Nikon

NIS-Elements

Photonics Instruments, Inc.
MicroPoint Module Instructions
(Part #97154)

(Ver. 1.03)

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I. Introduction

The Photonic Instruments Inc. MicroPoint is a system for creating a diffraction limited spot. The MicroPoint uses a nitrogen pumping laser to excite a dye pack. The resulting laser output is focused to a diffraction limited spot. The location of this diffraction limited spot is controlled by internal galvanometers. The power being delivered can be controlled by an internal and external ND Filter. The excitation wavelength is determined by the chemical composition in the dye pack.

II. Installation & Setup

A. Installation

In order to operate the MicroPoint with in NIS-Elements, it is necessary to install both the driver for the MicroPoint hardware and the NIS-Elements Module. It is assumed that the proper version of NIS-Elements (3.0 SP8 (Build 548)) is properly installed prior to installing the drivers and the Module.

i. Order of Installation

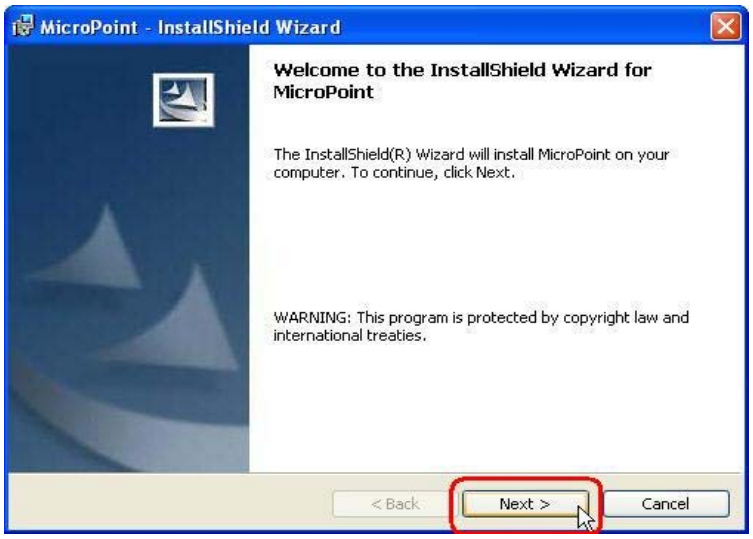
a. Install NIS-Elements

b. Install MicroPoint Module

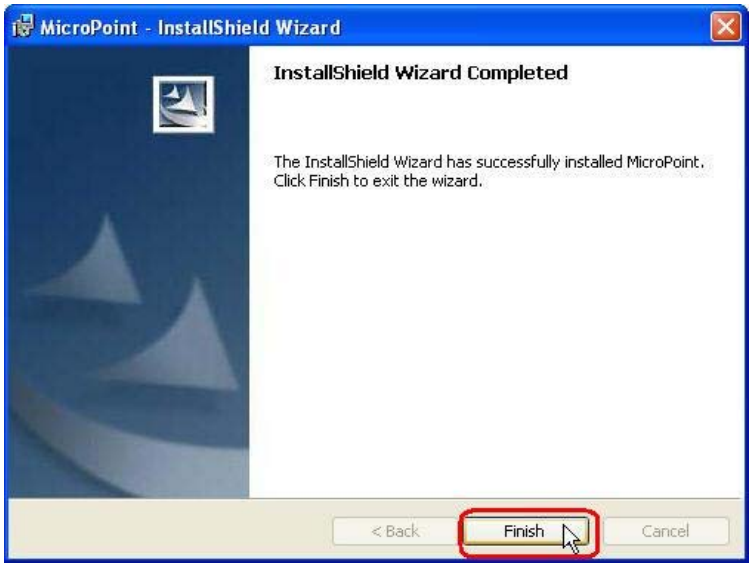
1. Copy the MicroPoint Installation file to a Local Directory. (i.e. C:\Nikon)

2. Double click on MicroPoint.msi

3. Press **Next** button.



4. Press **Finish** button.



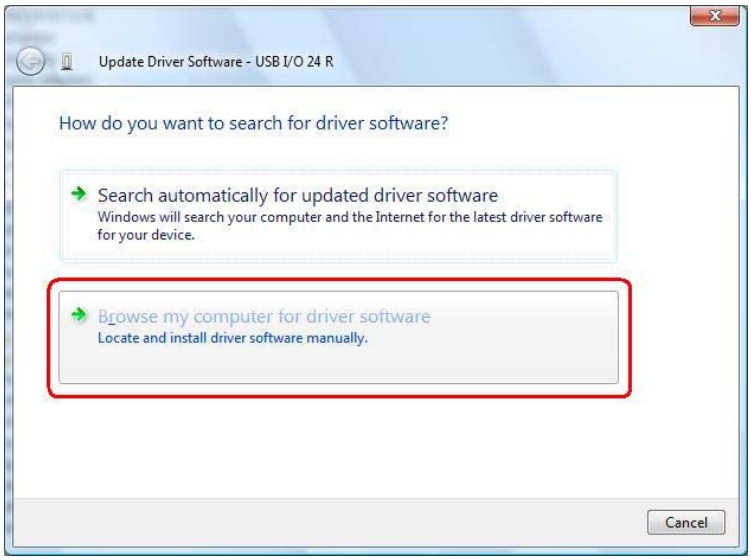
c. Install the MicroPoint Drivers

I. Vista 32 Installation

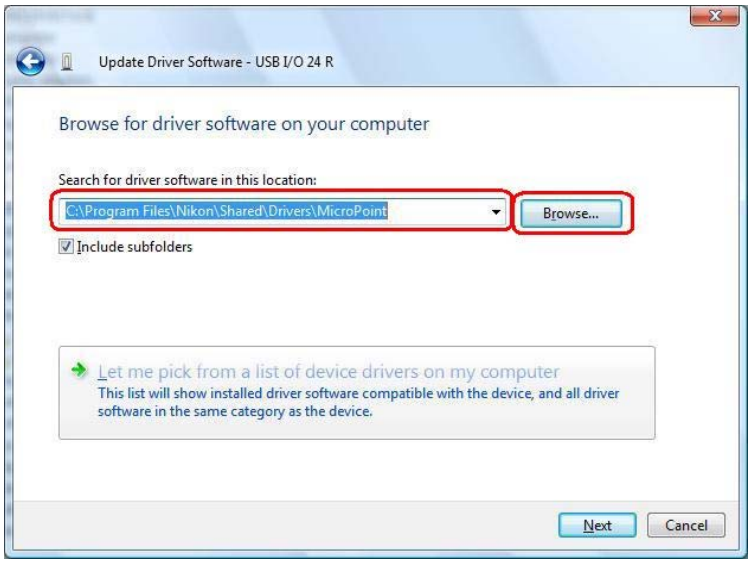
1. Plug the MicroPoint controlling box's USB cable into an open USB slot on the PC.

2. Turn on the MicroPoint. Windows will automatically find the new hardware. Select "Locate and install driver software (recommended)."

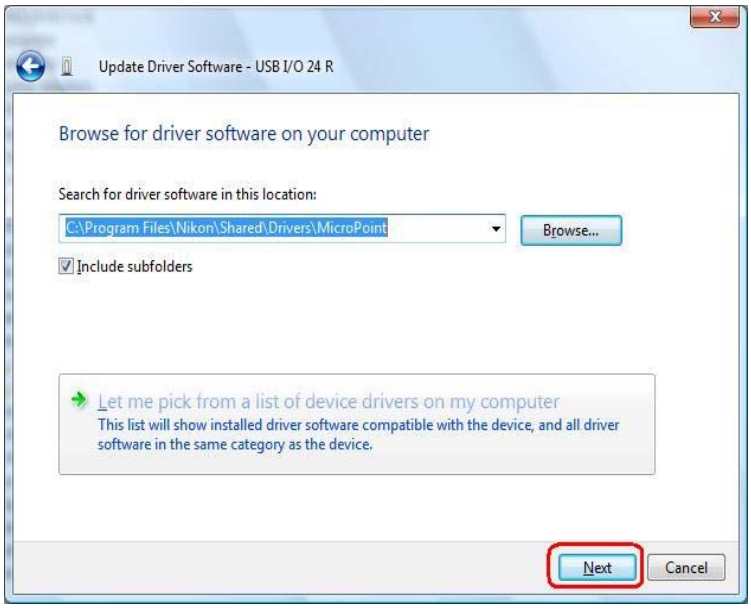
3. Select “Browse my computer for driver software.”



4. Press **Browse…** button and select the location C:\Program Files\Nikon\Shared\Drivers\MicroPoint.



5. Press **Next** button.



6. Select “Install this driver software anyway.”

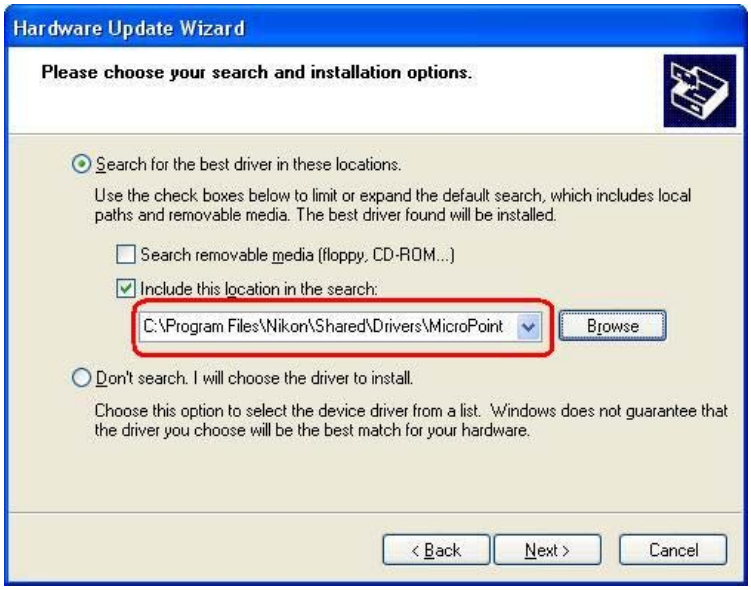
II. XP 32 Installation

1. Plug the MicroPoint controlling box’s USB cable into an open USB slot on the PC.

2. Turn on the MicroPoint. Windows will automatically find the new hardware. Select “Install from a list or specific location (Advanced).”

3. Press **Next** button.

4. Check **Include this location in the search**: and the location to C:\Program Files\Nikon\Shared\Drivers\MicroPoint.



5. Press **Next** button.

6. Press **Continue Anyway** button.

7. Press **Finish** button.

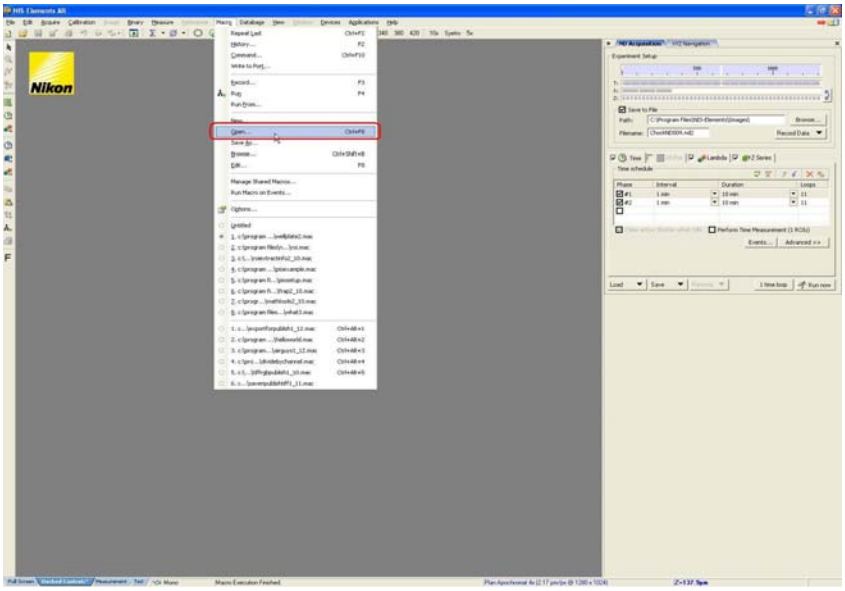
ii. Delivered Files and Locations

1. C:\Program Files\Nikon\Shared\dlls\MicroPointGUI.dll
2. C:\Program Files\Nikon\Shared\macros\MicroPoint.mac
3. C:\Program Files\Nikon\Shared\Drivers\MicroPoint\FTD2XX.DLL
4. C:\Program Files\Nikon\Shared\Drivers\MicroPoint\FTD2XXUN.INI
5. C:\Program Files\Nikon\Shared\Drivers\MicroPoint\FTD2XX.INF
6. C:\Program Files\Nikon\Shared\Drivers\MicroPoint\FTD2XX.SYS
7. C:\Program Files\Nikon\Shared\Drivers\MicroPoint\FTD2XXUN.EXE
8. C:\Program Files\Nikon\Shared\Drivers\MicroPoint\FTD2XX.LIB
9. C:\Program Files\ Nikon\Shared\docs\MicroPoint Instructions.doc
10. C:\Program Files\ Nikon\Shared\docs\ROI Help.pdf

B. Setup

i. Launching the MicroPoint Module Dialog.

1. Go to **Macro**->**Open** and select the MicroPoint Module (macro) file MicroPoint.mac. [Typically C:\Program Files\Nikon\Shared\Macros\MicroPoint.mac]

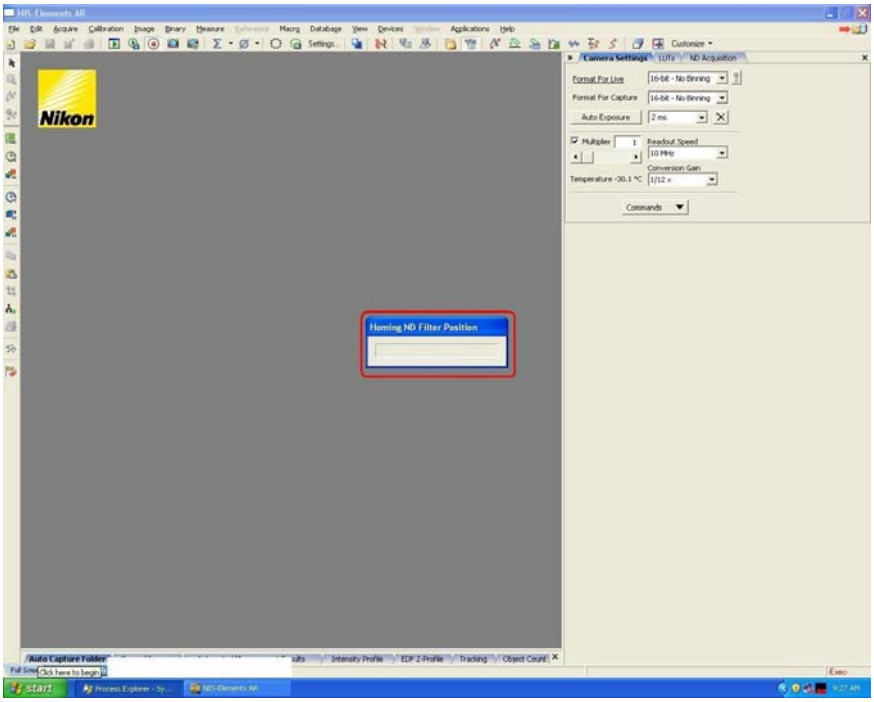


2. Go to **Macro**->**Run**.

This will launch the MicroPoint Module dialog. Upon start, the MicroPoint Module will check for Calibration Values.

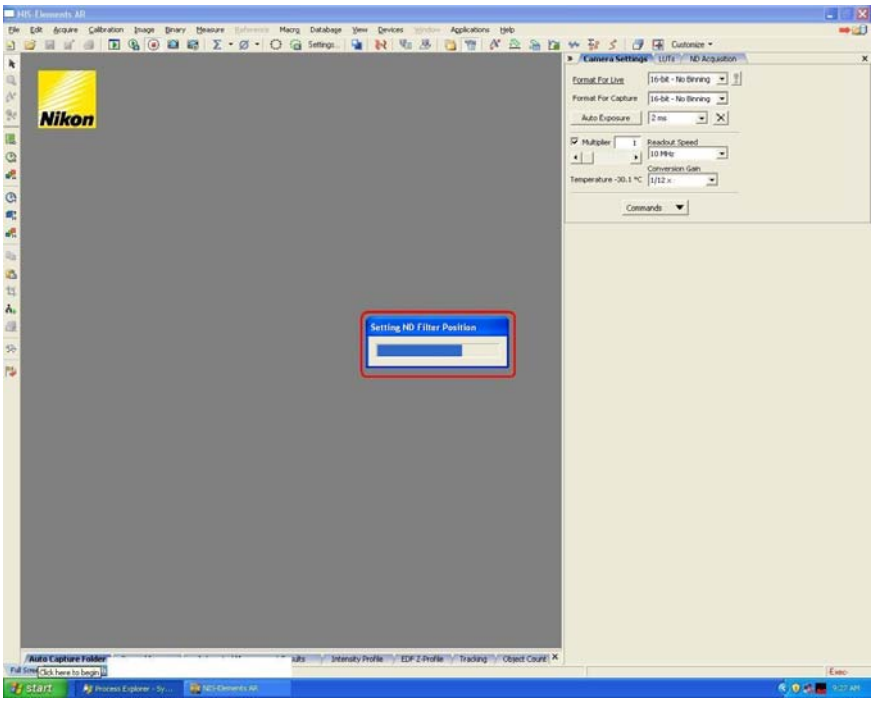
If no Calibration Values are found the MicroPoint Module will display a message that the “MicroPoint is not calibrated. Please calibrate.”

If the MicroPoint is Calibrated no message will appear. The MicroPoint Module will then home the internal neutral density filter.



Note: *The progress bar is an estimation only.*

Once the internal neutral density filter finds its home position, the MicroPoint will set the filter to the last set value for ND Filter Position (1-85).



