Quick Guide

ZEN 2012 SP2

Acquiring multidimensional images

We make it visible.

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1 Introduction

1 Introduction

This quick guide shows the most important steps that you must perform within the software to acquire multi-dimensional images. It is primarily concerned with understanding the workflow and get to know the software, not a detailed description of all available options.

We also limit to max. 2 dimensions, e.g. channels (multi-channel experiments), channels + positions (multi-position experiments) channels + time series (time series experiments). The correct configuration of all hardware components (e.g. camera, motorized stage, motorized filter wheels, etc.) in the **MTB 2011** (MicroToolBox) and the correct settings of the light path required in the **Locate** tab are explained in separate instructions. They are not part of this quick guide.

In the first chapter (**Setting up multi-channels experiments** [▶ 13]), you will learn how to create new experiments in the **Acquisition** tab. After that, the two variants will show you how to configure one or more channels for acquisition. This approach is the basis for all multi-dimensional experiments. To further your knowledge, in addition, the configuration of simple **multi-position** and **time series** experiments is described in the following chapters.

2 Acquiring Multi-Channel images

2 Acquiring Multi-Channel images

In the following chapters you will learn how to set-up and run multi-channel experiments with ZEN 2012 (blue edition) quick and easy.

i Info

Make sure that you work with a fully motorized microscope system. In advance all microscope components (e.g. objectives, filters, etc.) must be configured correctly in the Microtoolbox (MTB) software.

In principle there are two variants for setting up multi-channel experiments. The first variant uses Smart Setup, while the second variant uses the **Channels** tool. Both variants have similarities and differences, which are presented in the following overview:

Commonalities

- Fluorescent dyes and transmitted light techniques can be selected from a database.
- Hardware settings for motorized microscopes, which take the properties of the selected dye and the available microscope hardware into account, can be created automatically.
- Bases for experiments can be created using both variants and experienced users can optimize settings further.

Differences

Smart Setup	Channels tool
A maximum of 4 fluorescence channels and 1 transmitted light channel are available	No restriction on the number and type of channels
Offers up to 3 proposals of variants of the experiment (depending on the selected combination of dyes and available hardware)	-
Offers more optimization of experiment settings by using the Motif buttons	-
Graphic overview of the expected signal strength for the selected dyes	-

Smart Setup	Channels tool
Graphic overview of the expected spectral crosstalk with the selected dye combinations	-
Display of the excitation and emission spectra of the selected dyes	-
-	Channels can be configured for dyes that are not supported (or not supported sufficiently well) by the available hardware

2.1 Set up a new experiment

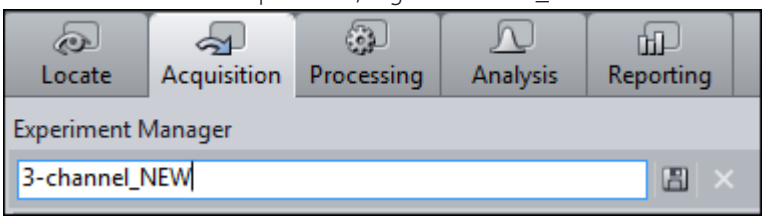
Prerequisites

- You have switched on and configured your microscope system and all components.
- You have successfully started the software.

Procedure

1. In the **Left Tool Area** click on the **Acquisition** tab.
2. In the **Experiment Manager** click on the **Options** button .

   The **Options** dropdown list opens.
3. To create a new, "empty" experiment, click on the **New** entry.
4. Enter a name for the experiment, e.g. "3-channel_NEW".



5. To create the experiment, click on the **Save** button .

You have created a new, blank experiment. All other settings are now stored in this experiment. If you make changes to the experiment, an asterisk (*) after the file name appears. This means that the experiment was modified and not saved. Save your experiments from time to time to ensure that your settings are not lost.

2.2 Variant 1: Configure channels by using Smart Setup

Procedure

1. Click on the **Smart Setup** button .

   The **Smart Setup** dialog opens.

2 To add a channel, click on the **Add** button in the **Configure your Experiment** section .

The **Add Dye or Contrasting Method** dialog opens.

