

Using the AutoAlign Application Module

Overview

MetaMorph[®] software, version 7.6 and above, now has available the AutoAlign Application Module. Compensate for stage shift, vibration or similar small whole field movement that can occur during a time-lapse. AutoAlign can be used on a stack of images or on a Multidimensional Data set. The user selects which wavelength to perform the alignment on and MetaMorph[®] software will apply the same translation to all the other wavelengths. For multi-Z data sets, the alignment is performed on the best focus image and each plane will be shifted by that amount. For data sets that contain multiple stage positions, the user will have the option of applying the shift to all stage positions based on a selected position. For rotational data sets, a single object can be aligned and adjusted for both the rotation and any slight translational motion over time.

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PRODUCTS
MetaMorph[®]

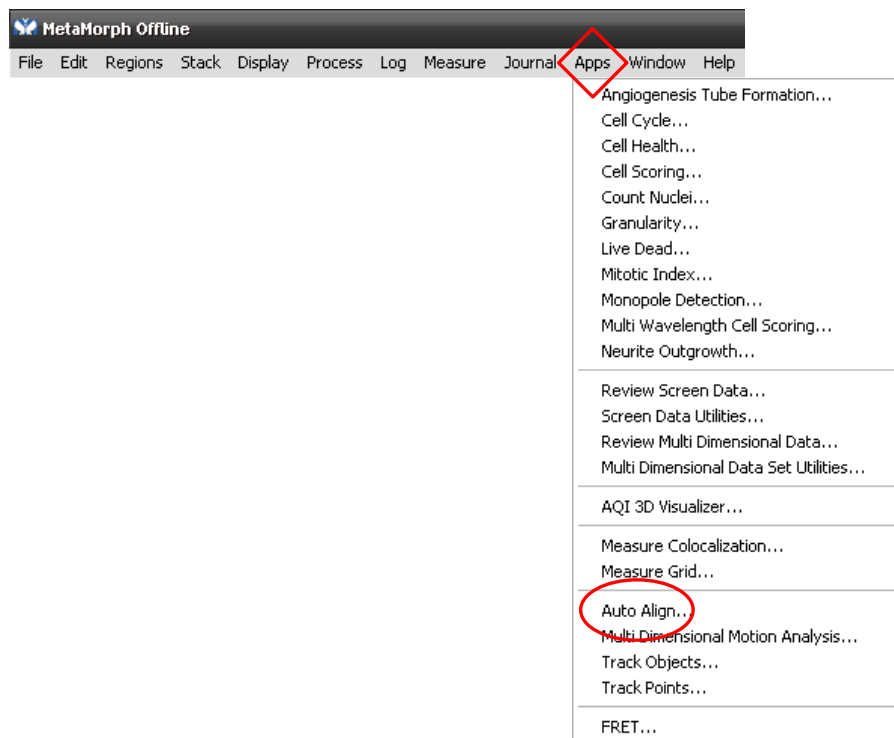
CREATED
17-June-2009

LAST UPDATED
7-July-2009

- The Auto Align application works with calibrated or uncalibrated images of any bit depth.