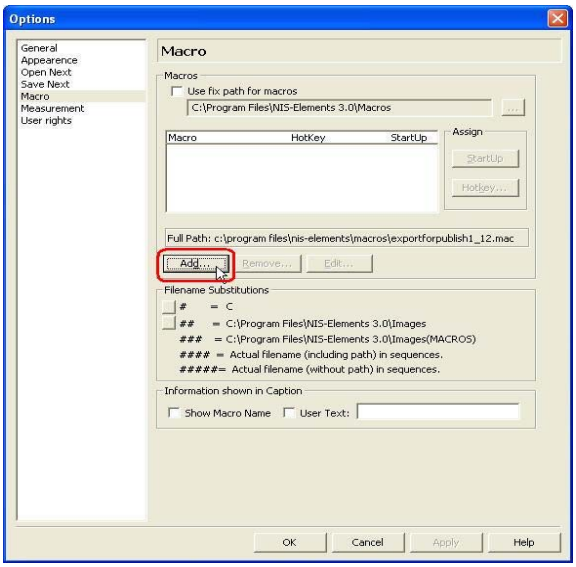
Note: *Upon running for the first time, the MicroPoint Module will leave the Neutral Density Filter at 1.*

The MicroPoint Module can either be run as a free floating dialog that is contained within NIS-Elements or it can be docked to either the Left or Right Docking Pane. Once NIS-Elements is closed, it will remember that last state of the Control panel. Then in the next session of NIS-Elements, after the MicroPoint Module is launched, the Control panel will automatically return to the MicroPoint Module dialog to its last position.

ii. Setting up automatic launching of MicroPoint Module Dialog

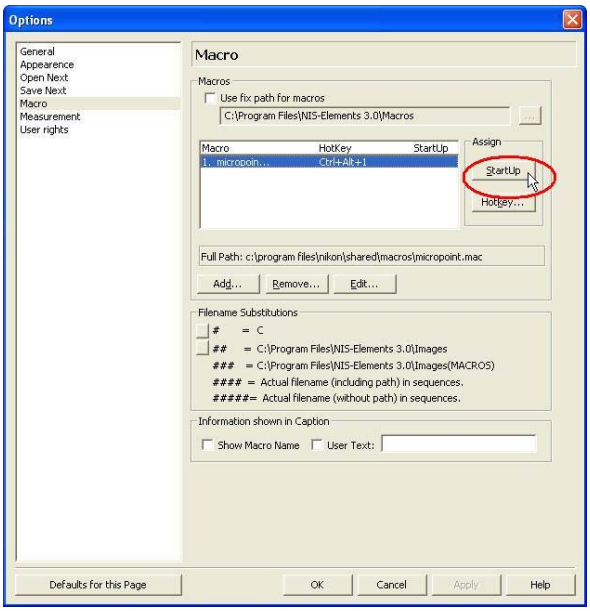
1. Go to **Macro**->**Options…**

2. Press the **Add…** button.



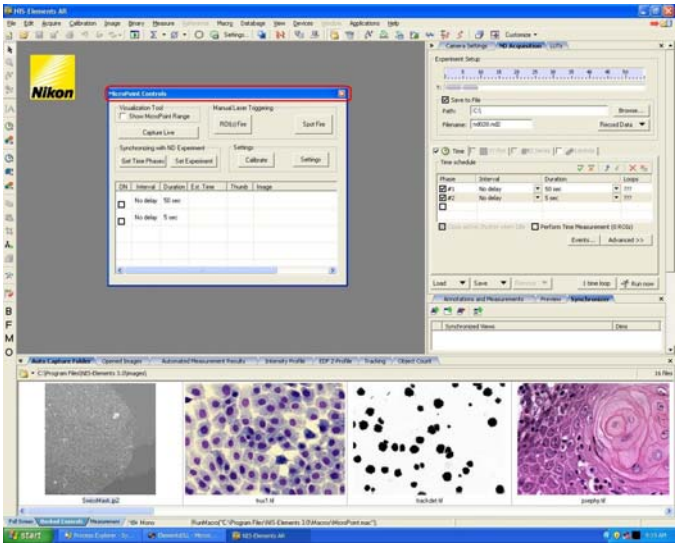
3. Select the MicroPoint Module (macro) file MicroPoint.mac [Typically C:\Program Files\Nikon\Shared\macros\MicroPoint.mac]

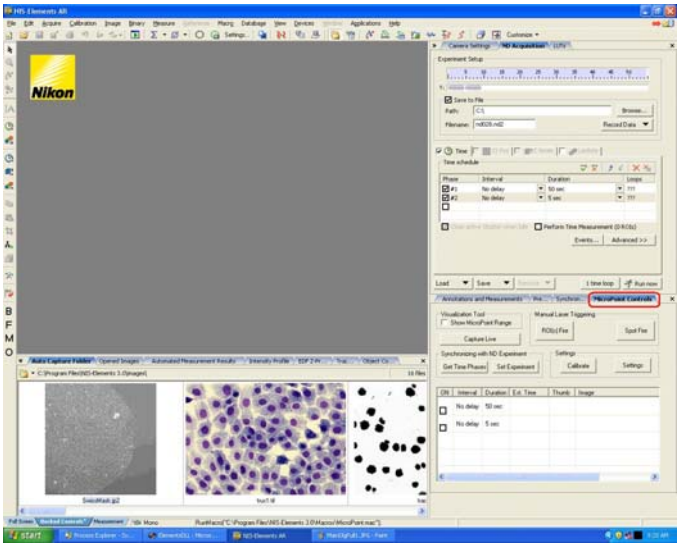
4. Highlight the MicroPoint.mac and then press **Startup** button.



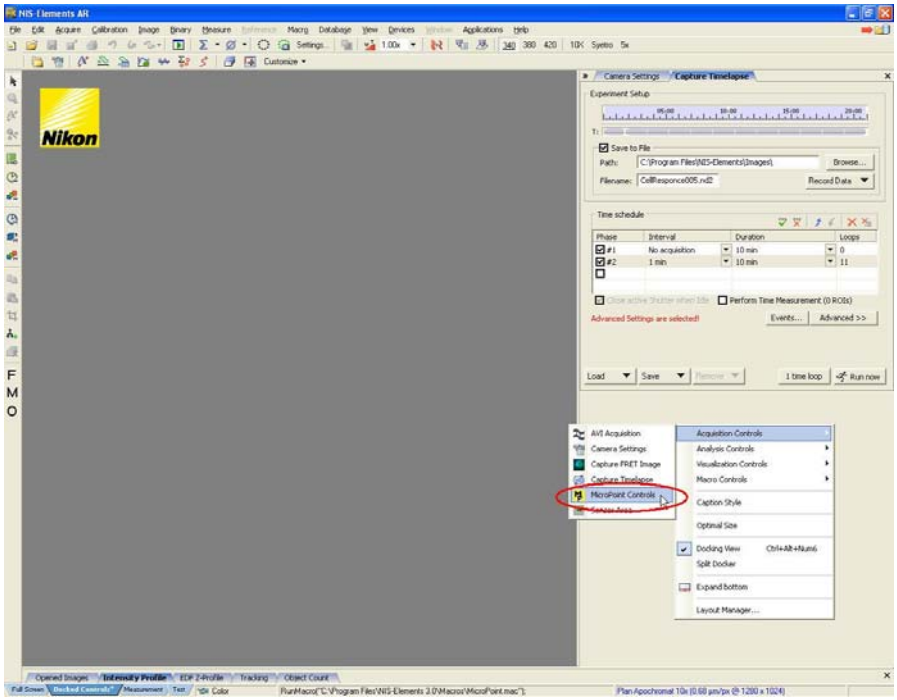
iii. Docking/Undocking MicroPoint Module Dialog.

The MicroPoint Module can be docked or undocked at anytime. Once the Module is launched/ opened, the MicroPoint Module dialog will appear. Double clicking on the title bar of the dialog will undock the MicroPoint Module control panel if it is docked or dock the MicroPoint Module control panel it is not already docked.





The MicroPoint Module can also be docked by right clicking in a docking panel and selecting **Acquisition Controls**->**MicroPoint Controls**.



III. MicroPoint Calibration Dialog

The MicroPoint Module is designed to be used with any NIS-Elements supported camera and any Nikon objective. The MicroPoint Calibration Dialog contains several tools to help enable the Calibration of the MicroPoint including Laser settings[1]. In order to calibrate, the MicroPoint Module collects 16 points in each axis. The collection of Calibration Points is designed occur automatically. However, there are tools for setting these calibration points manually. There can be several reasons to choose a manual calibration.

Notes:
All Calibrations are preformed with a Calibration Slide and transmitted light.

The image in the Live (from the camera) should be at the same focus as the image from the eyepiece. If an object is focused in the eyepiece and the Z needs to be changed to focus in Live, contact your Nikon representative. The difference in this focus will cause the laser to not be properly focused.

Warning: *Currently the MicroPoint Module has to be recalibrated for the following conditions:*

1. *The objective has been changed.*
2. *The capture area for the camera has been changed.*
3. *The Binning state has been changed. (Turned Off to On or On to Off)*

A. Finding Camera Direction and Rotation

For both the automatic calibration and the manual calibration, first allow the MicroPoint Module to find the direction and rotation of the camera. There are three basic parts to finding the Camera Direction and Rotation.

1. It is recommended to confirm that MicroPoint Module is generating a proper Calibration Point with a single laser trigger. The Calibration Points can be large for finding the Camera Direction and Rotation.
2. Start the Find Camera Direction and Rotation Process.
3. Defining a Calibration Point through thresholding.[2]

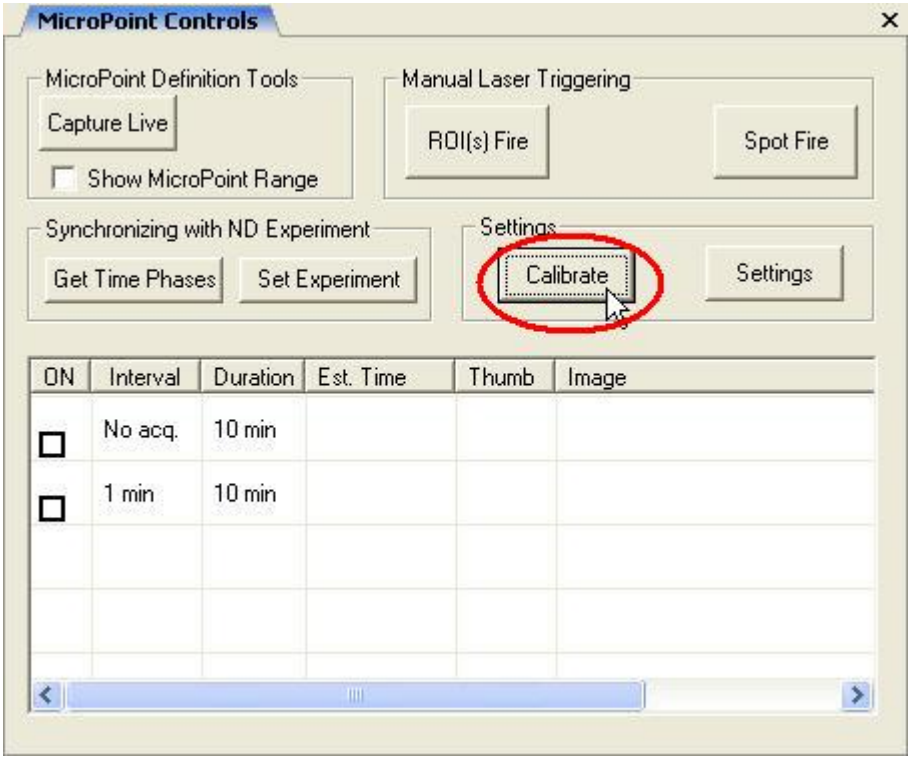
Camera Direction and Rotation only has to be successfully determined once. It may take a few attempts to determine what the proper combination of Camera Settings, ND Filter Position (the internal neutral density filter), and External Neutral Density Filter.

Note: *Automatic and Manual calibration are not available until the MicroPoint Module has successfully determine the Camera Direction and Rotation.*

[1] More information and detail on the common tools and function are included in Sections V & VI.
[2] Defining and thresholding a calibration point is detailed on Page 19 of this file.

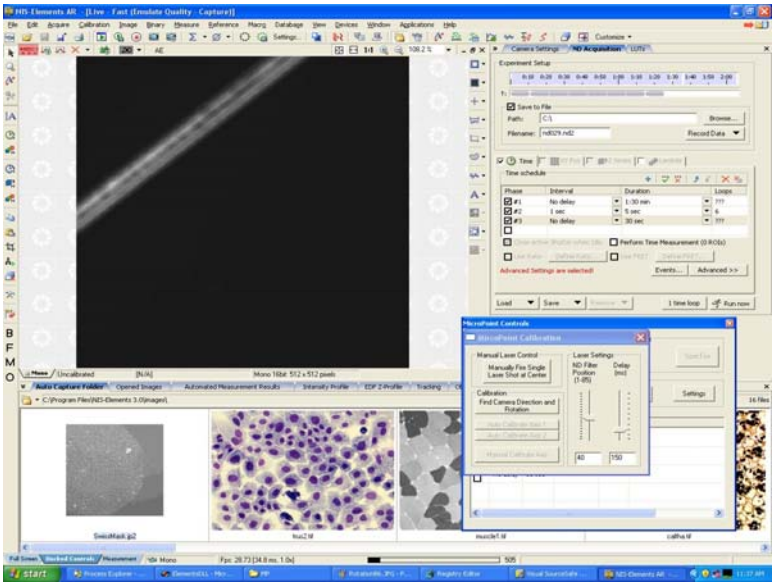
i. Typical Find Direction and Rotation.

1. To access the MicroPoint Calibration Dialog press **Calibrate** button on the MicroPoint Controls Dialog.

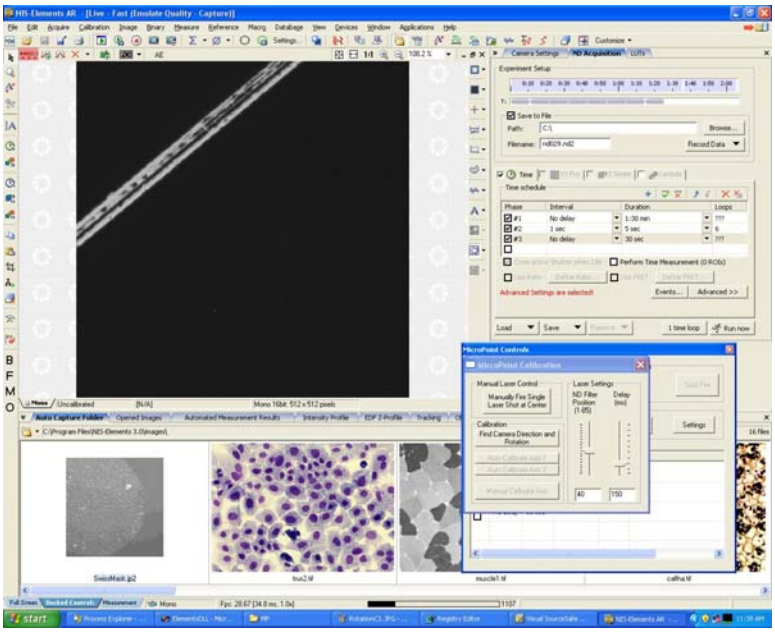


2. Place a Calibration Slide for viewing and ensure that the transmitted light source is On.

3. Locate and focus on scratch and/or landmark.

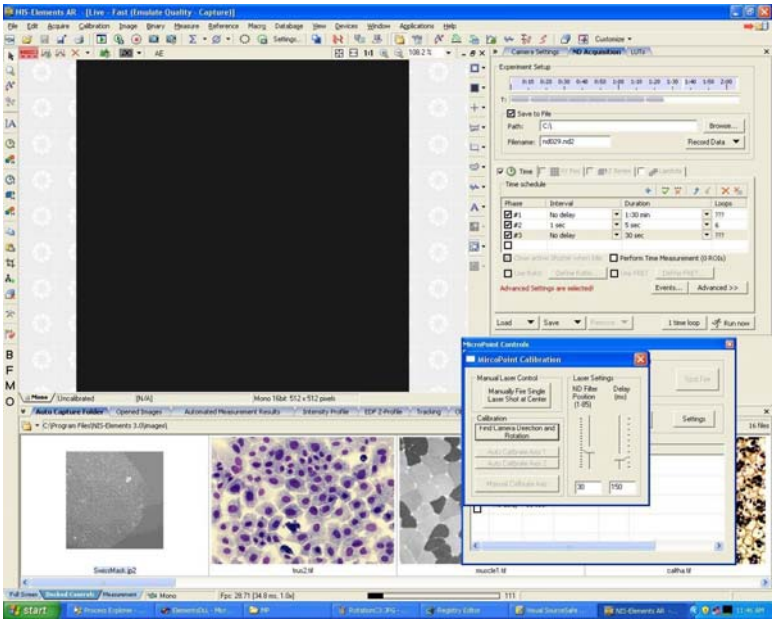


4. Focus.



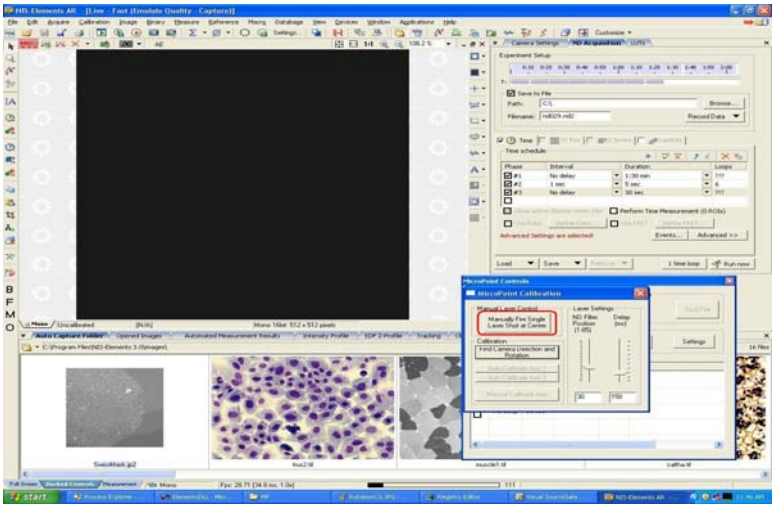
Note: *The camera settings may have to be adjusted. The setting should be adjusted so the Live image from the camera closely matches the image from the eyepiece. (i.e. If there are details inside of the scratch then the live image should reflect this and not be a solid line.)*

5. Move to a different field of view.

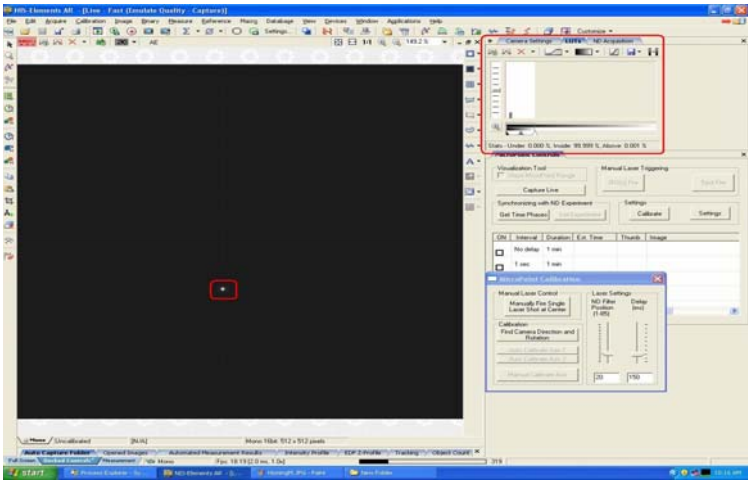


Note: *The different field of view does not have to be free of Calibration Points.*

6. Press **Manually Fire Single Laser Shot at Center** button.



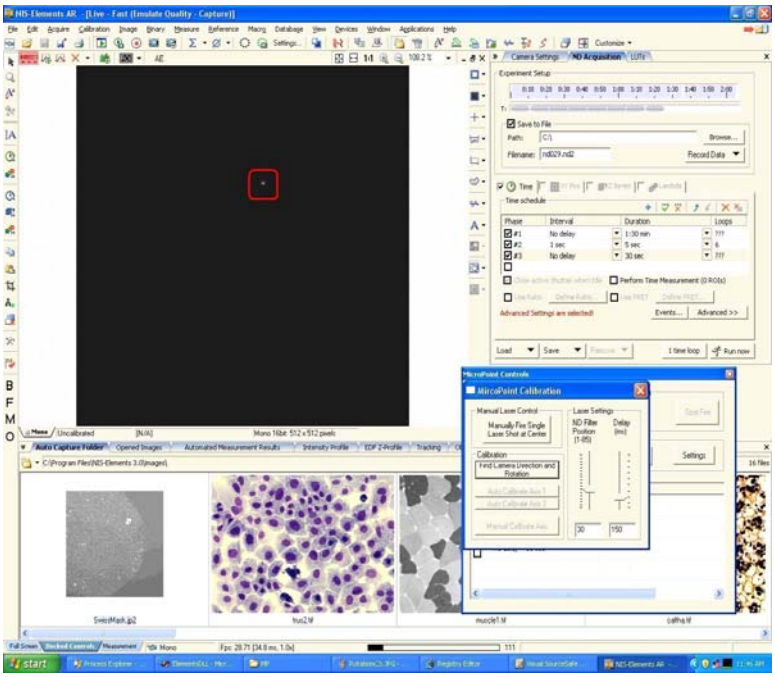
a. A Calibration Point may exist, but the Look Up Table (LUT) may have to be adjusted for Calibration Point easily visible in the Live window.