3 Select the desired dye or contrast method.

4 Click on the **Add** button. Alternatively you can double-click on the entry in the dye database. The dye is then adopted directly into the experiment.

You have added a channel to your experiment. To add further channels, repeat the last 2 steps.

Info

If you see the error message "**Smart Setup calculation failed**", it was not possible for Smart Setup to calculate any proposal. This may be because the filters and light sources available on the system do not allow an image of the dye to be acquired with a good signal strength or with little crosstalk. The channel for this dye or the contrast method cannot therefore be created. In this case, try selecting another, similar dye.

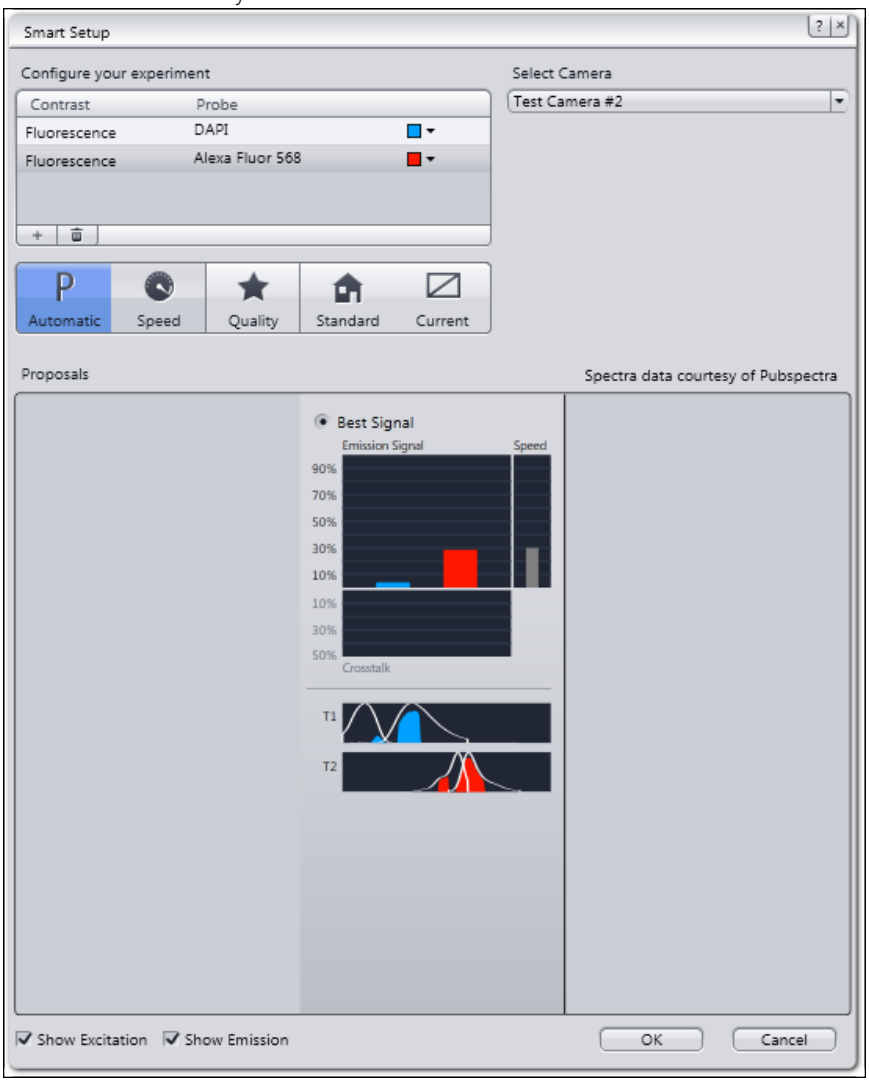
Should the error message be displayed for all dyes that you select, this may be due to one of the following causes:

- no light source has been configured or the light source is switched off

- no camera has been configured on the system, the camera is not connected or (on some models) has been switched off.

5 To return to **Smart Setup**, click on the **Close** button.

You will now see a graphic overview in the **Proposals** section. This displays the spectra of the dyes, the expected signal strengths per dye and the spectral crosstalk schematically.