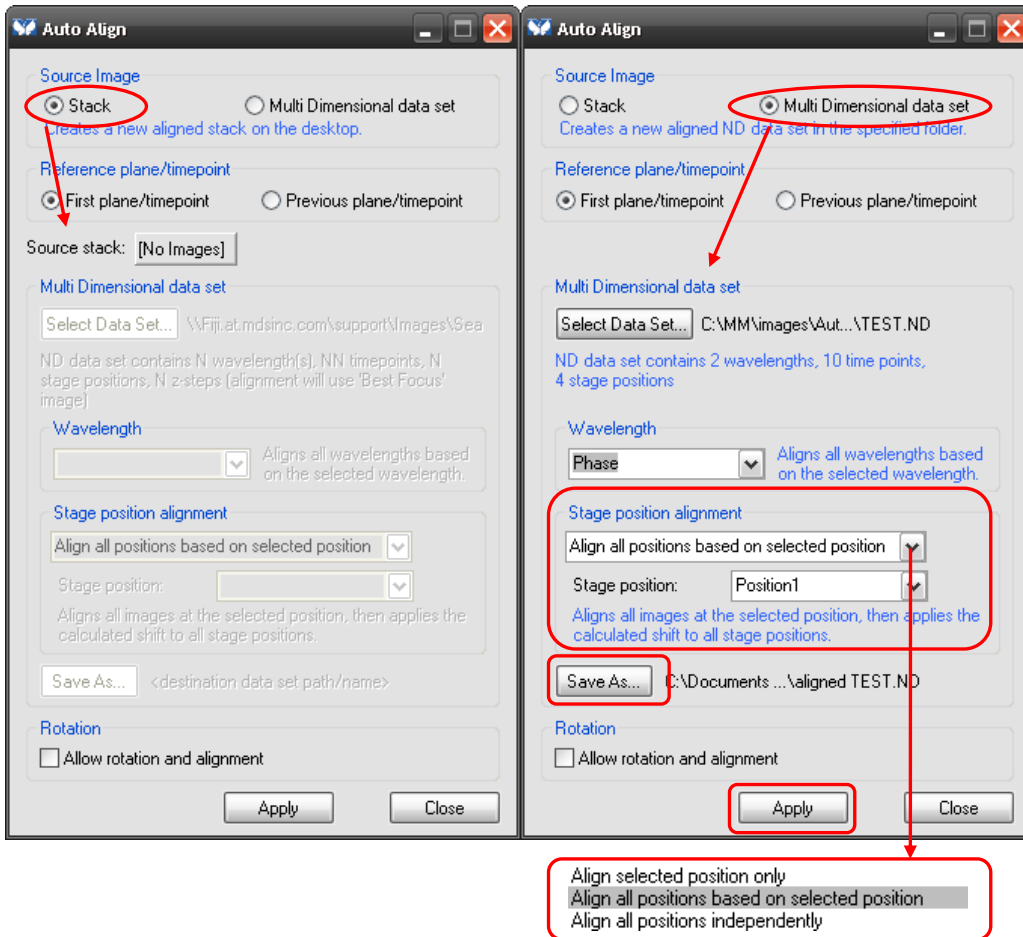
Instructions

AutoAlign can be found under the Apps menu.



Choose to align either a stack or a Multi Dimensional data set.

- For aligning a stack, the stack must be open on the MetaMorph[®] software desktop.
- For aligning a time lapse MD data set, the data is chosen from within the AutoAlign dialog.



- For aligning an MD data set that has multiple wavelengths, the wavelength chosen in the AutoAlign dialog will be the wavelength used to align all wavelengths
- For aligning an MD data set that has multiple stage positions, there are three possible choices:
 1. Align selected position only
 - Aligns images at the selected stage position. The new aligned data set will contain only the selected stage position.
 2. Align all positions based on selected position
 - Calculates alignment of images at the selected stage position and then applies that alignment to all other stage positions. The new aligned data set will contain all stage positions.
 3. Align all positions independently
 - Calculates alignment at each stage position. The new aligned data set will contain all stage positions, which may have images of different sizes.

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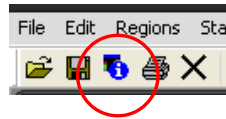
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When aligning a Multi Dimensional data set, a location and file name must be specified for the new data set being created by AutoAlign before attempting to align the source data set. Use the Save As button to perform this operation.

When you have finished entering the required information, hit the Apply button to start the alignment. The annotation for the image provides details about how the image was rotated (if applicable), shifted, and cropped. You can view the annotation for an open image by selecting **Edit > Image Info** or **Edit >**

Annotate Image. Image info is also available by pushing the “i” button on the menu bar as shown below.