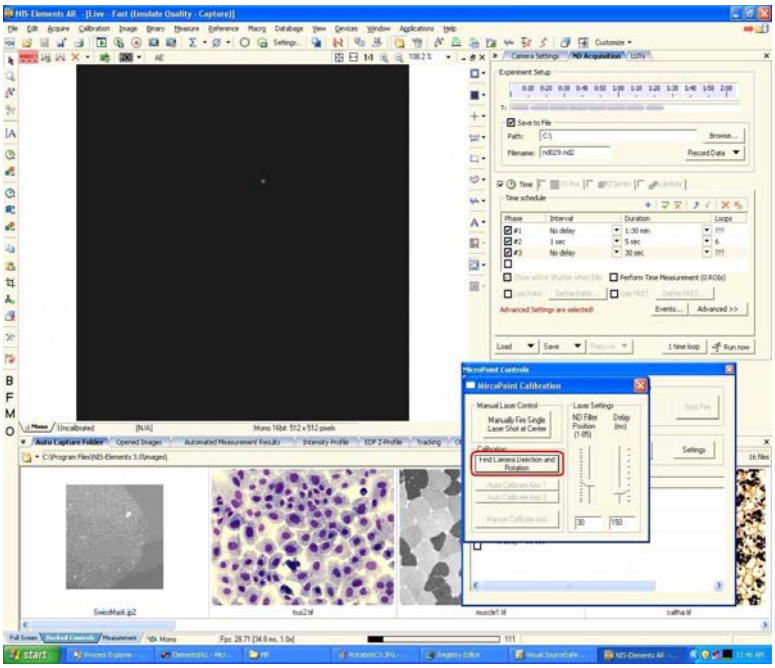
b. If needed, adjust power to ensure a Calibration Point [Higher to create a point if one does not exist, Lower if the point it large and/or uneven.]

> ***Notes:***
> *It is generally easier to start at a high power (Large ND Filter Position) and then try lower power.*
>
> *If ND Filter Position needs to be set to a value greater than 60 in order to create a Calibration Point, lower the External Neutral Density Filter.*

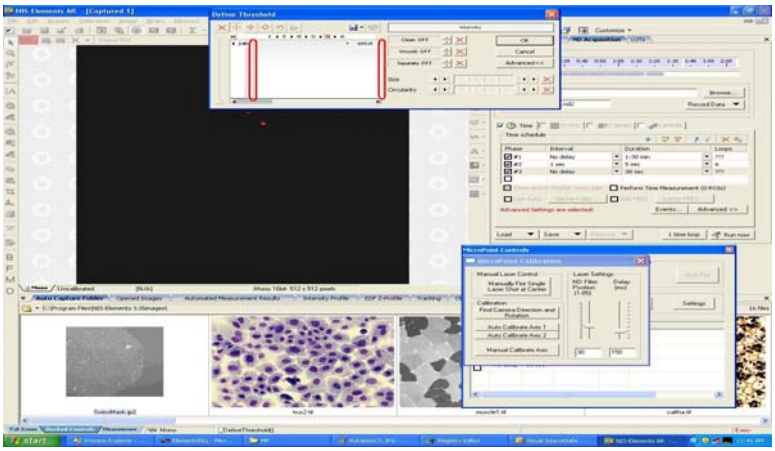
7. Repeat Steps 5-6 until the calibration point looks like a distinct round spot.



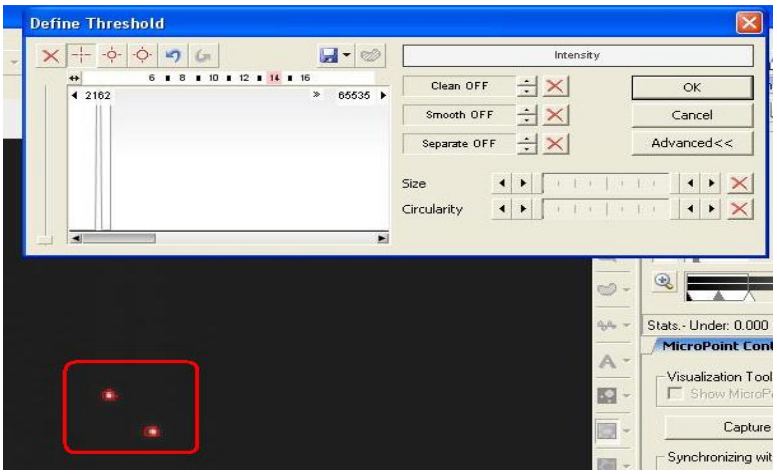
8. Press **Find Camera Direction and Rotation** button.



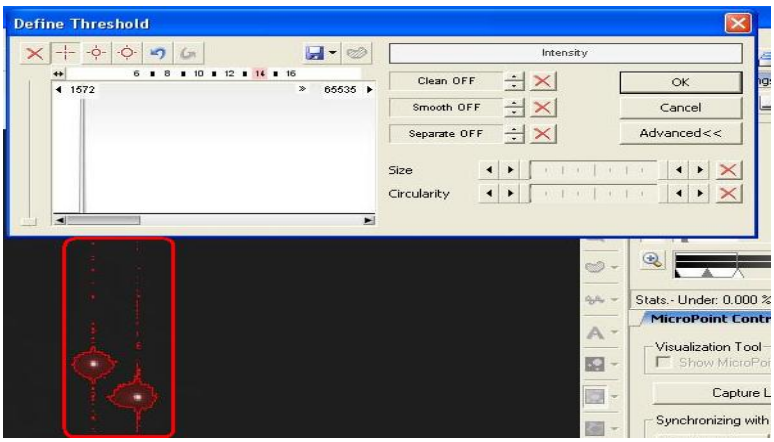
9. In order to define a calibration point, use the Thresholding Dialog to adjust the upper and lower limits of the histogram until the spot has been clearly detected.



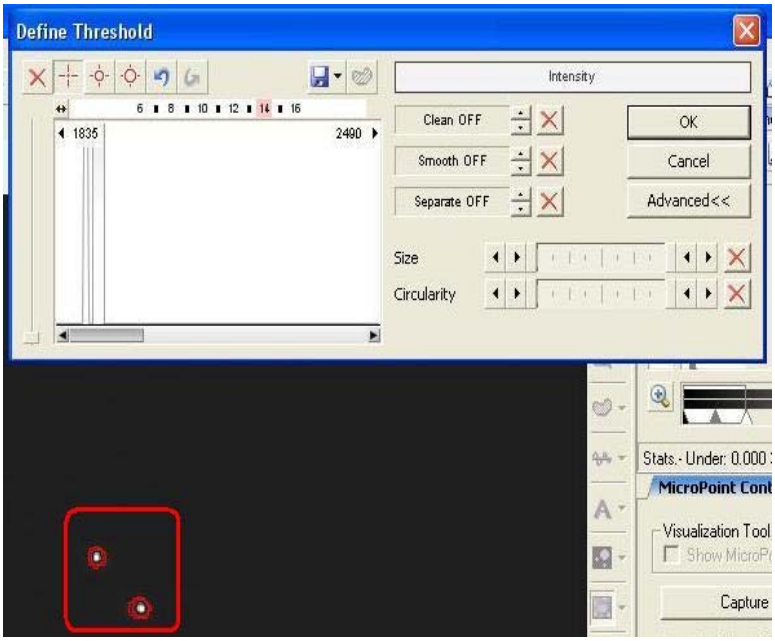
The goal is to have a single full circle around all Calibration Points in the field of view. The circle should be ideally round, but necessarily does not have to be, a long as it is solid.



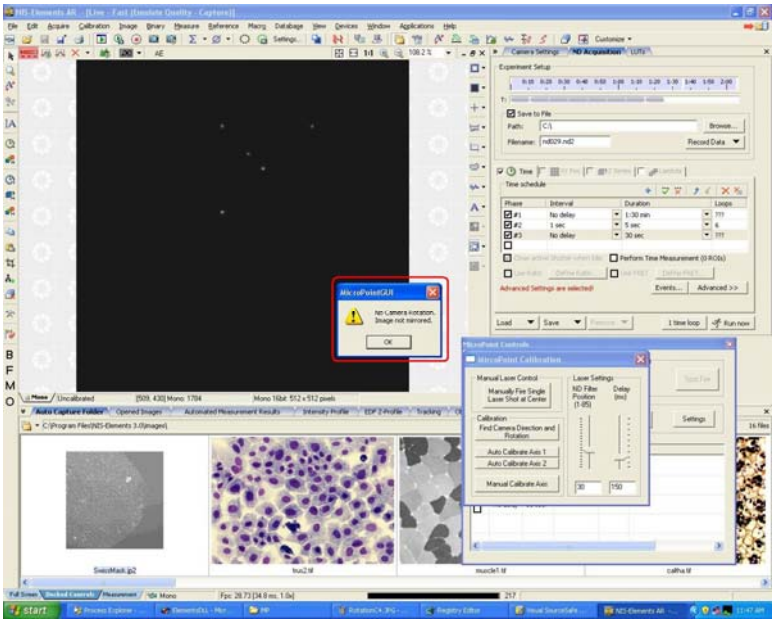
If there are random spots, the lower value for the thresholding is too low.



If the circles are not solid, the upper value for the thresholding is not high enough. Generally the upper value for the threshold should be set to the maximum.

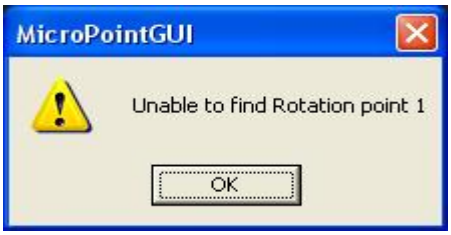


10. If successful, the MicroPoint Module will generate a message with the direction and rotation of the camera.



Note: The camera rotation can be 0, 90, 180, or 270 degrees. Camera direction can be 'Image not mirrored' or 'Image mirrored.'

If the MicroPoint Module was unable to find a Calibration Point, the Module will generate a message indicating which Calibration Point it was unable to find.



It will be necessary to repeat step 8-9 until the MicroPoint Module was successful in determining the direction and rotation. The following may have to be adjusted:

1. Camera Settings
2. Thresholding Values
3. ND Filter Position
4. External Neutral Density Filter

B. Automatic Calibration

The buttons for automatic calibration will not be enabled until the MicroPoint Module determines the camera direction and rotation. Once pressed, the Module will fire the laser into a non-Calibration area and then the Module will generate a prompt to threshold a Calibration Point. Once the Calibration Point characteristics have been supplied (through thresholding), the Module will fire 16 Calibration Points for the selected axis and locate their centers. Axis 1 may be the X or Y axis depending on the camera rotation. If the Module located all 16 centers, it will then calculate the corresponding galvanometer to pixel translation.

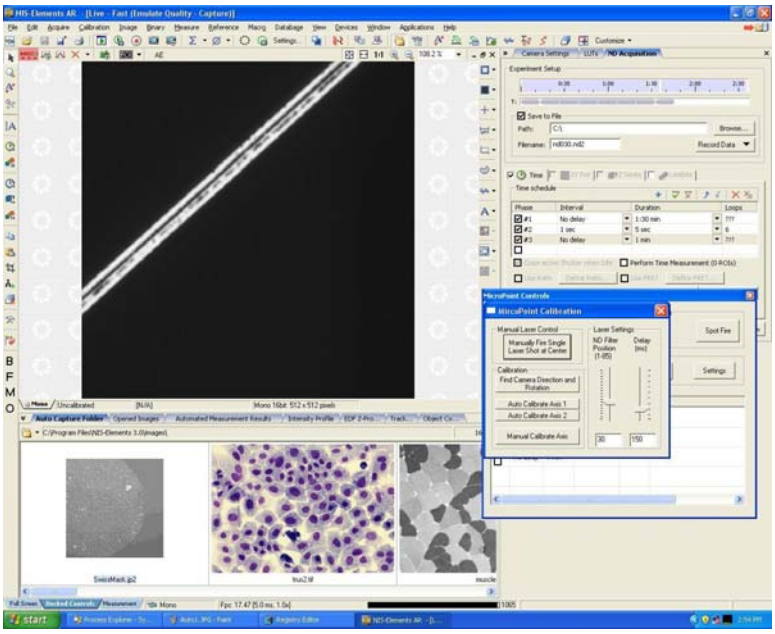
There are three basic parts to Automatic Calibration for Axis #.

1. Confirm that MicroPoint Module is generating a proper Calibration Point with a single laser trigger. The Calibration Points should be as small a possible. Smaller Calibration points will create a more accurate Calibration than larger Calibrations Points.
2. Start the Automatic Calibration for Axis # Process.
3. Define what a Calibration Point looks like for NIS-Elements (through thresholding).

Note: It is assumed that prior to Step 1 that the following has already been completed:
1. The Calibration Dialog is open.
2. The Camera Settings, ND Filter Position, and the External Neutral Density filter adjusted to ensure that a single laser triggers creates a satisfactory Calibration Point.

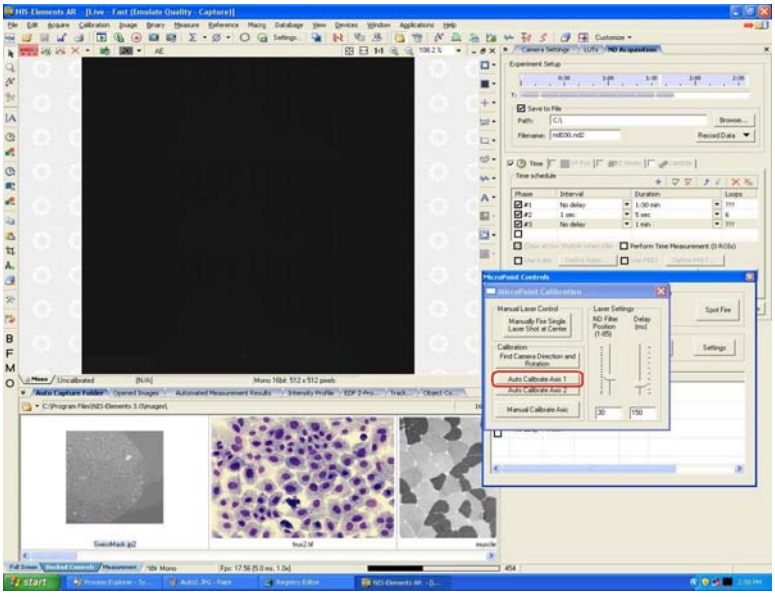
i. Typical Automatic Calibration.

1. Focus.



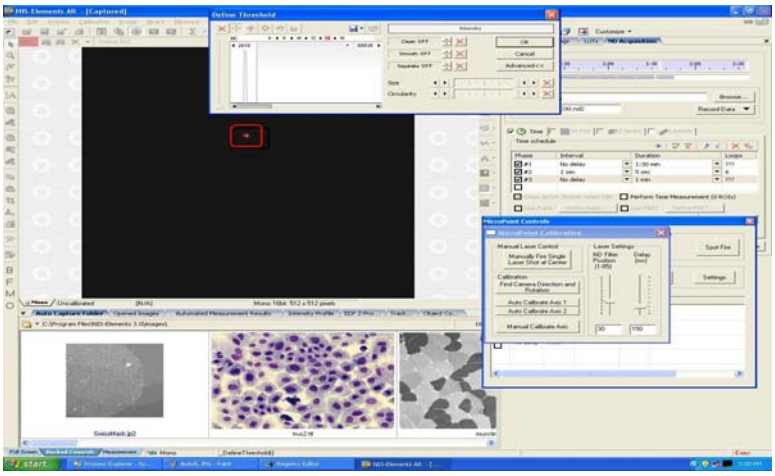
2. Move to a different Field of view.

3. Press Auto Calibrate Axis 1.



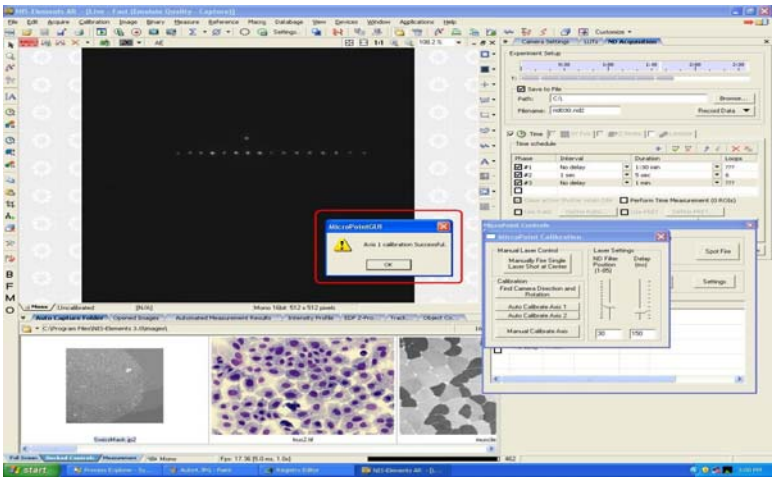
Note: The Axis can be calibrated in any order.

4. In order to define a calibration point, use the Thresholding Dialog to adjust the upper and lower limits of the histogram until the spot has been clearly detected.

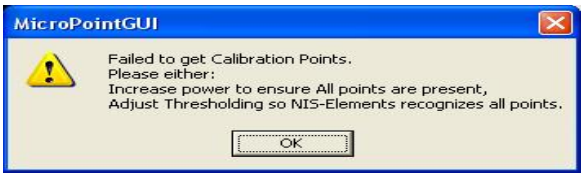


Note: *NIS-Elements will remember that last set Threshold. If no settings have changed since the previously found Calibration Point and the previously found Calibration point was satisfactory, then it is not necessary to adjust the thresholding and it is possible to simply press the* ***OK*** *button in the Thresholding dialog.*

5. If the MicroPoint Module finds all the Calibration Points, it will generate a message that the calibration was successful.