Info

Depending on which dye you have selected and the microscope hardware available, up to three different proposals (Best Signal, Fastest, Best Compromise) are displayed. These differ in terms of signal strength, crosstalk and speed. Select the proposal that best meets the needs of your experiment.

6 To select a proposal (if there's more than one), activate the **radio** button on top of the proposal.

7 To optimize experiment settings additionally, click on a **Motif** button. Automatic button is set as default setting.

Info

By the **Motif** buttons you can optimize acquisition parameters and camera settings automatically either for a high quality (**Quality** button) image or a faster acquisition but reduced image quality (**Speed** button). Find a more detailed description of **Motif** buttons in Smart Setup dialog.

8 To optimize experiment settings, adopt the suggestion and leave **Smart Setup**, click on the **OK** button.

The added channels are adopted automatically into the **Channels** tool.

9 Click on the **Set Exposure** button in the **Action buttons** bar on top of the **Acquisition** tab.

The exposure time is now measured for all three channels one after the other. This is adopted into the settings for the channels. Following the measurement of the exposure time, the multi-channel image is acquired automatically and displayed in the **Center Screen Area**.

10 To save the experiment together with all the settings, click in the **Experiment Manager** on the **Options** button .

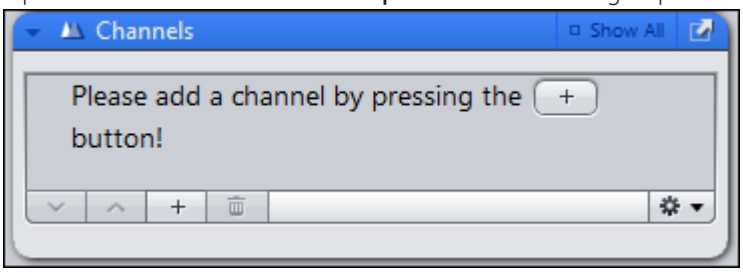
11 In the **Experiment Manager** click on the **Save** entry in the dropdown list.

You have set up the multichannel experiment using **Smart Setup**, executed it and then saved the configuration. This means that you can repeat the experiment as often as you like using the same settings.

2.3 Variant 2: Configure channels by using Channels tool

Procedure

1 Open the **Channels** tool in the **Acquisition Parameter** group.



2 To add a channel, click on the **Add** button .

The **Add Dye or Contrast Method** dialog opens.

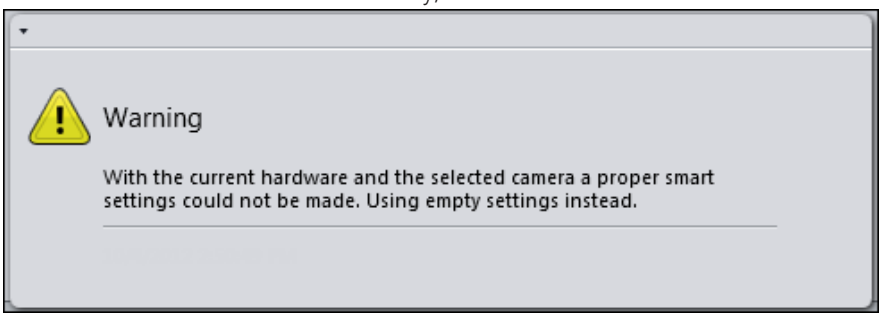
3 Select the desired dye or contrast method.

4 Click on the **Add** button at the bottom of the dialog.

You have added a channel to your experiment. To add more channels, repeat the last 2 steps.

Info

If you see this warning for a few seconds (see below), the software is unable to calculate a suggestion for the acquisition of this dye or contrast method. This may be because the filters and light sources available on the system do not allow an image to be acquired with a good signal strength or with little crosstalk. The channel for this dye or contrast technique is nevertheless created and can be found in the **Channels** tool. The appropriate acquisition settings cannot be created for this automatically, however.



Try selecting another, similar dye or edit the available suggestion manually in the **Acquisition Light Path** tool.

5 Click on the **Close** button.

You will see the added channels in the **Channels** tool.

