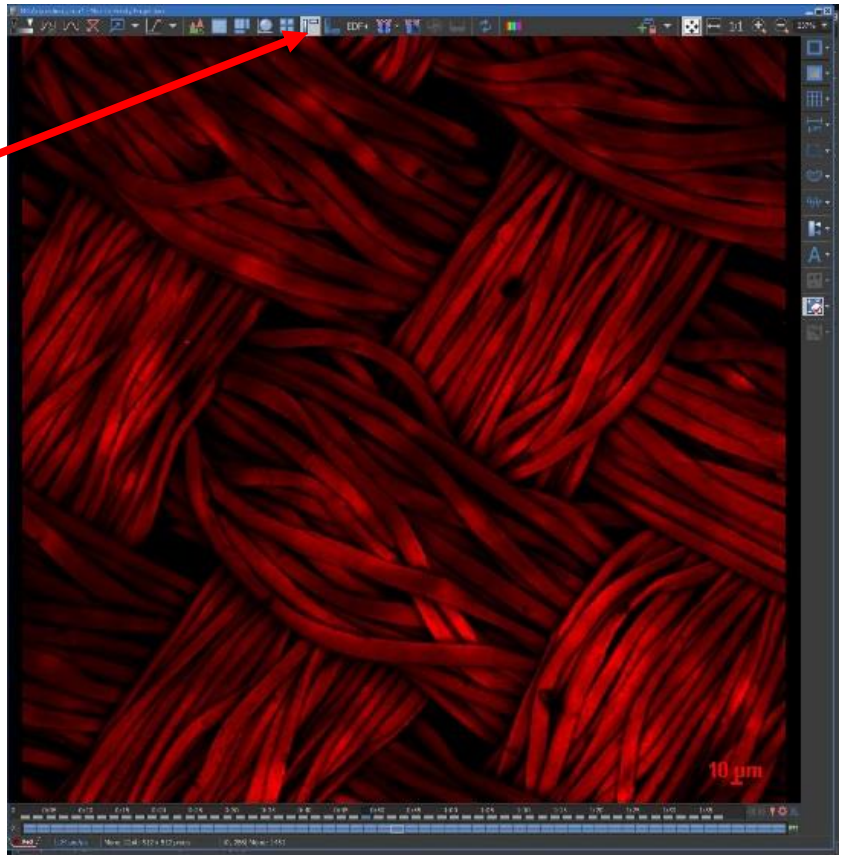
Blending Mode
- Alpha
- MIP
- Depth Coded Alpha
- Depth Coded MIP



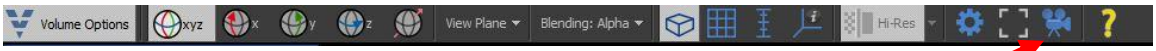
Tiled View



Maximum Intensity Projection



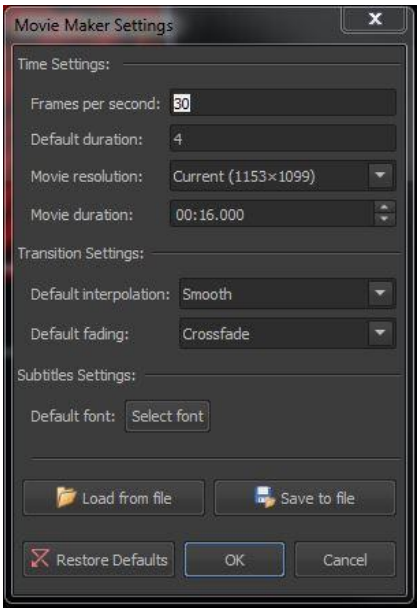
3D Movie Maker in the Volume View.



Movie Maker Option

1) Select the display orientation for your first image in the movie and click on the "**+**" sign.
2) Change the display to the second orientation in the movie and click on the "**+**" sign.

3) Select the green arrow to preview the movie or select the red X to delete the movie series.
4) Select the blue cog for the Movie Maker Settings options.
5) Select the film clip icon to generate the movie.
6) Select **File / Save As** to save the movie in an **AVI** format.



Preset Options
Drag and drop icon into the movie stream.