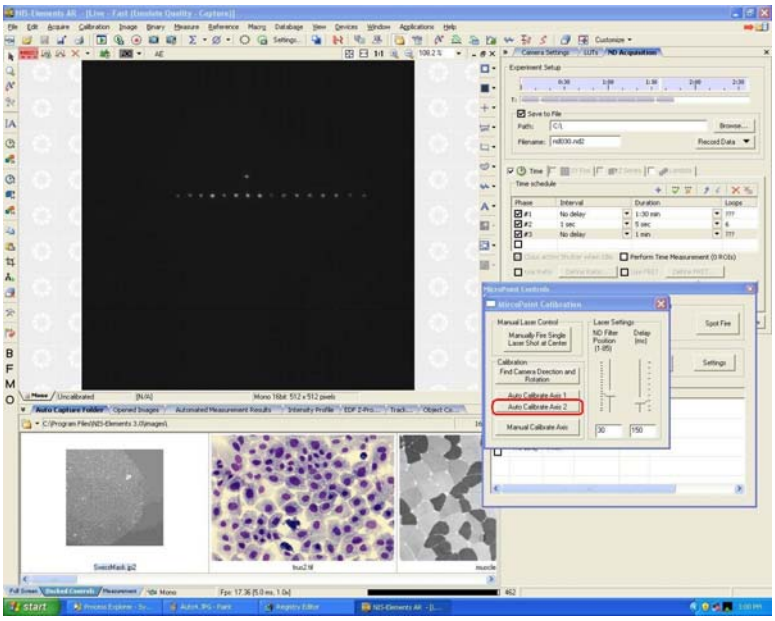
If the MicroPoint Module cannot find all the Calibration Points, a warning will appear and it will be necessary to repeat Steps 2-4.



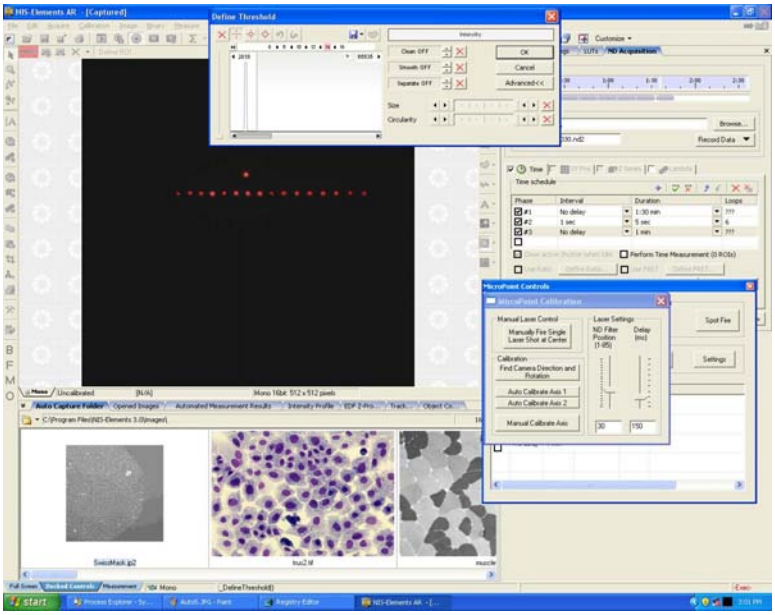
6. Move to a different Field of view.

> ***Note:*** *This may not be necessary if the Calibration points are small and the threshold definition is satisfactory.*

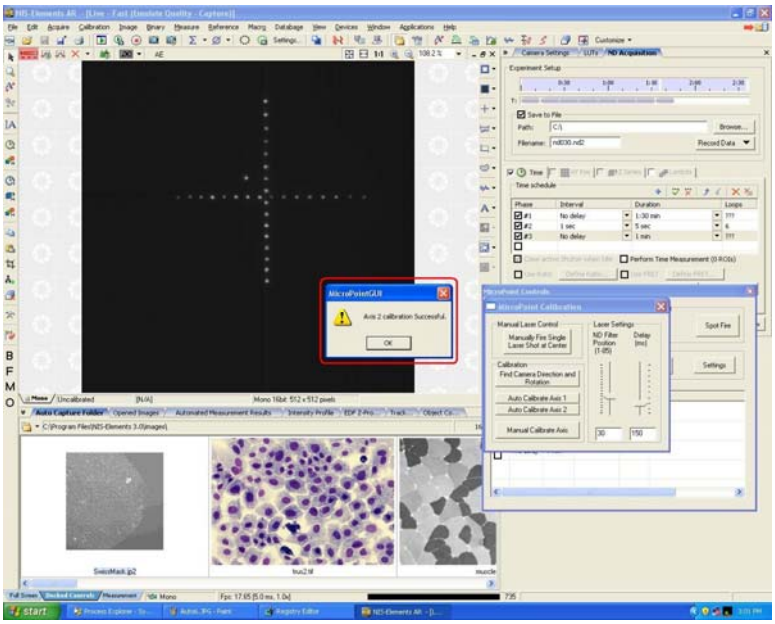
7. Press Auto Calibrate Axis 2.



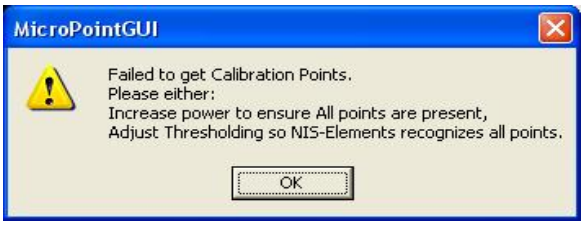
8. In order to define a calibration point, use the Thresholding Dialog to adjust the upper and lower limits of the histogram until the spot has been clearly detected.



9. If the MicroPoint Module finds all the calibration points, it will generate a message that the calibration was successful



If the MicroPoint Module cannot find all the Calibration Points, a warning will appear and it will be necessary to repeat Steps 6-8.



C. Manual Calibration

The **Manual Calibrate Axis** button will not be enabled until the MicroPoint Module determines the camera direction and rotation. Manual Calibration is only intended for cases in which automatic calibration fails to locate all Calibration Points or if there is an interest to try to improve the MicroPoint Module's calculated Calibration.

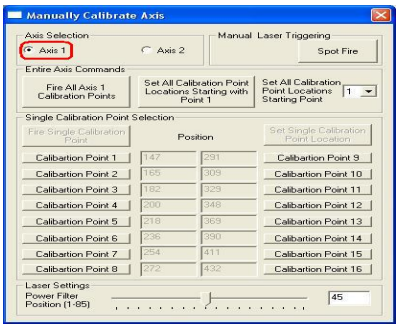
Tip: *Poor Calibration typically occurs because of unsatisfactory thresholding and/or camera limitations.*

There are two basic parts for Manual Calibration:

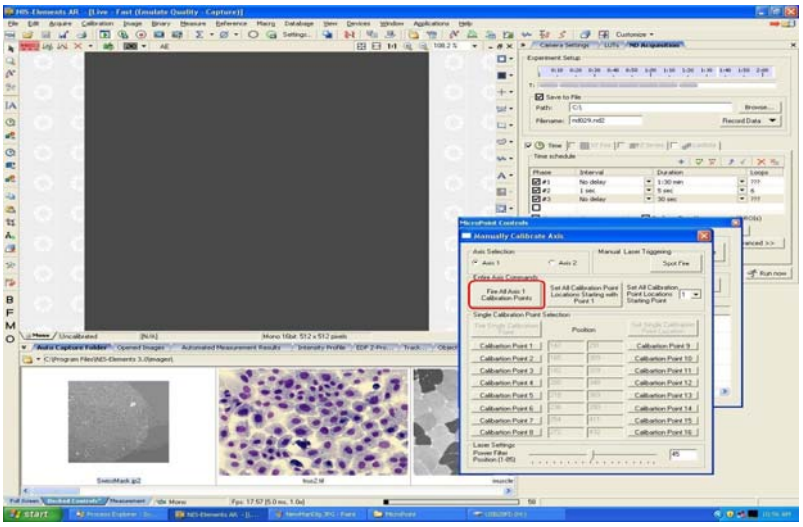
1. Create a Calibration Point.
2. Set the Position of Calibration Point.

i. Typical Manual Calibration.

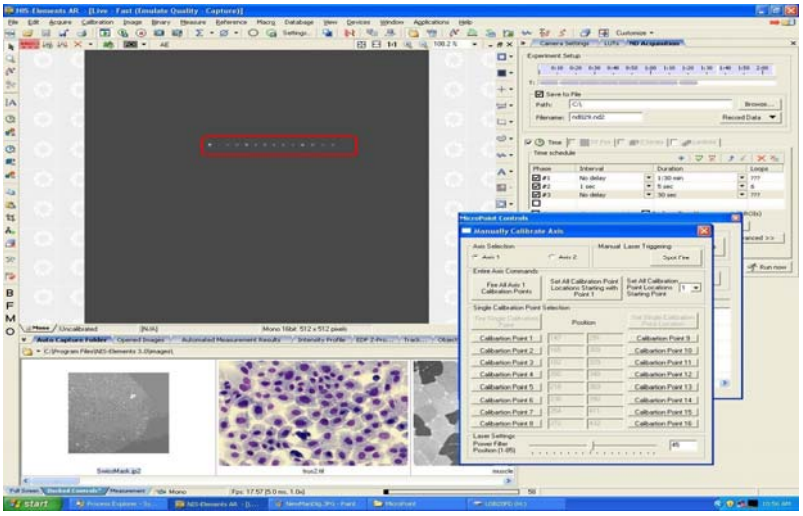
1. Select an Axis.



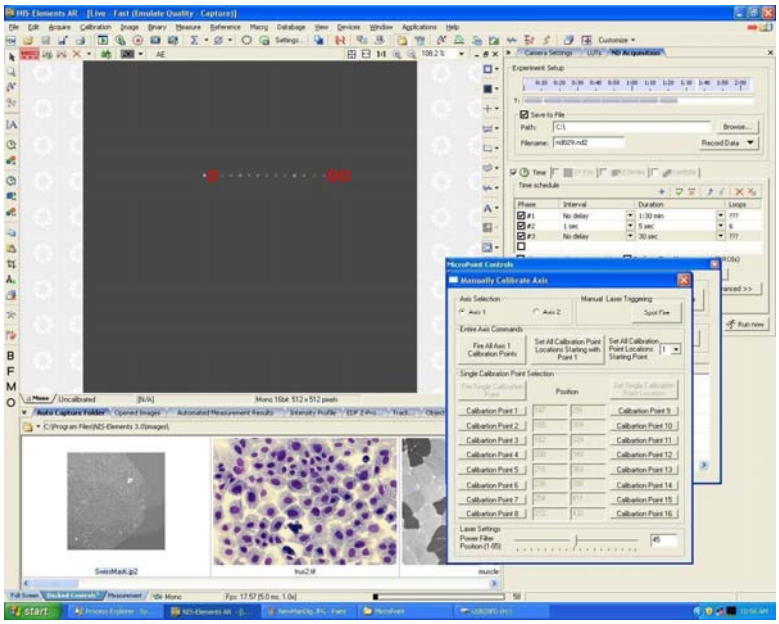
2. Fire All Axis # Calibration Points.



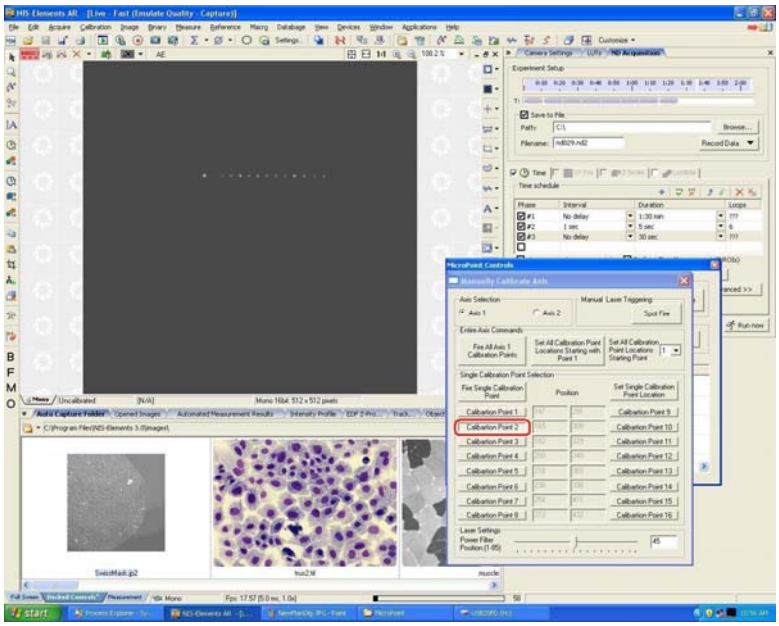
3. Visually confirm all 16 Calibration Points exist.



i. Select the Calibration Point that was not generated.



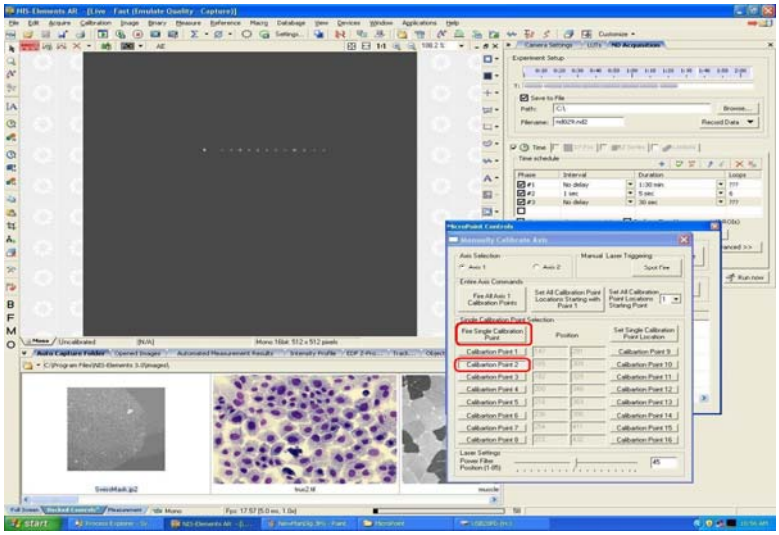
Note: It may not be apparent which points are missing.



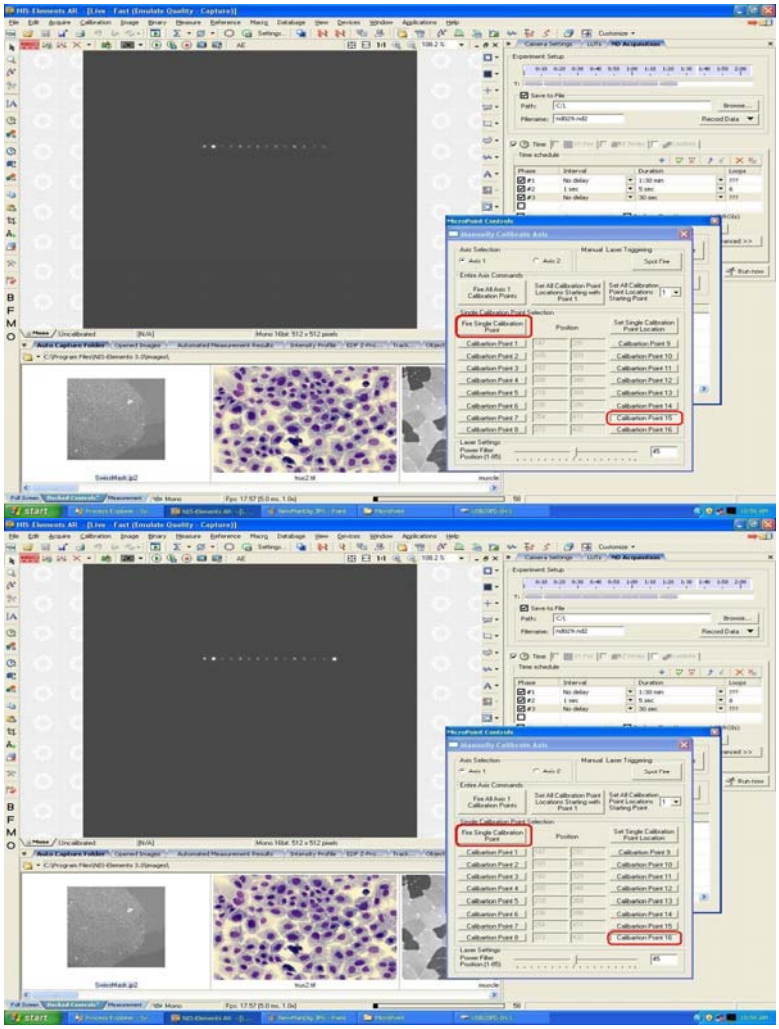
ii. Make sure the Calibration Slide is properly focused.

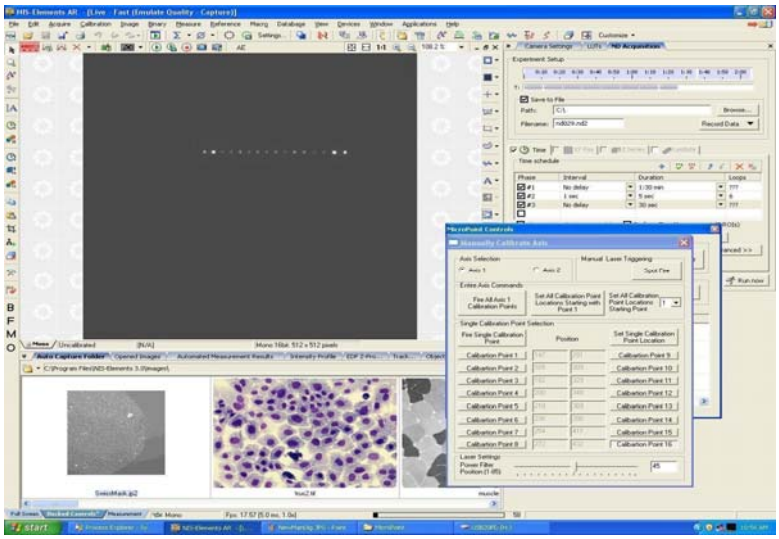
iii. Adjust the power. [Higher to create a point if one does not exist, Lower if the point it large and/or uneven.]

iv. Press **Fire Single Calibration Point** button.