6 Click on the **Set Exposure** button in the **Action buttons** bar on top of the **Acquisition** tab.

The exposure time is now measured for all three channels one after the other. This is adopted into the settings for the channels. Following the measurement of the exposure time, the multi-channel image is acquired automatically and displayed in the **center screen area**.

7 To save the experiment together with all the settings, click in the **Experiment Manager** on the **Options** button .

8 Click on the **Save** entry in the dropdown list.

You have set up the multichannel experiment using the **Channels** tool, executed it and then saved the configuration. This means that you can repeat the experiment as often as you like using the same settings.

3 Acquiring Time Series images

3 Acquiring Time Series images

Prerequisites

- To set up **Time Series** experiments you need to license the **Time Series** module.
- You have set up a **new experiment** [▶ 14], at least **defined one channel** [▶ 13] and adjusted focus and exposure time correctly.
- You are on **Acquisition** tab.

Procedure

1. Activate the **Time Series** tool by activating the **Time Series** checkbox in the **Acquisition Dimensions** section.

   The **Time Series** tool appears in the **Left Tool Area**.

2. Open the **Time Series** tool.
3. Set length of your time series by the **Duration** slider. You are able to select an interval (days, hours, minutes, seconds) or the cycles (1-n) e.g. 10 cycles.
4. Set interval of your time series by the **Interval slider**, e.g. 5 s.
5. Click on **Start Experiment** button.

The time series experiment will be started. You`ve successfully learned the basics of how to set up time series experiments. In our example in 10 cycles after each 5 seconds an image is acquired. The time Series image also contains 10 single images.

4 Acquiring Multi-Position images

4 Acquiring Multi-Position images

⚠ CAUTION

Damage to the device because of missing calibration

A missing or false calibration of stage, focus and sample carrier can lead to damage on your microscope system.

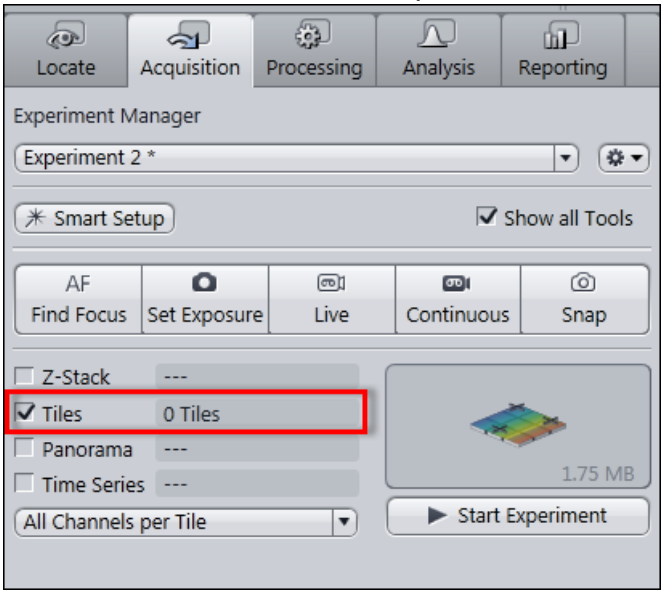
Calibrate **stage**, **focus** and the **sample carrier** before you set up or execute a **Tiles** or **Position** experiment.

Prerequisites

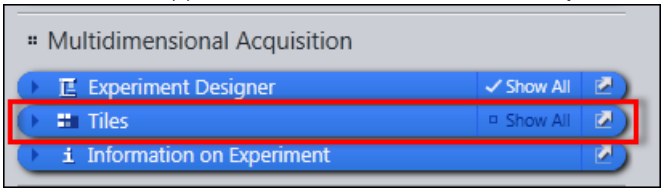
- You are on the **Acquisition** tab.

Procedure

1. Set up a new experiment in the **Experiment Manager**, see **Set up a new experiment** [▶ 14].
2. Configure at least one channel for image acquisition either by using **Channels** tool or **Smart Setup**.
3. Click on **Set Exposure** button in the **Action buttons** bar on top of the **Acquisition** tab. This will calculate exposure time automatically.
4. Click on **Find Focus** button. This will focus your sample automatically. If necessary adjust the fine focus of the sample manually by moving the z-drive of the microscope via **Live** image.
5. Activate the **Tiles** checkbox in the **Acquisition Dimensions** section.

