

Multi Dimensional Acquisition:

Auto Focus Dialog

Abstract

The Multi Dimensional Acquisition (MDA) dialog box provides tools and options for most multidimensional applications. This note will describe the Auto Focus dialog. For further information on Multi Dimensional Acquisition see T20117, T20121, T20122, T20123, T20124, T20126, T20127, T20128 and T20129.

ARTICLE #T20125

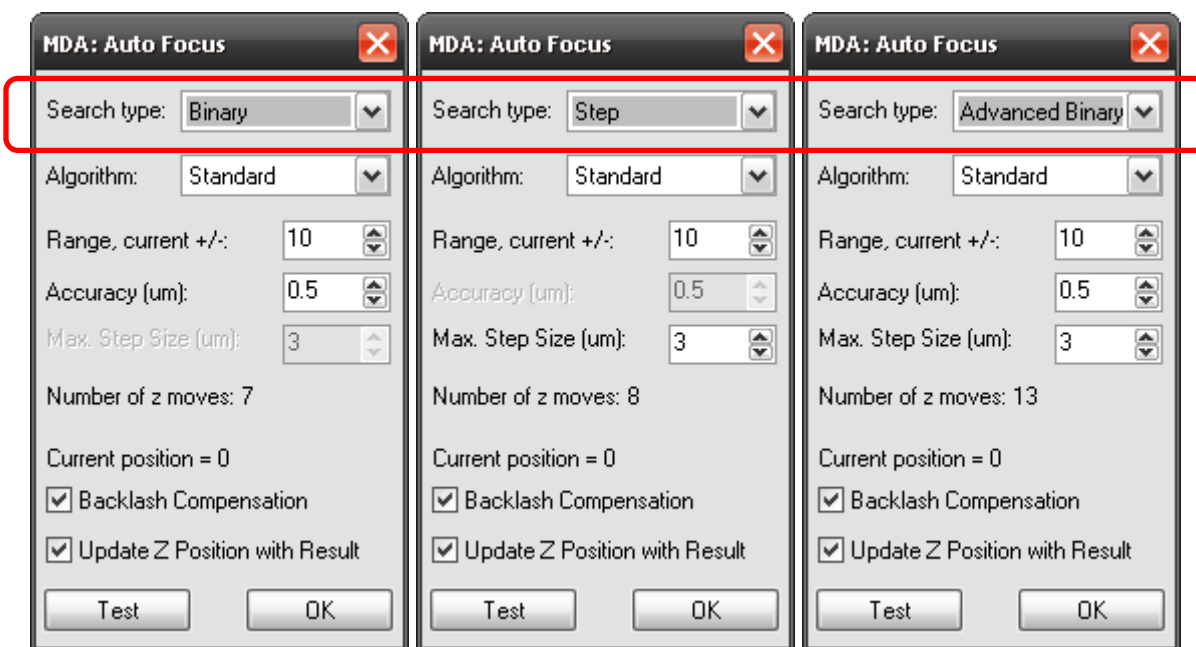
PRODUCTS
MetaMorph®
Version 7.0 or above

CREATED
27-August-2009

LAST UPDATED
27-August-2009

The Auto Focus Dialog

The Auto Focus dialog is where Auto Focus is configured for the multidimensional experiment. This dialog will only be displayed if the appropriate hardware is installed and detected by the MetaMorph® software.



Binary

→ This focusing option attempts to find the best focus position in the fewest number of steps possible. When using this option, MDA takes an image at both the lowest Z position and the highest Z position of the range specified and determines which image is better focused. MDA then compares the image with the better focus to an image in the middle of the range specified, and determines which of these two images is better focused. Then, the range is recursively halved between the position of the image in the middle of the new range and the position of the image with the best focus. These steps are repeated until the distance between the positions of the two images is within the desired range of accuracy. This option uses the same algorithm that is used with the Devices>Find Focus menu option and the Software>Auto-Focus menu option.

Step

- This focusing option attempts to find the best focus position by taking images at fixed intervals (steps) within a specified range of Z positions. MDA uses the position of the image with the best focus as the in-focus position for subsequent image acquisition. This option uses the same algorithm that is used with the Devices>Find Focus menu option and the Software>Auto-Focus menu option.

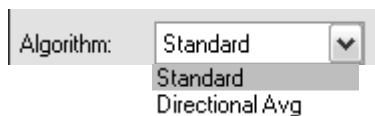
Advanced Binary

- This focusing option is a combination of the Step and Binary methods. First, as in the Step method, MDA takes images at fixed intervals (steps) within a specified range of Z positions, and determines the position of the image that is best focused. Then, MDA performs binary focusing to adjust the Z position until the distance between the positions of the two images is within the desired range of accuracy.

NOTE: To assess the best focal position in the Binary, Step, and Advanced Binary options, MDA uses the Brenner algorithm to measure a "focus value" or "focus score." The Brenner algorithm is based on the sum of squares of the intensity difference between a pixel and a pixel that is two pixels away from it. This calculation provides a rough estimate of image resolution such that, the higher the value at a given pixel, the sharper the grayscale transitions must be surrounding that pixel. When making a comparison of this value for a pixel across several focal planes, the image with the highest focus value will be the sharpest. When the best focus value is determined, the focus motor is moved to the corresponding focal plane (or Z position).

Algorithm

- Specifies the algorithm that you want to use for autofocus. Use **Standard** for acquiring images of normal brightness using lower magnification objectives; use **Directional Avg.** for acquiring images of reduced brightness that can result from using objectives with magnification of 20X and greater.



Range, Current +/-

- Specifies how far above and below the current Z position that the Z motor will be moved while acquiring focus test images. This distance is specified in steps, as defined by the Focus command.

Accuracy: Step(s)

- Specifies the Z distance to move. Changing this value changes the Number of Z Moves.

Number of Z Moves

- Indicates the total number of acquisitions that will be made, based on the settings selected with the Range, Accuracy and Max Step Size fields.

Backlash Compensation

- Enables the Backlash Compensation Z motor movement protocol which directs the focus motor to move to a Z axis position slightly below the target position and then move against gravity to the target position. This ensures that the Z motor gears are fully engaged, avoiding drift due to slippage. This option has no effect on focus devices with built-in backlash compensation.

Update Z position with Result

- Resets the Z position to the best focus point.

Test

- Activates auto focus at the current position.

OK

- Saves settings and closes the dialog box.

If there are any further questions, please contact Technical support at 800-635-5577 x1820 or support.dtn@moldev.com.