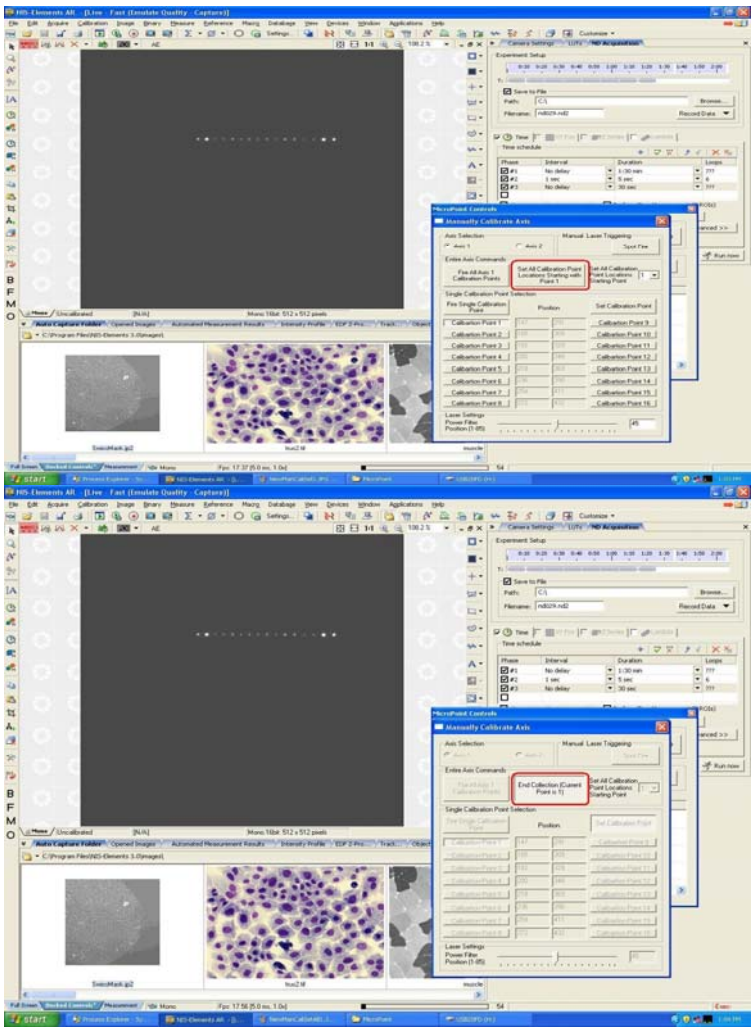


v. Repeat steps 3.i.-3.iv until all points are generated.

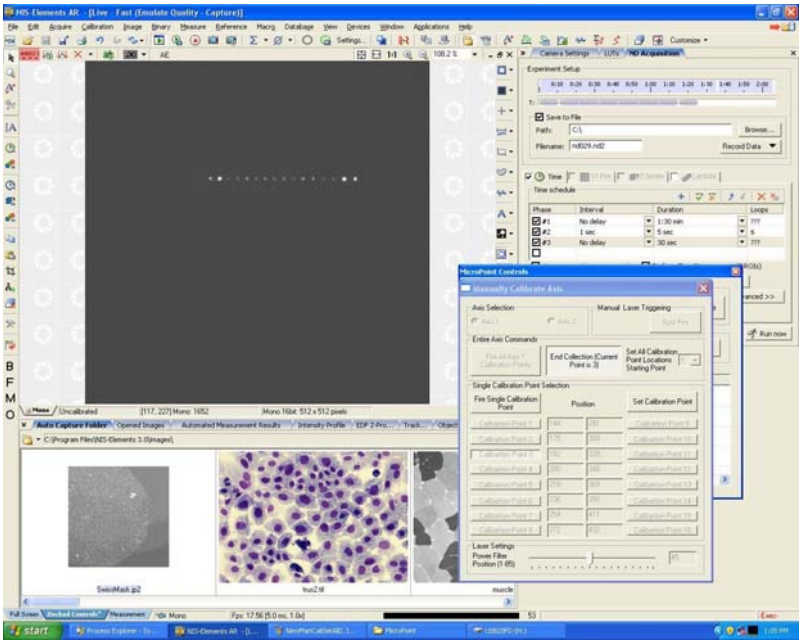




4. Press **Set All Calibration Point Locations Starting with Point** # button.



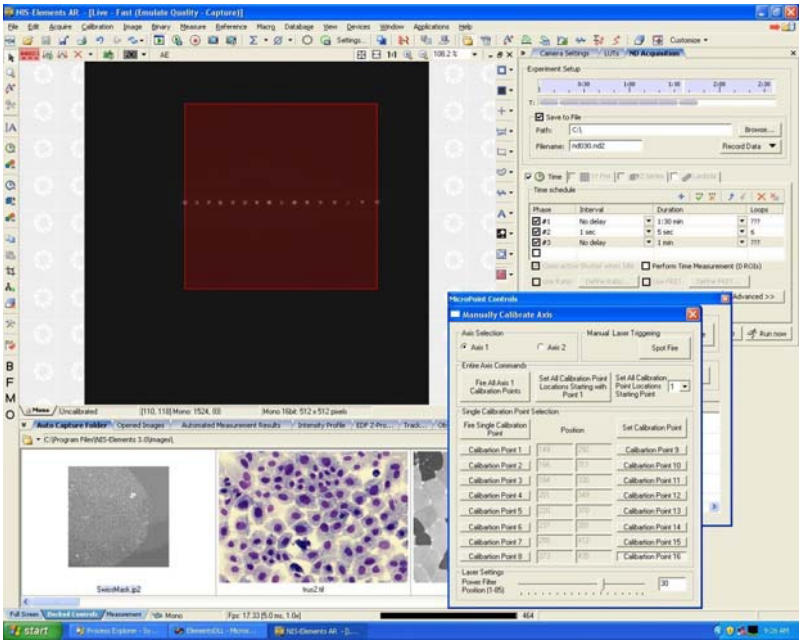
5. Double-Click in the center of each calibration point.



6. Select other Axis.

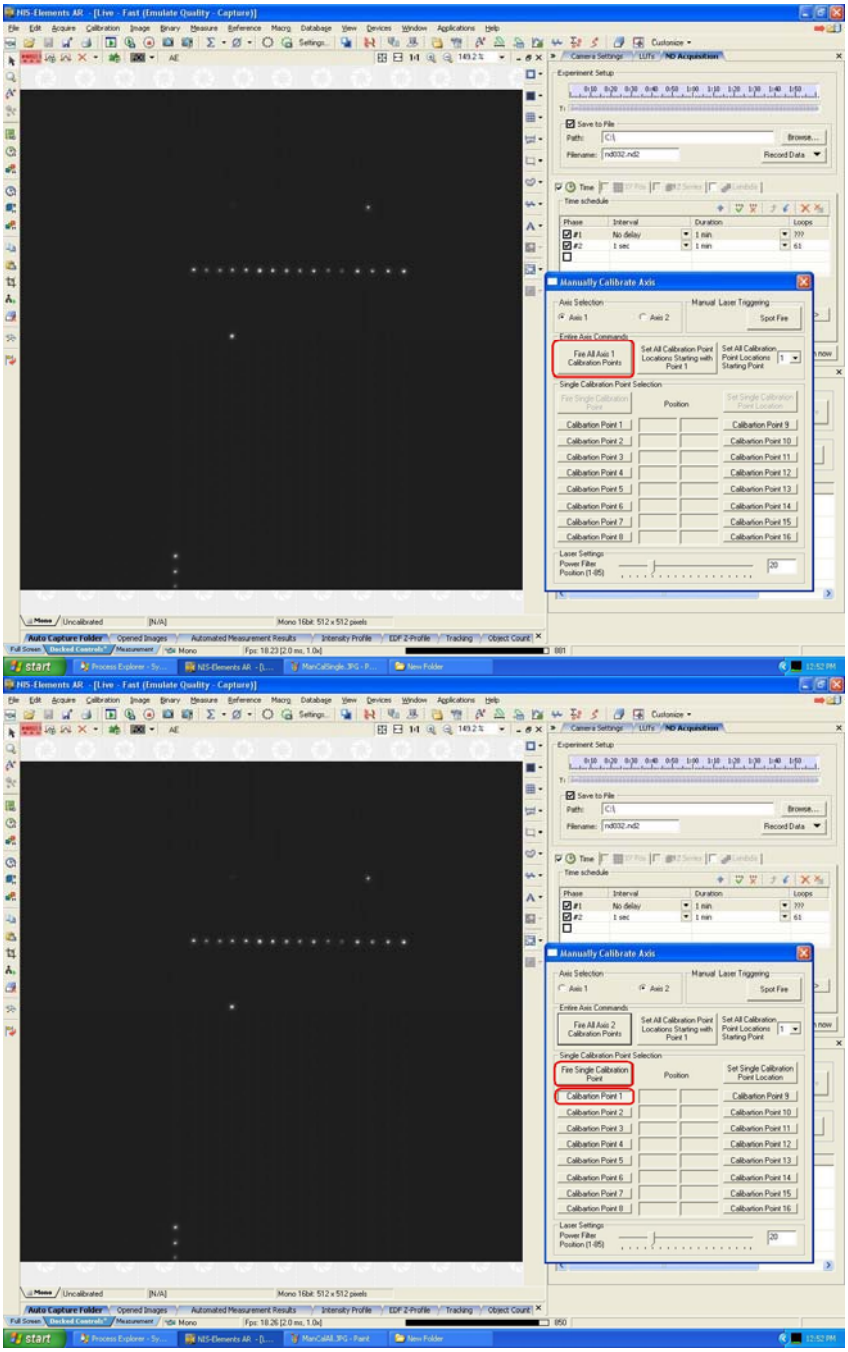
7. Repeat Steps 2-5.

8. Once the MicroPoint Module has all calibration points (from both Axis) it will calculate and display the MicroPoint Range.

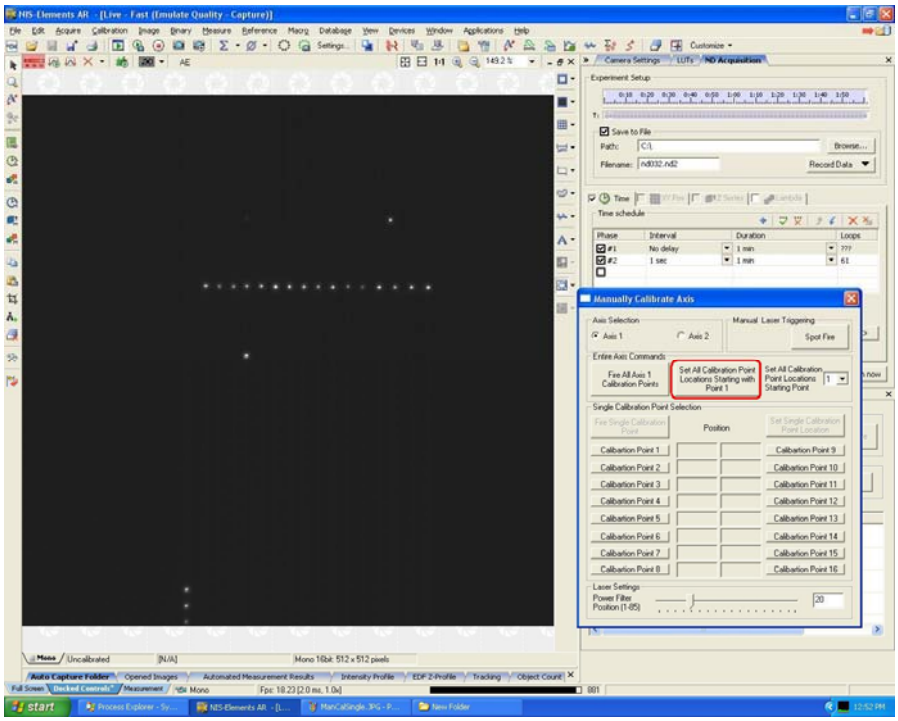


ii. Manual Calibration Dialog.

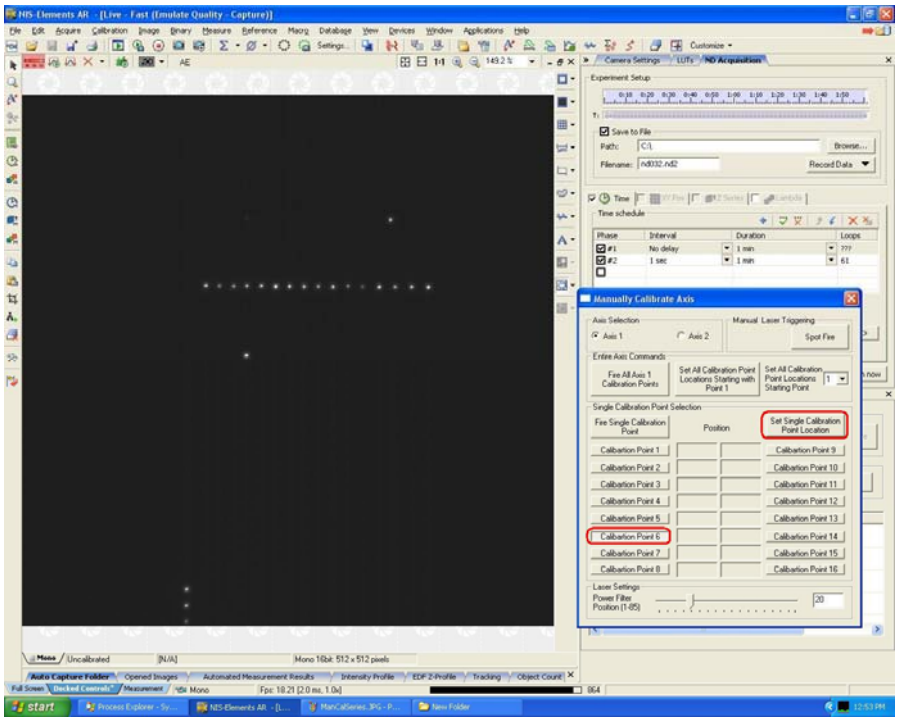
The Manual Calibration Dialog contains several tools for creating and setting calibration points. It is possible to create all of the calibration points for an axis at one time or select individual Calibration Points to create.



It is possible to set the position of a series of Calibration Points.

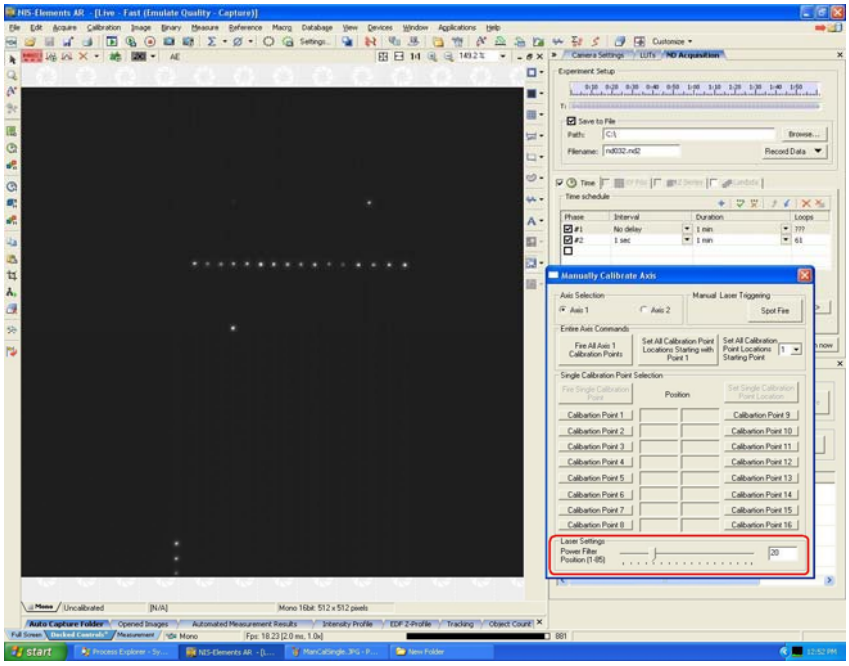


It is also possible to set the position of a Calibration Point one at a time in any order.

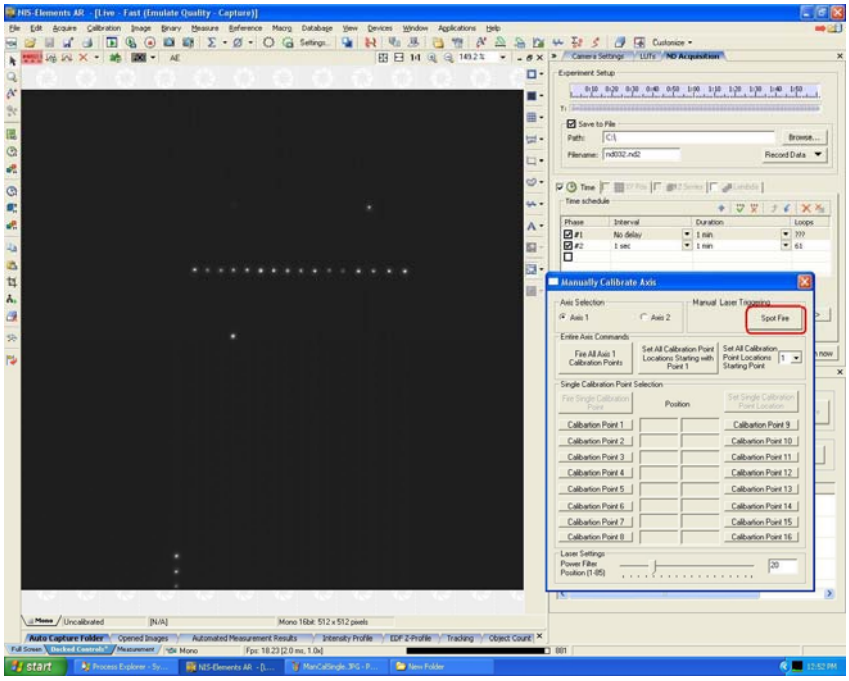


The Calibration created with the Manual Calibrate dialog is not saved until prompted. This allows for the freedom to experiment with different

calibrations without the risk of having to keep a less accurate Calibration. In addition, is it is also possible set the Laser Power in order to ensure there is proper power being delivered to create a Calibration Point.



The Spot Fire Tools is available to test the accuracy[3].

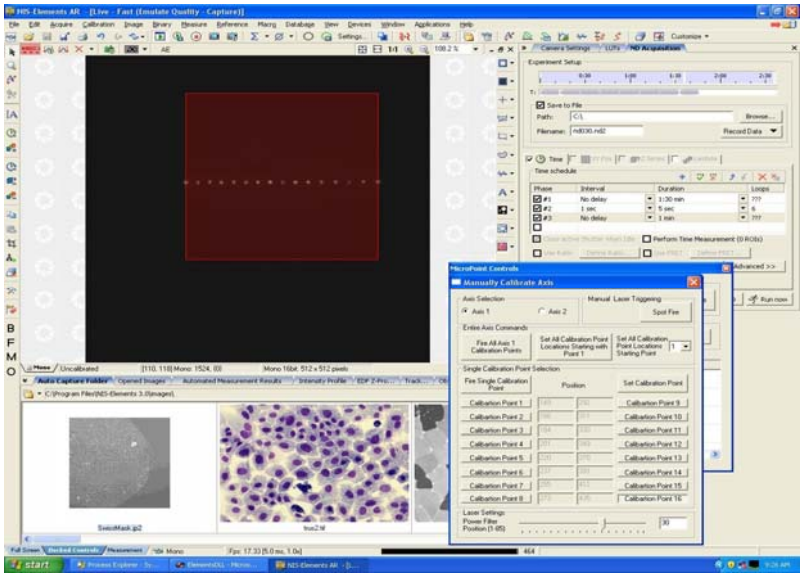


[3] Detailed explanation of Spot Fire is located in Section V-A.