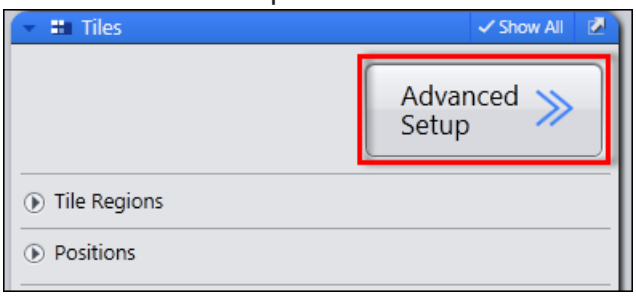


The **Tiles** tool appears in the **Multidimensional Acquisition** tool group.



6 Open the **Tiles** tool window.

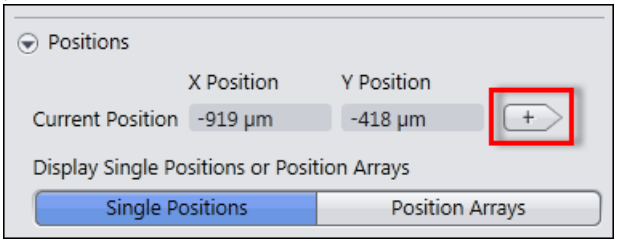
7 Click on **Advanced Setup** button.



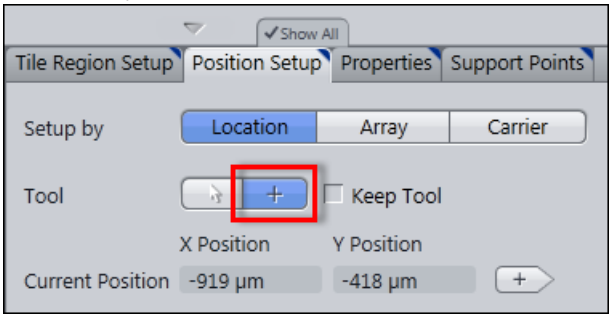
Advanced Tiles Setup (ATS) view opens. You see the **Live** image from the camera in the **Live Navigator** tool.

8 Move **Live Navigator** tool to a position where you want to add a position by simply double clicking at the position.

9 Click on **Add** button in **Tiles** tool | **Positions** section, to add this position to your experiment.



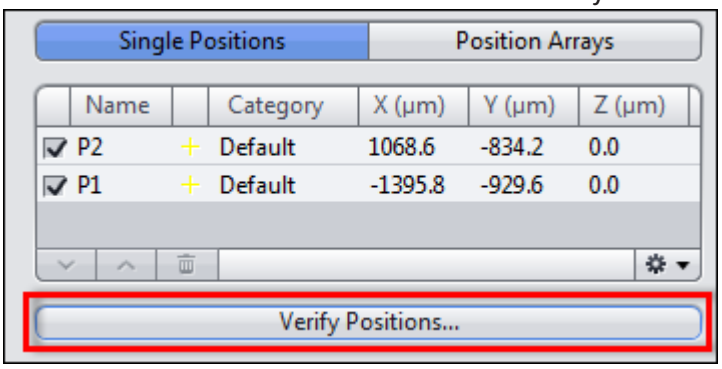
10 Alternatively click on **Add** button in **Position Setup** view control tab.



