

Image Scaling and Autoscale

Overview

A 16-bit image consists of 65536 possible gray levels. However, due to experimental conditions, much of the important image information may be contained within a narrow range towards the lower end of the grayscale. This is particularly the case for low light level experiments. Additionally, most computer monitors can only display 256 gray levels. The Scale Image command allows you to select a range of gray values in a 16-bit image which can then either be displayed in the same image window or copied as a new 8-bit image. This will effectively rescale the intensities in the original image, allowing you more easily to see differences of grayscale values that might otherwise be impossible to discriminate visually.

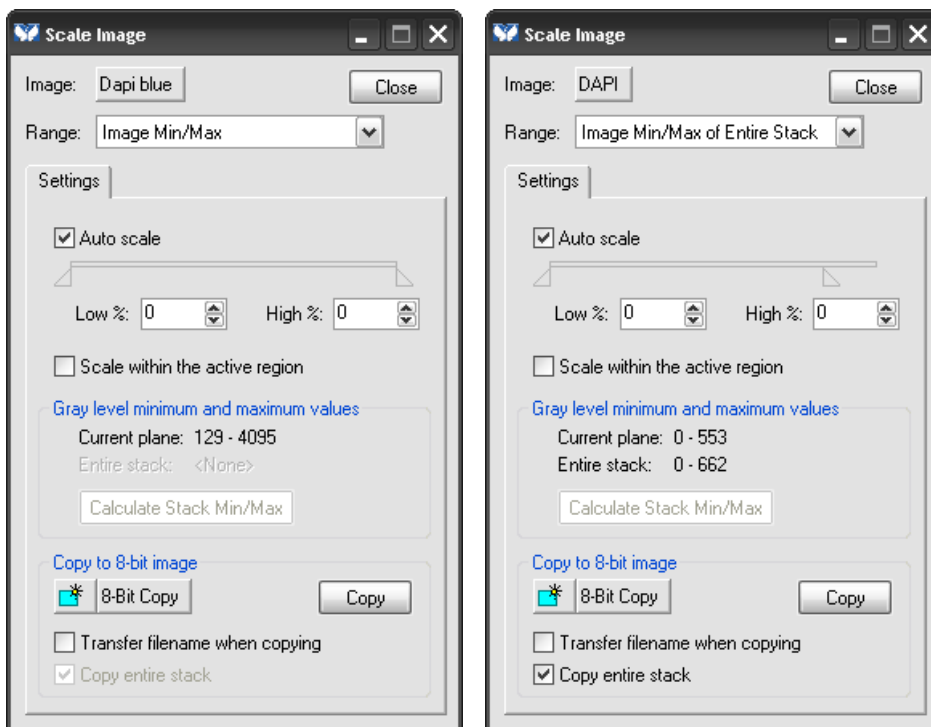
ARTICLE #
T20136

PRODUCTS
MetaMorph®
MetaFluor®
MetaVue®

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NOTE: This command does not affect your image data. It only affects the display of the data on your monitor.



Scale Image for an individual image.

Scale Image for a stack of images.

WARNING: If you use the *Copy* command button while *Transfer Filename When Copying* is enabled, and then save the image, you will overwrite the original 16-bit Source image because the copied 8-bit image will have been given the same name as the original image file. As a result, your 16-bit data may be irretrievably lost. You should also note that the image's file name is not necessarily the same as the name displayed in the title bar of the image window.

The initial default range of gray levels will be those between the image's lowest gray level and highest gray level (the *Image Min/Max* option in the *Range* drop-down list).

Image Min/Max	Image Min/Max of Entire Stack
8-Bits (0-255)	8-Bits (0-255)
10-Bits (0-1023)	10-Bits (0-1023)
12-Bits (0-4095)	12-Bits (0-4095)
14-Bits (0-16383)	14-Bits (0-16383)
16-Bits (0-65535)	16-Bits (0-65535)

Thus, you cannot set the *Image Min/Max* slider to a value below the image's minimum value or above its maximum value. However, if you select one of the other *Range* options (10-bit, 12-bit, 14-bit, or 16-bit depth), you can change the initial default range to include the entire gray levels available in an image's depth type. You can then set the range for the scaling manually to a range of your preference.

NOTE: Pixels equal and above the High intensity value are White and pixels equal and below the low intensity value are Black

If you do not want to scale the image manually, you can use “*Auto-Scale*”. The MetaMorph software will base the scaling range on the image's lowest and highest gray levels. If your image contains outlying “hot” pixels (either oversaturated or undersaturated), you can exclude a selected percentage of the pixels in the image using “*Auto-Scale*”, but not the actual gray levels in its histogram, at the lower and upper end of the image's grayscale range by adjusting the *Low %* and *High %* spin boxes in the *Scale Image* dialog box. For example, you could exclude the bright nucleus of a cell in a fluorescence image by setting the low spin box to 1% and the high spin box to 10%. Scaling will then be based on the lowest and highest remaining grayscale values. If you enable “*Scale Within Active Region*”, autoscaling will work within an active region of interest. This will allow you to place a small active region of interest over the object(s) you want to see, and the image will be scaled based on the grayscale values of that region. This can be particularly useful when working with a stack because “*Auto-Scale*” recalculates within the active region for each plane in the stack.