D. Visualization

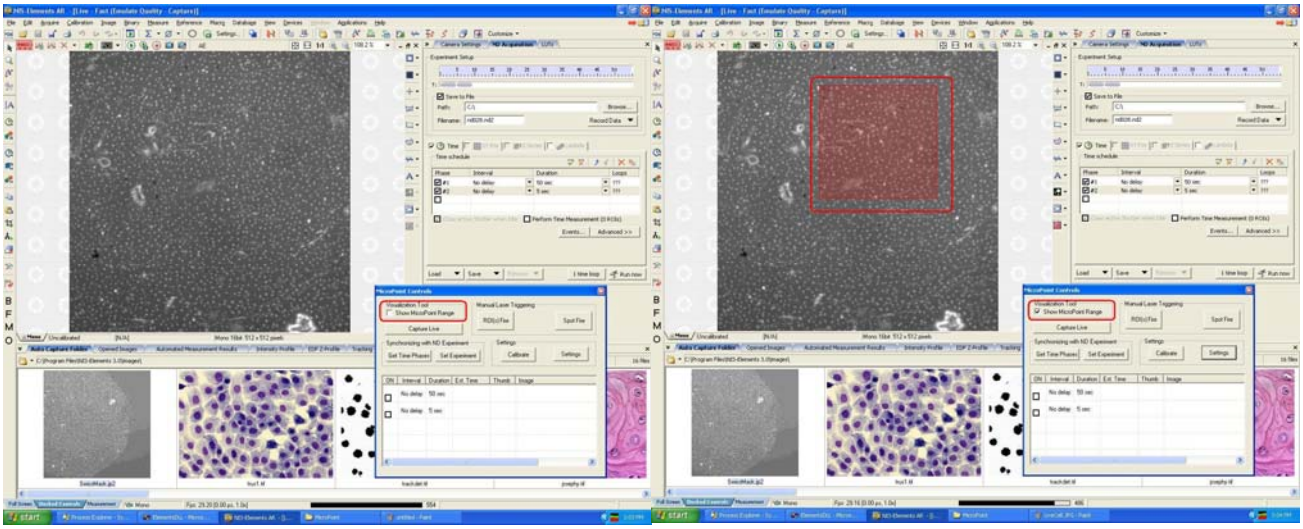
The range (where the MicroPoint galvanometers can physically move) of the MicroPoint is usually much smaller than the area captured by the camera CCD. Once calibrated, the MicroPoint Module can display the range of the MicroPoint. There are two ways to make this range appear.

1. A position of a Calibration Point has been set and Calibration Point positions of all other Calibration Points have already been set.



2. Click the **Show MicroPoint Range** check box.

The **Show MicroPoint Range** check box can be used to give a visual reference to where the laser can be triggered. The **Show MicroPoint Range** check box will only be enabled if the MicroPoint Module has been calibrated.



IV. Generating Patterns

The Mosaic Module converts ROIs into patterns. These generated patterns can be created either within an ND Experiment or during a Live image.

A. ND Experiment Pattern Generation

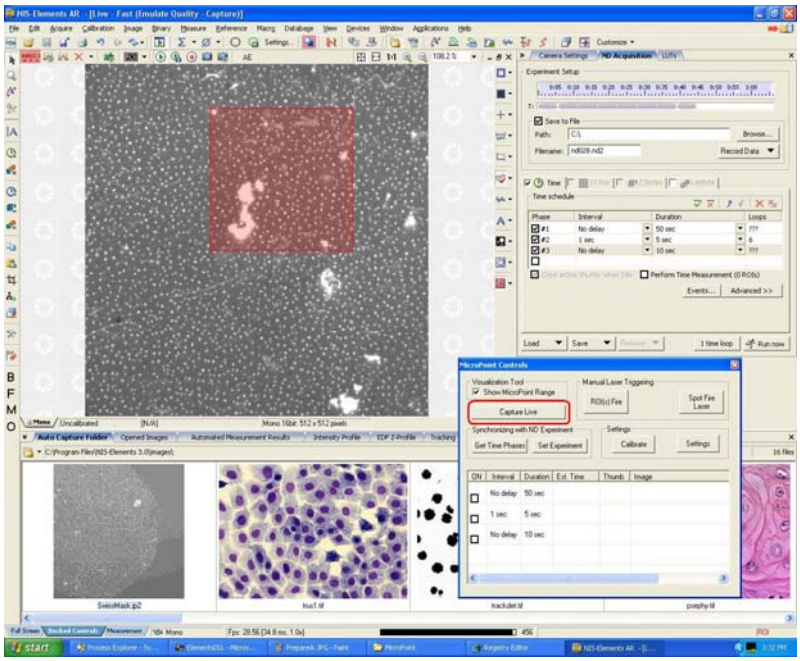
The goal of generating patterns within an ND Experiment is to capture the response within a time-lapse. The MicroPoint Module will start generating the pattern at the start of the time phase.

Warning: *The ND experiment must contain more than one time phase.*

i. Defining the Pattern(s).

During this step the user will be defining ROIs. These ROIs along with the Settings (**Number of Triggers Per ROI Point** and **Decimation**)[4] will create patterns.

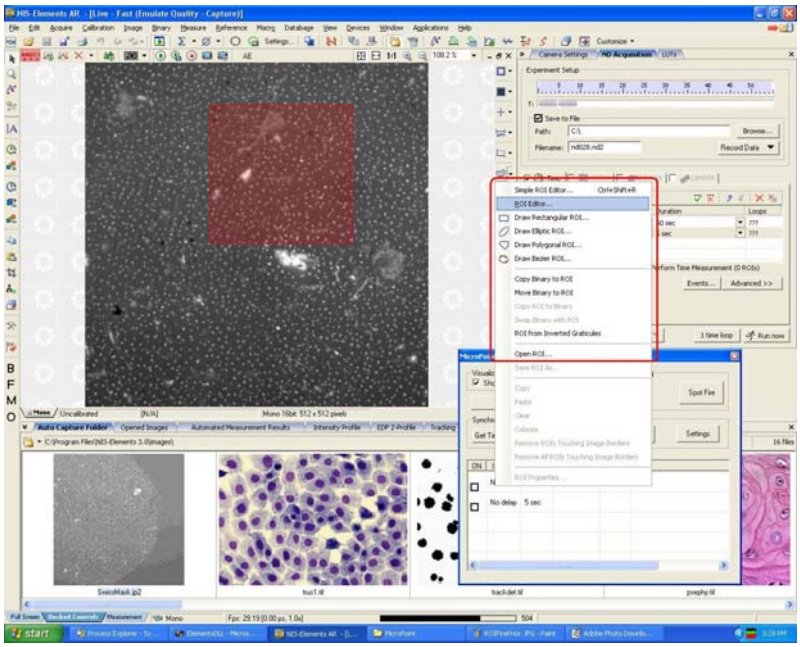
1. Press **Capture Live.**



Note: *A separate image will have to be captured for each time phase that requires a pattern generation.*

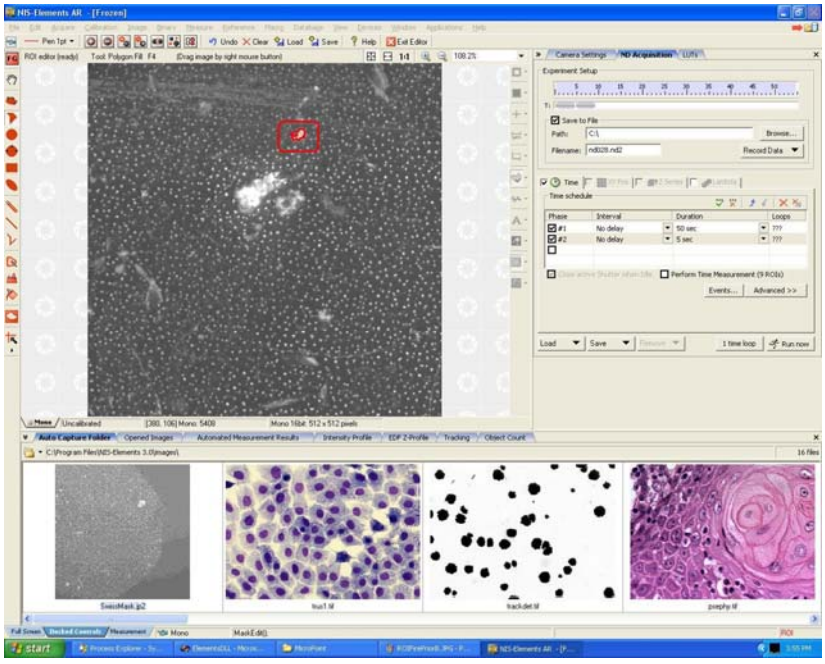
[4] *Detailed explanation of* ***Number of Triggers Per ROI Point*** *is located in Section VI. Detailed explanation of* ***Decimation*** *is located in also located in Section VI.*

2. Right Click the ROI box (kidney bean icon) and select any ROI definition Tool (including Open ROI…).



Note: *Detailed explanation of creating ROIs is included in the PDF C:\Program Files\Nikon\Shared\docs\ROI Help.pdf.*

3. Use ROI Tools to draw ROIs.

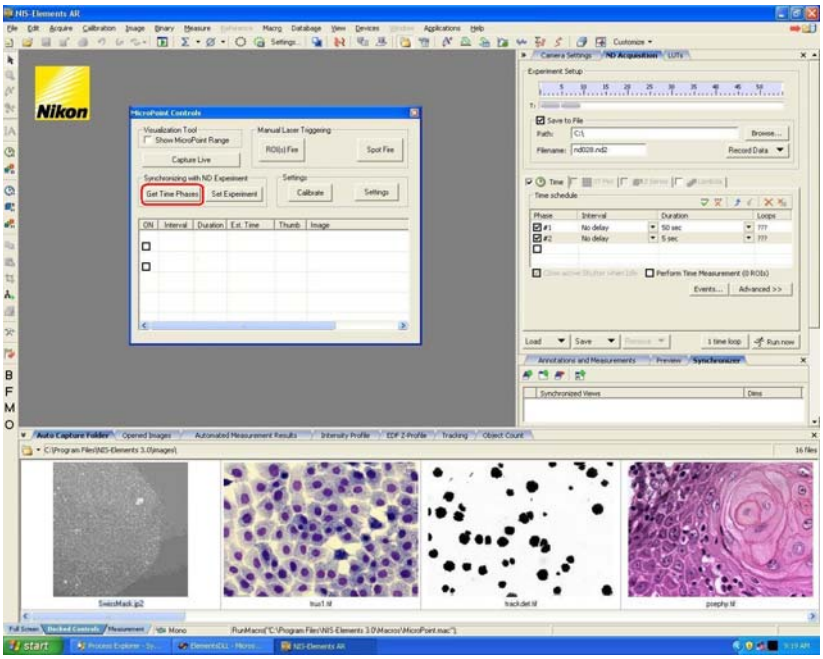


Note: *MicroPoint Effective Area is not shown while in ROI editor.*

ii. Synchronizing the MicroPoint Module with ND Experiment.

The MicroPoint Module only receives the ND experiment information on start up or when the **Get Time Phases** button is pressed.

> ***Warning:*** *Since pressing* ***Get Time Phases*** *will clear all loaded patterns and exposure times it is recommended to synchronize prior to loading patterns.*



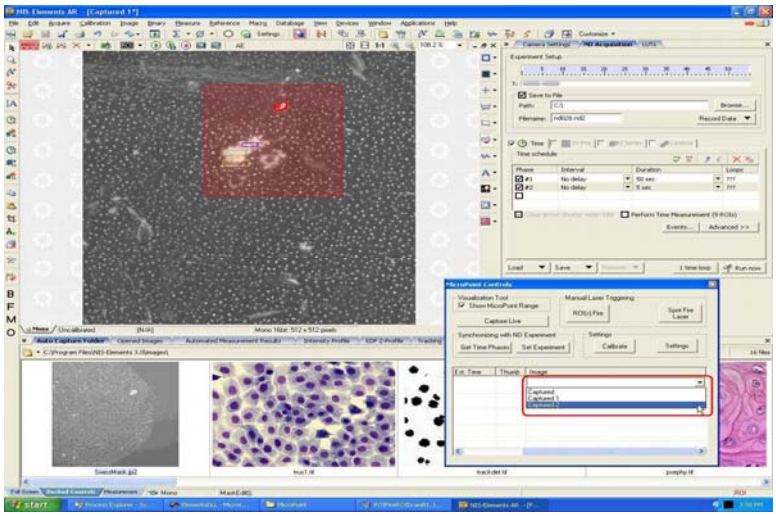
1. Configure the parameters of the ND experiment using the ND Acquisition Dialog.

2. Click **Get Time Phases**. This will load the interval, duration and number of loops for each phase of the time-lapse.

iii. Loading a ROI(s) for the ND Experiment.

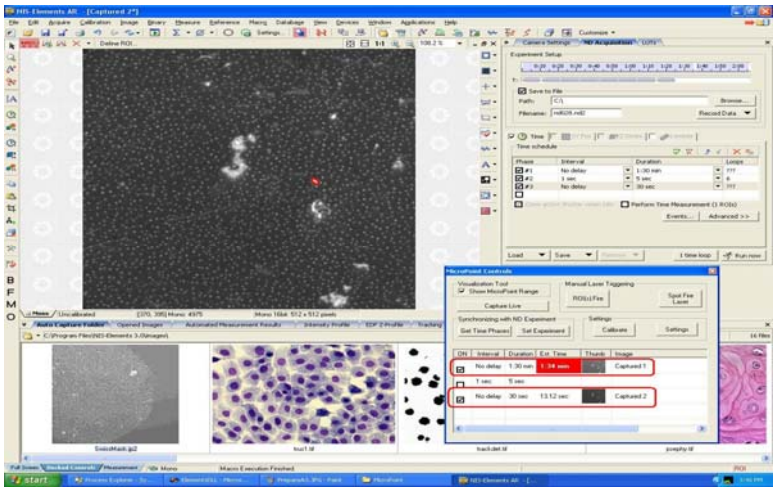
In this step, different patterns that are selected to generate are transferred to the MicroPoint Module. This allows the generation of the different patterns during the ND experiment.

1. In the MicroPoint Module Dialog, click in the Image Column that corresponds to the Time Phase for pattern to be generated.



Note: *A drop down box corresponding to the selected phase will appear. This drop down menu will list all the open images*.

2. Select the name of the image with the desired ROI(s). After this selection, NIS-Elements will generate the patterns.



Notes:

The selected Time Phase will automatically be selected for Frap/Stimulation during the experiment.

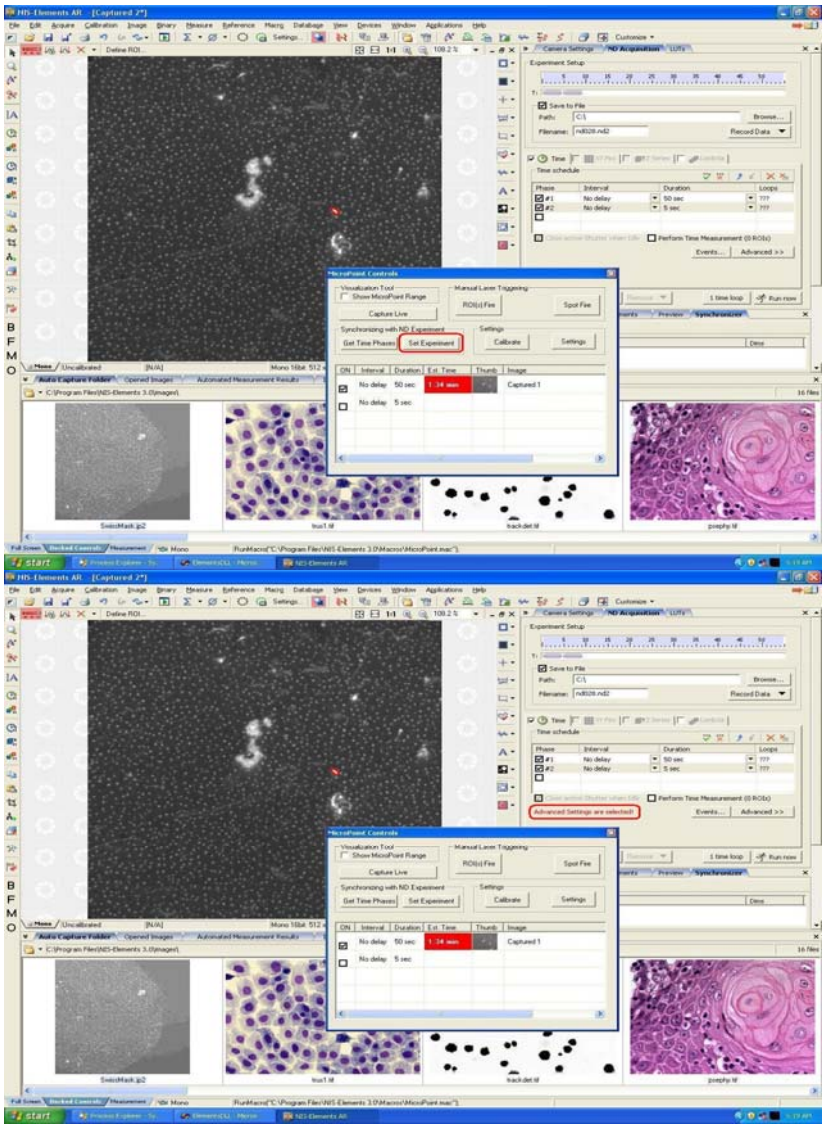
This Est. Time is how long the MicroPoint estimates for generating a pattern. The estimated time can be greater than the duration of the actual phase. If the pattern is estimated to take longer to generate than the length of the actual phase, then the phase will appear in red.

3. Repeat Steps 1-2 until all desired patterns have been loaded.

iv. Synchronizing ND Experiment with MicroPoint Module

The MicroPoint Module and the NIS-Elements ND Dialog require synchronization in order to determine which Phases in the ND experiment it will be starting to generate the patterns. This should occur once all patterns for selected time phases have been loaded.