11 To add further positions, repeat the last 3 steps.

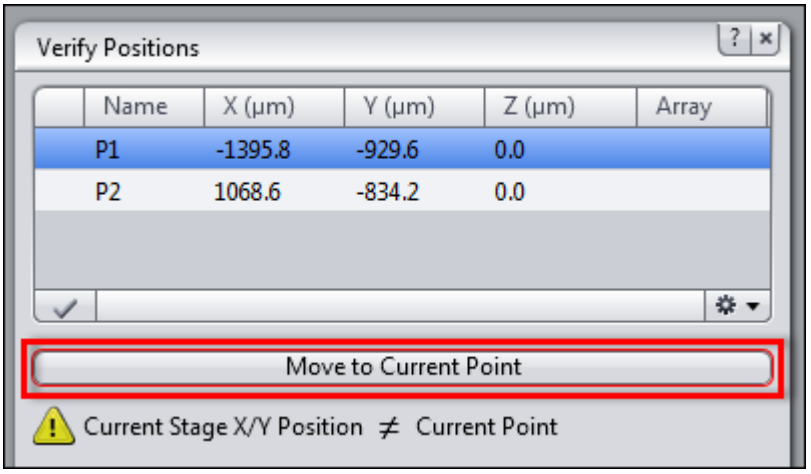
As you have added several positions to your experiment you can go on verify the focus values of each position whether automatically or manually. Proceed as follows:

12 Click in **Tiles** tool in the **Positions** section on the **Verify Positions** button.



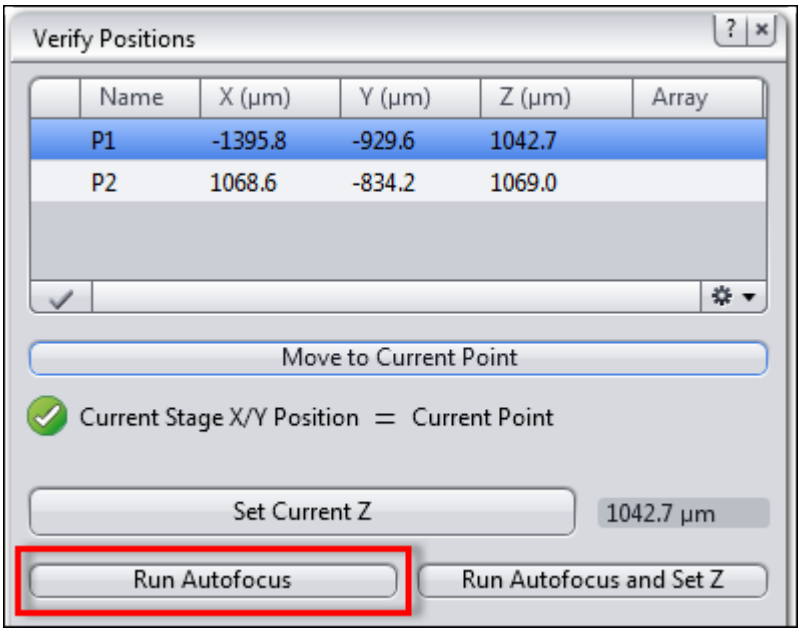
The **Verify Positions** dialog opens.

13 Click on **Move to Current Point** button.



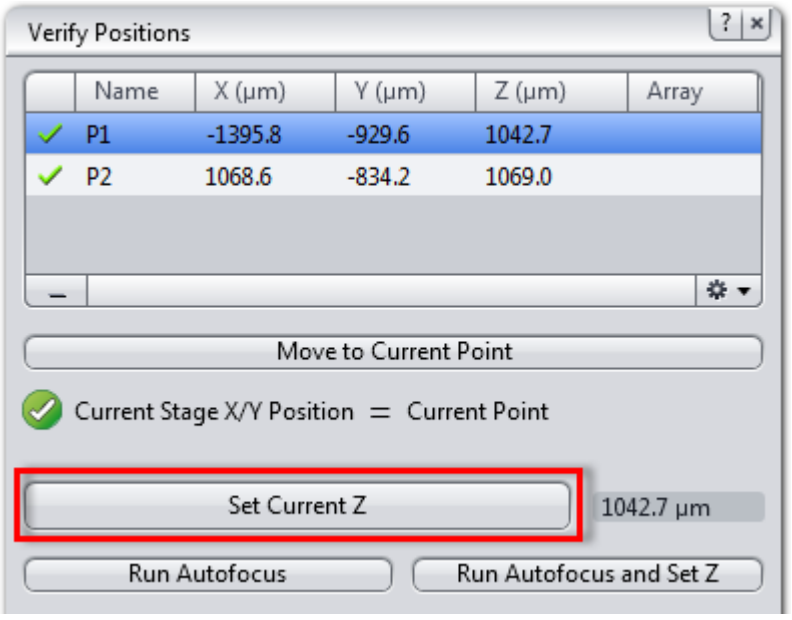
The stage moves automatically to the blue highlighted position (e.g. P1) in the positions list. Alternatively double click on the list entry to move the stage to that position.