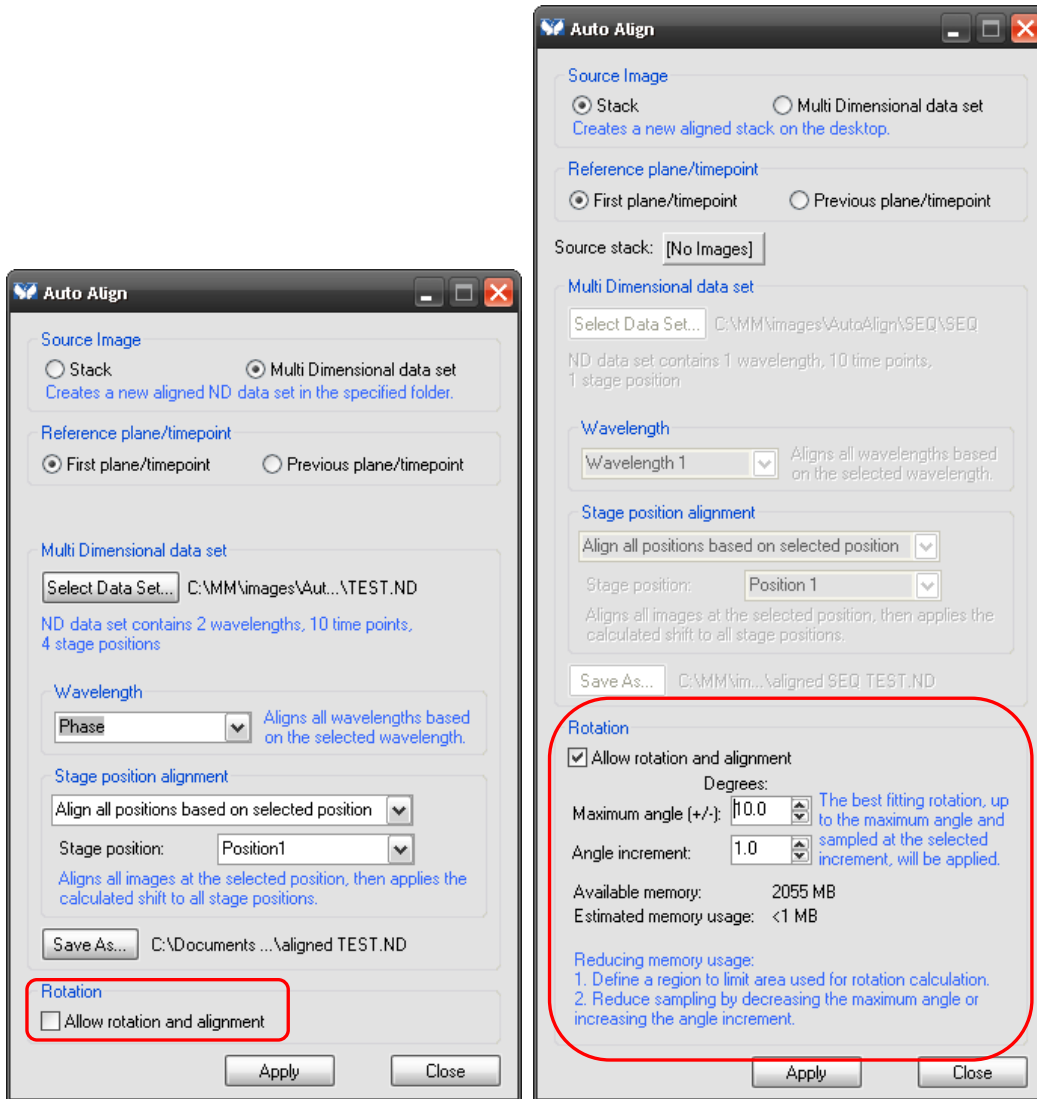
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For Rotational analysis, click on the check box labeled "Allow rotation and alignment".



There are two numbers that can be chosen to be applied to the images, either stacks or time-lapse MultiDimensional data sets – Maximum angle and Angle increment.

1. Maximum angle (+/-)
 - A range value that, in conjunction with the *Angle increment* field, specifies the angles at which rotation sampling should occur. The value in the *Maximum angle* field is both the angle less than zero degrees and the angle more than zero degrees. For example, if you

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enter 10 in the *Maximum angle* field, the rotation samples will be at increments between -10 degrees and 10 degrees.

2. Angle increment

- Specifies at what angles the rotation samples will occur within the range value that you entered in the *Maximum angle* field. For example, if you enter 10 in the *Maximum angle* field and 5 in the *Angle increment* field, the rotation samples will be at -10, -5, 0, 5, and 10 degrees.

NOTE 1: It is recommended that if the object of interest takes the entire field of view an X-Y shift alignment should be performed before the rotational alignment.

NOTE 2: If the field of view contains multiple objects, it is recommended that a maximum projection be performed to determine the size and location of the region to use during rotational analysis. It is also recommended that at least two objects be within the region of interest. The region can then be copied into the AutoAlign stack or Multidimensional image for alignment purposes.

NOTE 3: Auto Align shifts the whole field of view to align images. If the image stack contains more than one rotating object, Auto Align matches the best fitting rotation angle based on the entire contents of the image. Also, when images in a stack or a multi dimensional data set are aligned, the aligned images are cropped to show just the shared area. If the alignment is for rotation of an object, any blank pixels around the edges of the image are filled in with 0 intensity pixels (black).

IMPORTANT: The rotation alignment process can be time-consuming. Aligning an object that rotates may require a significant amount of computer memory. If the amount of memory needed is close to or exceeds the memory available, a message appears to inform you. You will not be able to align the images until you make adjustments to the settings to reduce the amount of memory required. Follow the suggestions that appear on the dialog box to reduce the required memory.

For further assistance please contact Meta Imaging Series® technical support at 800-635-5577 option 3-2-2 or email support.dtn@moldev.com.

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