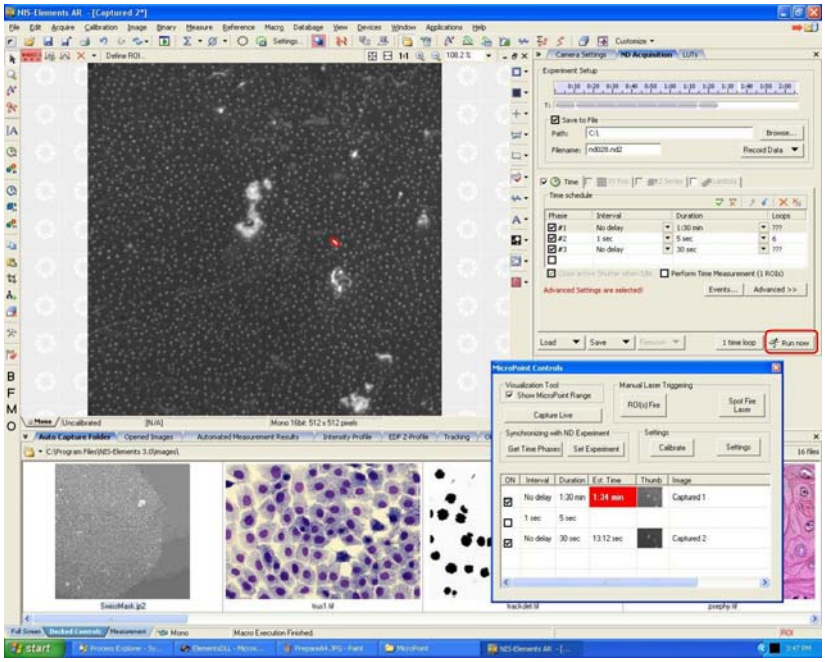
1. Press **Set Experiment.**



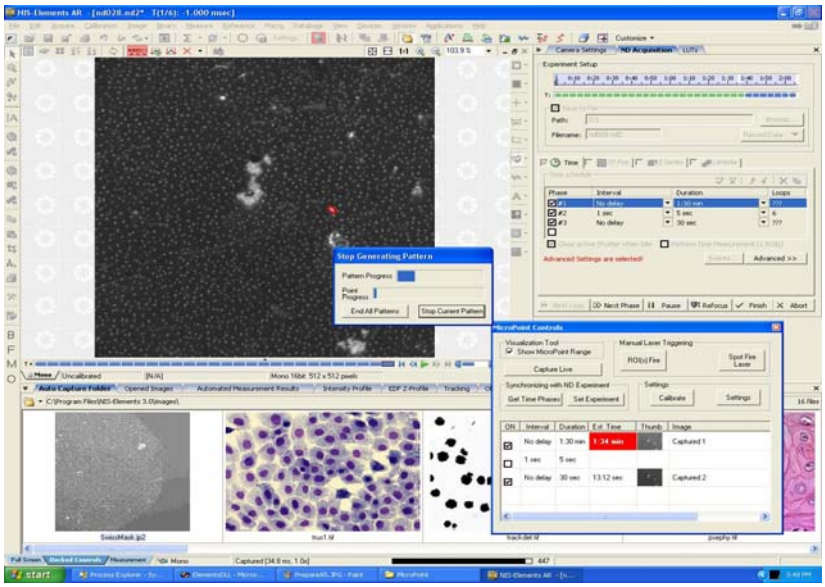
Note: *After* ***Set Example*** *is pressed, 'Advanced Settings are selected!' will appear. It is normal for 'Advanced Settings are selected!' to be present prior to pressing* ***Set Example****. This indicates a prior experiment has been set.*

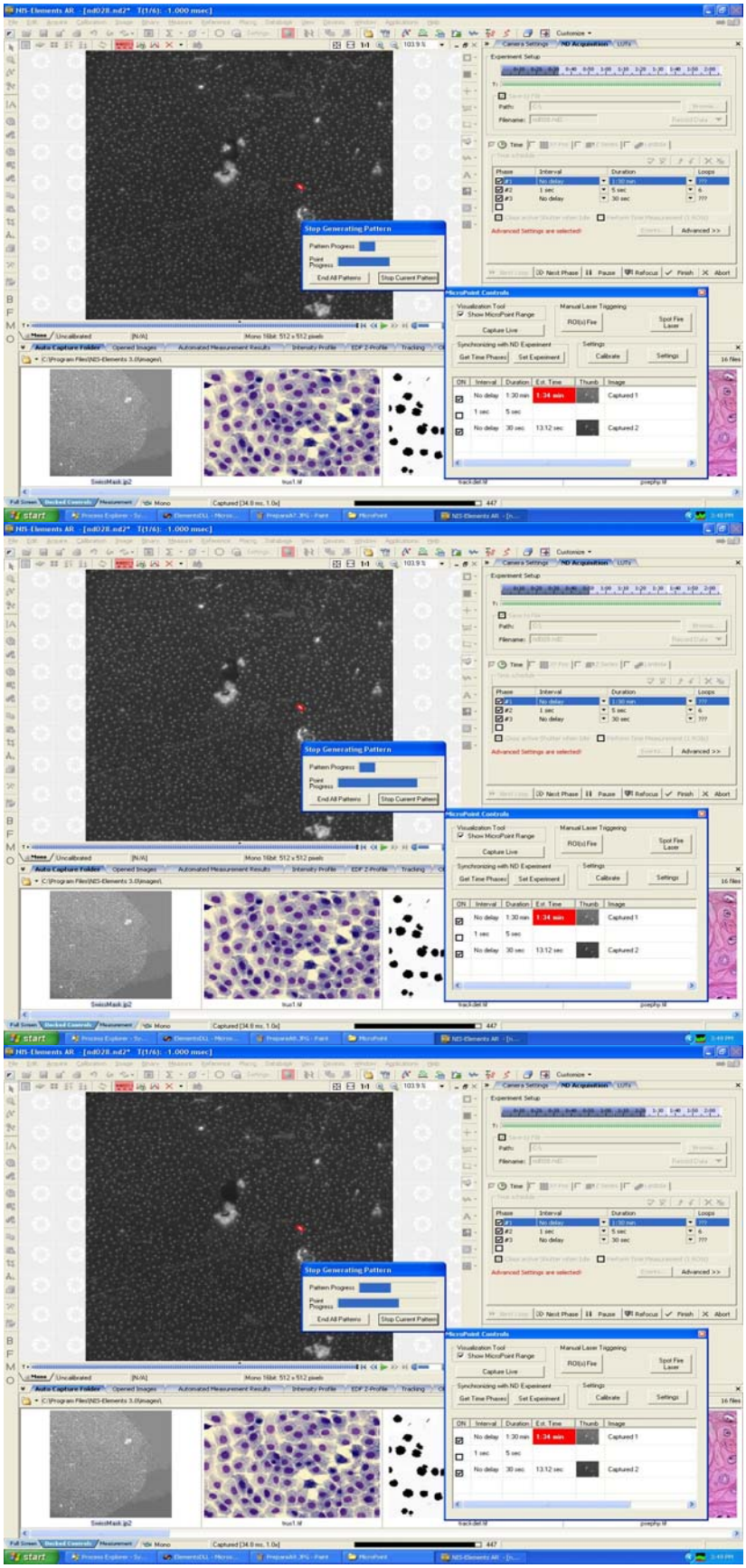
v. Running ND Experiment

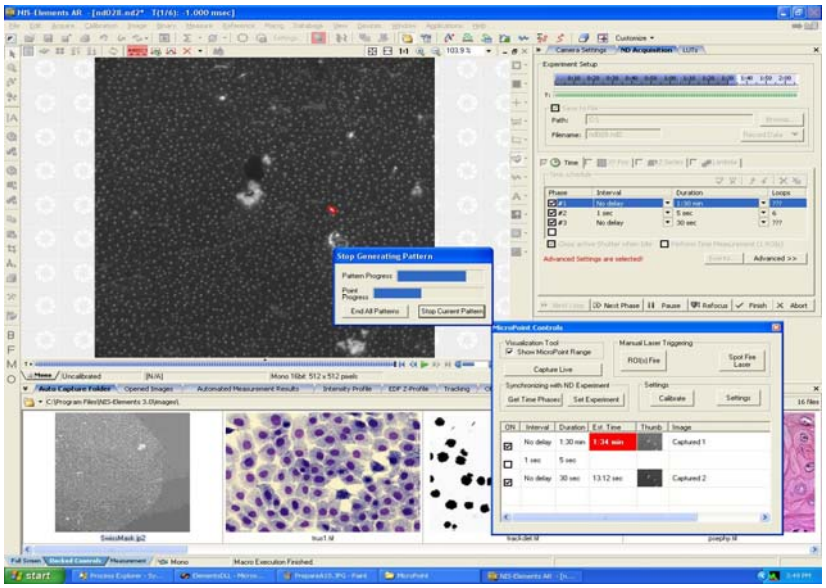
1. Press **Run now**.

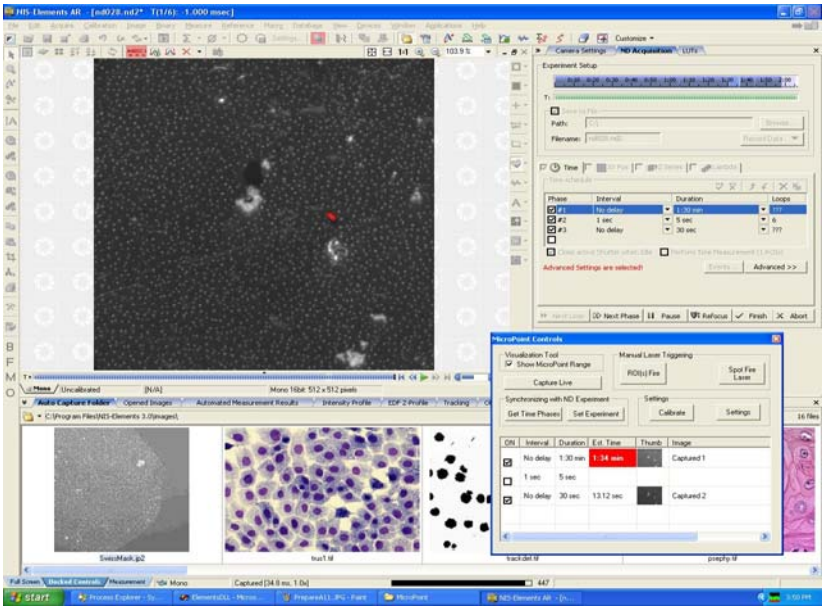


> ***Note:*** *After **Run now** is pressed, the experiment with pattern generation will begin.*



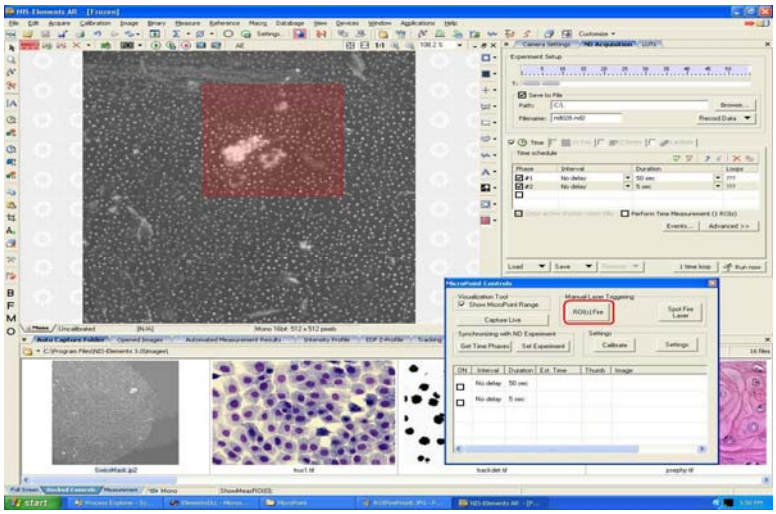

Stop Generating Pattern
Pattern Progress
Point Progress
End All Patterns
Stop Current Pattern
MicroPoint Controls
Visualization Tool
Show MicroPoint Range
Capture Live
Manual Laser Triggering
ROI(s) Fire
Spot Fire Laser
Synchronizing with ND Experiment
Get Time Phases
Set Experiment
Calibrate
Settings


Stop Generating Pattern
Pattern Progress
Point Progress
End All Patterns
Stop Current Pattern
MicroPoint Controls
Visualization Tool
Show MicroPoint Range
Capture Live
Manual Laser Triggering
ROI(s) Fire
Spot Fire Laser
Synchronizing with ND Experiment
Get Time Phases
Set Experiment
Settings
Calibrate
Experiment Setup
Advanced Settings are selected!
Advanced >>
Captured 1
Captured 2


MicroPoint Controls
Visualization Tool
Show MicroPoint Range
Capture Live
Manual Laser Triggering
ROI(s) Fire
Spot Fire Laser
Synchronizing with ND Experiment
Get Time Phases
Set Experiment
Settings
Calibrate
Experiment Setup
Advanced Settings are selected!
Advanced >>
Captured 1
Captured 2

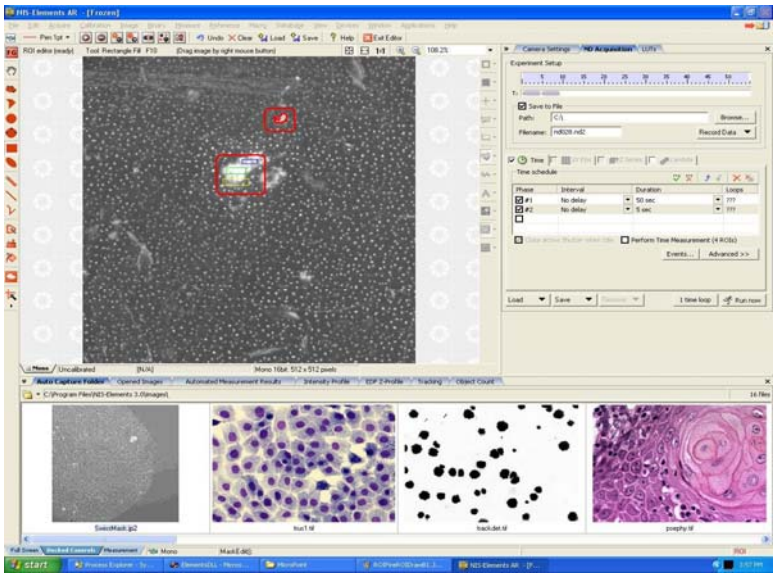
B. Live Pattern Generation

The Manual Laser Triggering section contains tools for using the MicroPoint outside of an ND Experiment. ROI(s) Fire and Spot Fire will only be enabled if the MicroPoint Module is calibrated.[5]

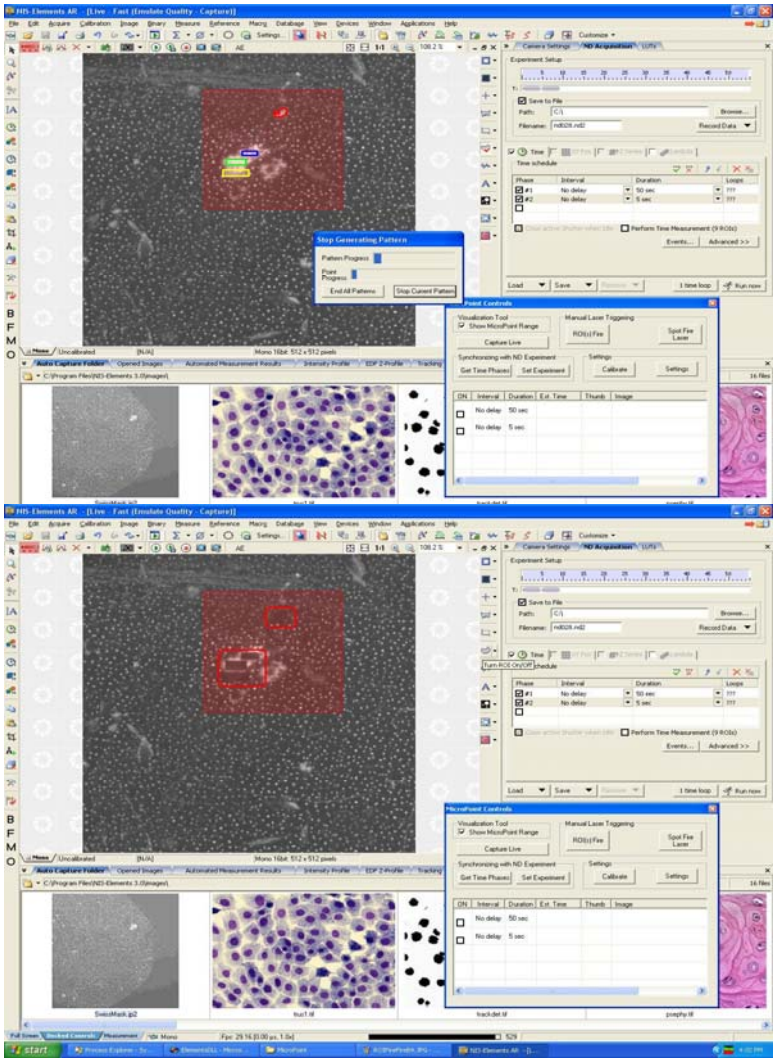


i. ROI(s) Fire

ROI(s) Fire button directs the MicroPoint Module to Stimulate/Bleach spots within the current ROI or ROIs on the screen. The actual number of spots and the locations of those spots are calculated from the Calibration, the Laser Settings[6], the ROI Settings[7] (Laser Triggers Per ROI Point), and Decimation[8].

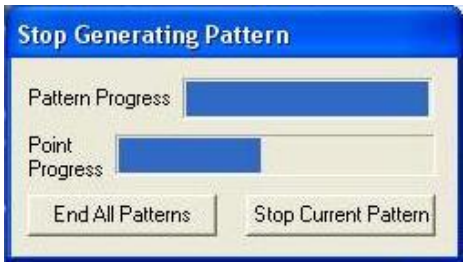


[5] Spot Fire is detailed in Section V-A in this file..
[6] Laser Settings are detailed in Section V-B in this file.
[7] ROI Settings (Laser Triggers Per ROI Point) are detailed in Section VI-A-i.
[8] Decimation (# of Adjacent Point to Skip) is detailed in Section VI-A-ii in this file.

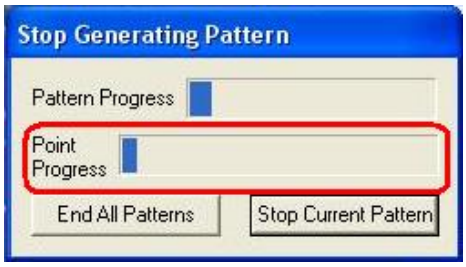


C. Stop Generating Patterns

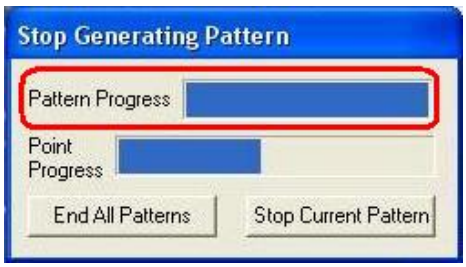
Regardless of whether patterns are being generated manually or as part of an ND experiment, they can be stopped.



There are two Progress bars. Point Progress shows what percentage of the current pattern has been generated.



Pattern Progress shows the current pattern of total patterns to be generated. When the progress bars is 100%, it is generating the last pattern of either an ND Phase or **Fire ROI(s)** button.



There are two options for stopping the pattern:

1. The pattern being generated can be stopped with **Stop Current Pattern**.
2. All pattern generation can be stopped with **End All Patterns**.