14 Click on **Run Autofocus** button.



An autofocus search will be started. You can also set the fine focus manually by moving the z-drive of the microscope. Make sure that the sample is focused now.

15 Click on **Set Current Z** button.



The position will be marked as verified in the list by a checkmark.

16 Click on **Move to Next Point** button.

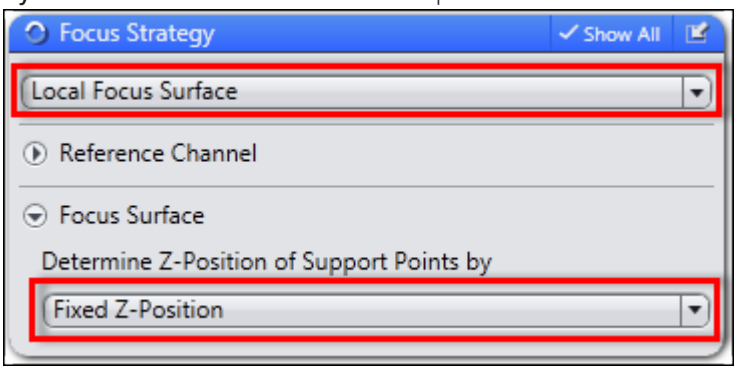
The stage moves to the next position automatically. Repeat the last 3 steps until you have verified all positions in the list. The message **All points have been verified** appears.

17 Close the **Verify Positions** dialog.

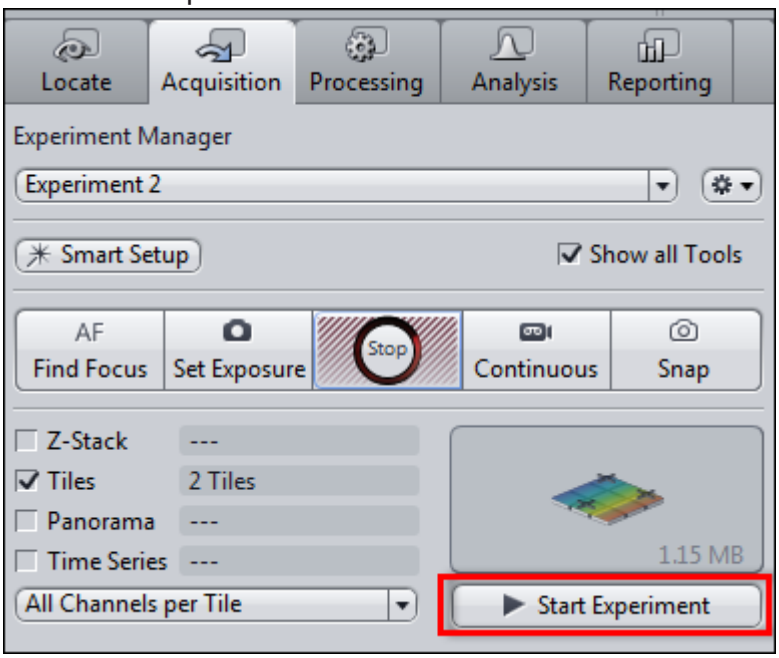
18 In the **Acquisition Parameter** tool group open the **Focus Strategy** tool.

19 Select **Local Focus Surface** as focus strategy from the dropdown list.

20 In the section **Focus Surface** under **Determine Z-position of Support points by** select **Fixed Z-Position** from the dropdown list.



21 Click on **Start Experiment** button.



The multi-positions image will be acquired now.

22 To save the experiment together with all the settings, click in the **Experiment Manager** on the **Options** button .

23 Click on the **Save** entry in the dropdown list.

You have successfully set up a multi-position experiment, verified the positions focus and acquired the multi-position image.

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