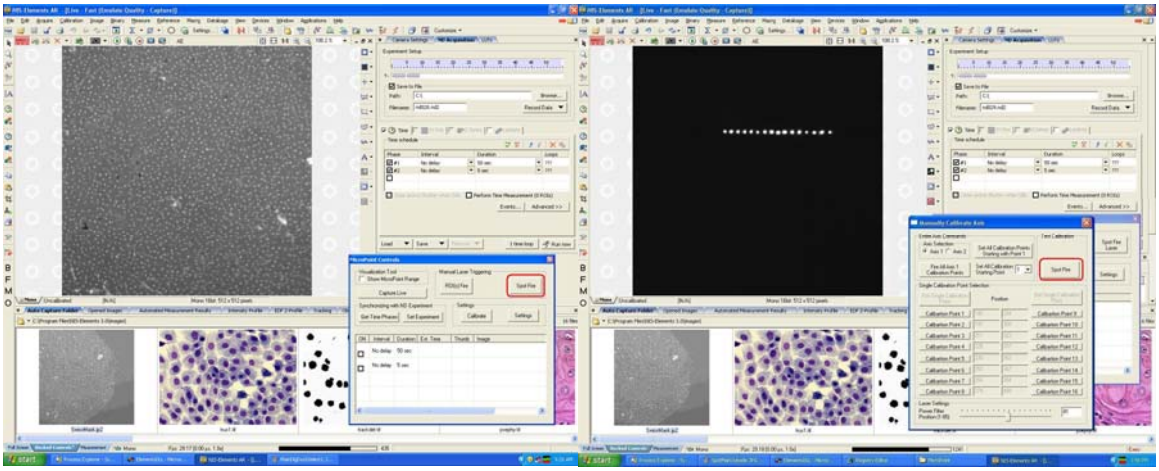
Note: *If **End All Patterns is selected** during an ND experiment, it will end all patterns for the current Time Phase **only**.*

V. Common Tools/Functions

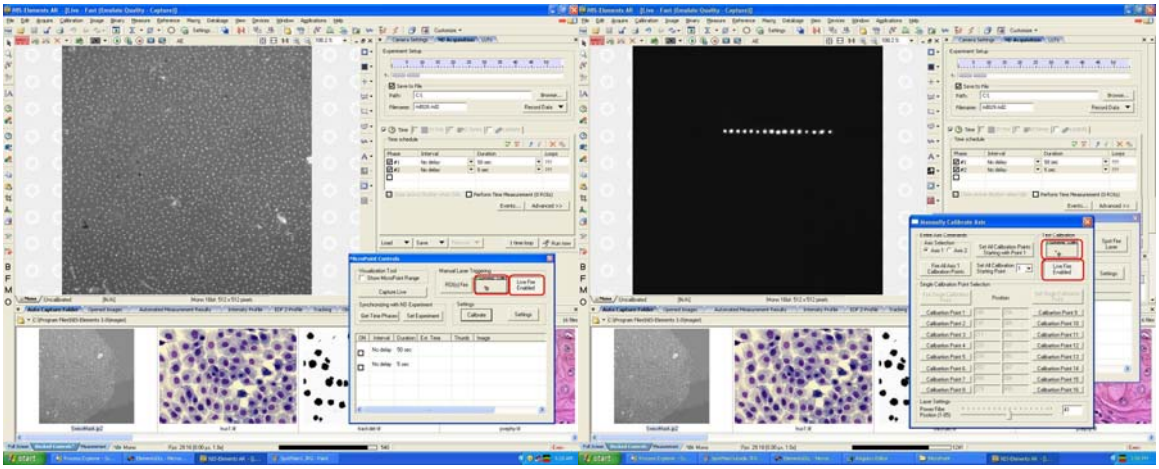
Some controls or tasks, such as Spot Fire, can appear in different MicroPoint Module Dialogs. When the control has the same name it will perform the same action regardless of where it appears. (i.e. The **Spot Fire** button in the MicroPoint Controls Dialog performs exactly like the **Spot Fire** button in the Manually Calibrate Axis Dialog.)

A. Spot Fire

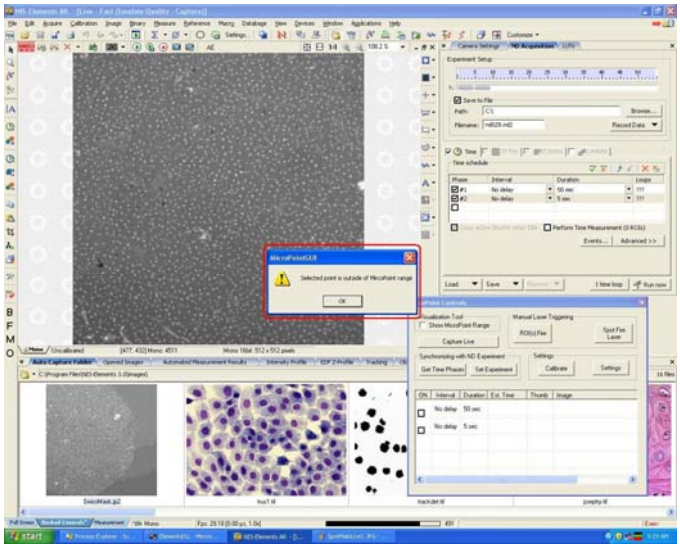
Spot Fire allows for a selection of a spot within the live window to aim the galvanometers and Fire the laser.

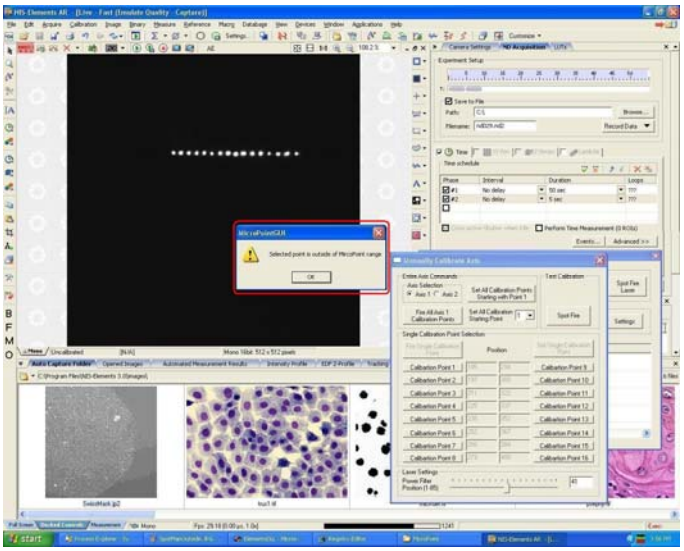


Once the **Spot Fire** button is pressed, the Module enters Live Fire Mode. In this Mode there will be an animation that provides a prompt that the MicroPoint Module is waiting for a double click inside the Live window. In addition, the text on the button will change to **Live Fire Enabled**. After the MicroPoint Module recieves a double click inside the Live window the Module will activate the laser to fire a spot at the location in the image of the double click. If the MicroPoint Module has not received a double click in the amount of time set in the **Spot Fire Timeout,** Live Fire Mode will end.



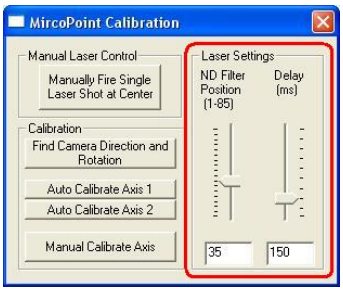
If the double click occurs outside of the MicroPoint's Range (Full description in section IV.A.), a message will indicate that the double click was outside of the MicroPoint's Range and the laser will not fire.

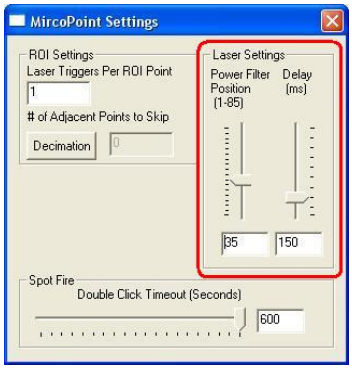


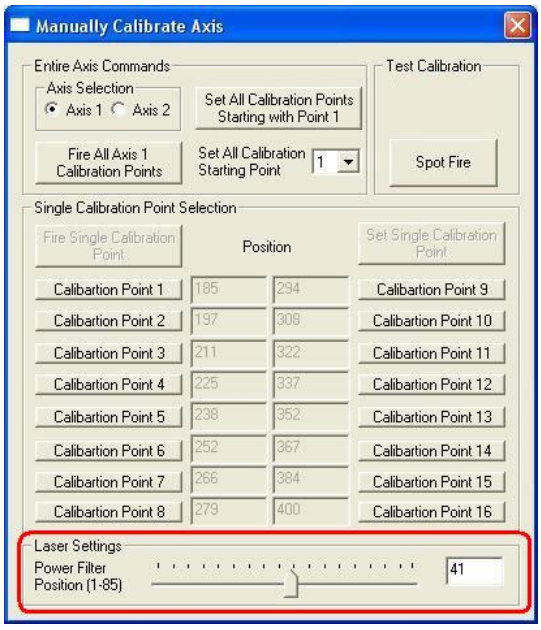


B. Laser Settings

Laser Settings are available for adjusting the internal neutral density filter, (affecting the laser power delivered) or the delay between laser pulses (affecting the accuracy of quickly generated patterns). Laser Settings can be controlled from three different dialogs: the MicroPoint Calibration Dialog, the Manual Calibrate Axis Dialog, and the MicroPoint Settings Dialog.







i. Power Filter Position (1-85)

The **Power Filter Position** is the current location of a circular neutral density filter located inside the MicroPoint. While the position of this neutral density filter has a direct correlation to the power delivered, a specific neutral density filter position does not solely contribute to the power delivered. Several factors affect the power delivered.

1. The power output of the nitrogen pumping laser.
2. The position of the external neutral density filter. (On the nitrogen pumping laser.)
3. The type of dye being used.
4. The focus of the MicroPoint.
5. The magnification of the current objective.

ii. Delay (ms)

The galvanometers need time to move from their current position to their new position. The delay adjusts the time from when the galvo positions are set until a trigger laser command is issued. Typically 150 ms is needed to move from any galvo position to any new galvo position. If there are only small movements, this delay can go as low as 60 ms. However, the shorter the delay, the less accurate the MicroPoint becomes.

VI. MicroPoint Settings Dialog

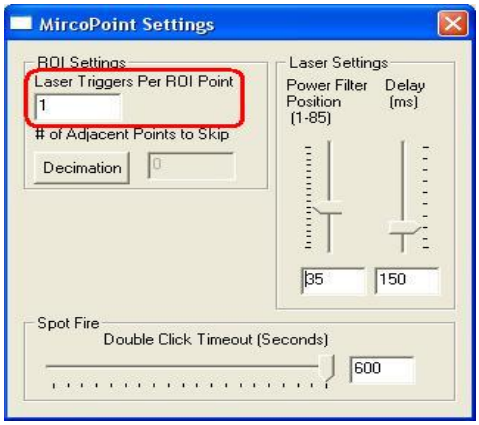
The dialog contains the options for ROI Settings (pattern generation), Laser Settings, and Spot Fire timeout.

A. ROI Settings

ROI Setting provides some control over how a pattern is generated. There is no assumption made on the size of the spot created by the Laser firing. The size of the spot is affected by magnification, focus, and the Power Filter Position.

i. Laser Triggers Per ROI Point

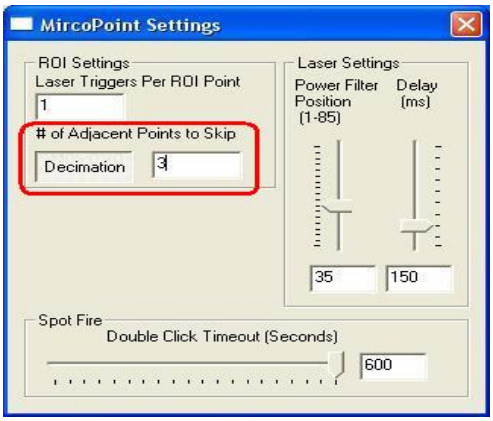
Laser Trigger Per ROI Point is a method for delivering a level of power while ensuring that the spot created by the laser is as small as possible.



The value in the **Laser Triggers Per ROI Point** edit box determines the number of times the Laser will fire. (i.e. a 3x3 ROI Box with a value of 4 in **Laser Triggers Per ROI Point** edit box will fire the laser 36 times [(9 points in the ROI box) * (4 Laser Triggers Per ROI Point)])

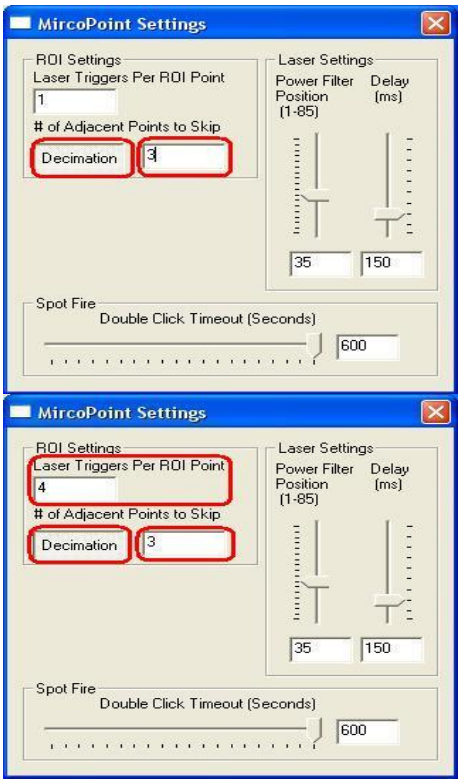
ii. Decimation (# of Adjacent Points to Skip)

Decimation allows for the ability to spread out the location of the Laser triggers. If at the current power level the spot created takes up the area of three pixels on the specimen, it may be a preference to skip every two pixels so the specimen does not receive the energy from the adjacent laser strikes.



To skip adjacent points, use the **Decimation** Option. The value in the **Decimation** edit box determines the number of ROI points the MicropPoint Module will skip when generating the pattern. (i.e. a 4x4

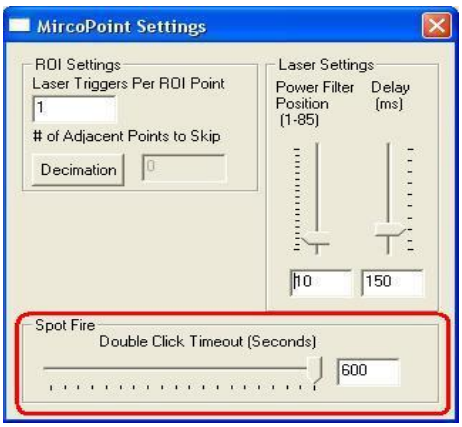
ROI box with a value of value of 3 in # **of Adjacent Points to Skip** edit box will fire the laser 4 times [it skips every three points so it fires every fourth, (12 points in ROI box) / (Fires every fourth)].



***Note:* Laser Triggers Per Point** *and* **Decimation** *can be used at the same time. If above two examples were combined, then the laser would fire 16 times. There would be 4 points left in the decimated box and 4 Triggers per laser.*

B. Spot Fire Timeout

The **Double Click Timeout** adjusts how long the MicroPoint Module will wait for a double click in the live window. The MicroPoint Marco can wait as short as 30 seconds or as long as 10 minutes.



VII. Frequently Asked Questions

1. **I never installed the MicroPoint Module in Vista, but when I plugged the MicroPoint into my PC, it never asked me to install the driver. The device works, why?**

   The MicroPoint is an FTDI device. Some devices such as Shutter Filter wheel also use FTDI. Vista is smart enough to realize it can use drivers install for a different device fro the MicroPoint.

2. **Why won't NIS-Elements remember the last position of the MicroPoint Dialog?**

   Check the NIS-Elements Build number and confirm that is it Build 548 or higher.

3. **Occasionally I get the following dialog box:**

   Why?

   When the MicroPoint Module was trying to collect the location of a double-click on the live window it instead got a single click or an accidental keyboard stroke instead. Generally the MicroPoint Module is smart enough to ignore these inputs but occasionally it will try to interpret this input and then display the above dialog box. It is recommended to press the **Cancel** button. This should be no ill effects cause by selecting **Cancel**.

4. **Why is Auto Calibrate not working?**

   They could be several different reasons for why the Auto Calibrate would not be working, but the most common reasons are that the calibration slide is not in focus. Just because one field of view is in focus, it does not necessary mean that the image will still be in focus several fields of views away. Sometimes it is believed that the best focus was achieved while focusing a calibration point. It is not uncommon for sharp edges to form on a Calibration Point when it is out of focus but for there to be a halo around a calibration point when it is in focus. This is why it is recommended to find a landmark, such as a scratch, to focus on for this calibration process.

5. **It seems like I have to increase the Power Filter Position to get the same power delivered. Why?**

   They could be several different reasons for why powered delivered is decreasing. One of the most common reasons is that the Dye Pack needs to be changed.

6. **Occasionally when Show MicroPoint Region is check the MicroPoint Region is not shown or the MicroPoint Region is shown without Show MicroPoint Region being checked. Why?**

   When one chooses to perform some actions through NIS-Elements and not through the MicroPoint Module, this can happen. This issue is easily solved by clicking the **Show MicroPoint Region** check box.

7. **I do not see MicroPoint encoded in the HASP Key. Why?**

   The MicroPoint Module is encoded as NIS Special No. 5

8. **When installing NIS-Elements I do not see the install option for the MicroPoint. I have NIS Special No. 5 encoded on my HASP Key. Where is the Module and why is it not working?**

   The MicroPoint Macro is not included in the core package of NIS-Elements. There should be a separate CD included with NIS-Elements that contains this software. If the CD is missing please contact your Nikon representative to obtain the Module.

9. **Can I Fire the laser at the same time as I image?**

   Yes, if the interval of the phase of an ND experiment is shorter then the Est. Time, the laser will be firing at the same time as the image capture. (Not necessarily the exact time but the two will be happening concurrently.)

10. **Can I make just a single phase time-lapse that will generate the ROI(s) and image at the same time?**

    No, while it is possible to generate a pattern in a single phase, the ND experiment itself must contain at least two time phases. It is okay if the first time phase is a “No Acquisition Phase” for a very short period of time.

11. **After I install the Module (using the installation program), is there anything else I need?**

    Yes, the NIS-Elements HASP key must be programmed (NIS Special

No.5) for this Module (as any other module). Part #97154.

12. I know the MicroPoint Module is HASP key protected and the customer must pay for it, but how do I demonstrate it?

The Module is automatically enabled in the demo HASP keys.

13. Does this Module work with Vista 64?

No. Current the MicroPoint Module only works with XP and Vista 32.

14. What versions of NIS-Elements will this work for?

This Module will work with Version 3.0, service pack 8 (build 548) or later

15. Will this MicroPoint Module work in NIS-Elements BR?

Yes, the MicroPoint Module will work in NIS-Elements BR with the condition that the Advanced Interpreter (API) module (part # MQS42510) is purchased as well.