Dialog Box Options

Image

→ Selects the 16-bit Source image that you want to scale.

Close

→ Closes the dialog box.

Range

→ Selects the range of gray levels available for scaling. The default setting of *Image Min/Max* sets the range between the image's minimum and maximum gray levels. You can also specify gray levels that are outside of the default range to include all of the levels available in the following image types:

- 8-Bits (0-255)
- 10-Bits (0-1023)
- 12-Bits (0-4095)
- 14-Bits (0-16383)
- 16-Bits (0-65535)

Settings

→ Auto Scale

This will allow the MetaMorph software to scale the image based on the lowest and highest gray levels in the image. A specific percentage of pixels can be excluded from the lower and upper end of the scaling range with the *Low %* and *High %* spin boxes. When the *Auto Scale* check box is selected, the *Low Scale* and *High Scale* options are disabled.

→ Low%

This will exclude the lowest selected percentage of pixels in the image (not the percentage of gray levels in the histogram) from the range of values being autoscaled. This option is available only when the *Auto Scale* check box is selected.

→ High%

This will exclude the highest selected percentage of pixels in the image (not the percentage of gray levels in the histogram) from the range of values being autoscaled. This option is available only when the *Auto Scale* check box is selected.

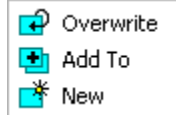
- Low Scale
This selects the darkest gray level for the scaled image. This option is available only when the *Auto Scale* check box is not selected.
- High Scale
This selects the brightest gray level for the scaled image. This option is available only when the *Auto Scale* check box is not selected.
- Scale Within the Active Region
This directs the MetaMorph software to use the maximum and minimum intensity values in the active region of interest when *Auto Scale* is selected. The entire image will then be displayed using the values determined from the active region of interest.

Gray Level Minimum and Maximum Values

- Displays the minimum and maximum gray level values for the current plane and the entire stack.

Copy to 8-bit Image

- Destination
Choose to overwrite, add to, or create a new file for the resulting image of the *Copy* command.




- Copy
This command copies the scaled image to a new 8-bit image. If your 16-bit image was thresholded, the 8-bit image will be saved with the 16-bit thresholding state scaled to an 8-bit thresholding state.
- Copy entire stack
This command copies all planes in a stack to a new 8-bit stack. If your 16-bit stack was thresholded, the 8-bit stack will be saved with the 16-bit thresholding state scaled to an 8-bit thresholding state.
- Transfer filename when copying
This command will transfer the file name of the original 16-bit image to the new 8-bit image when you use the *Copy* command button.

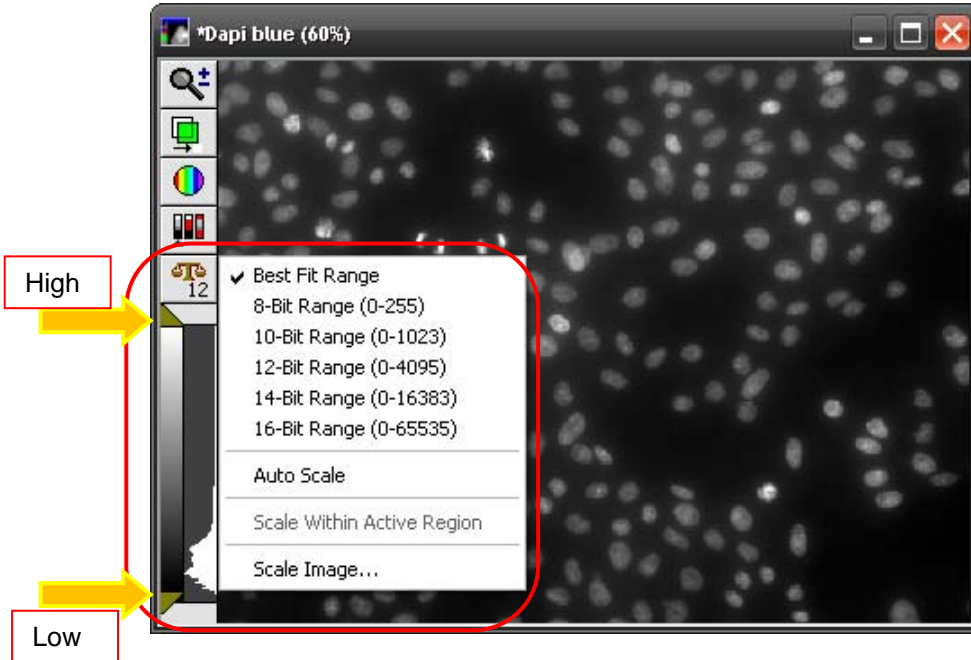
Scaling a 16-Bit Image

To scale a 16-bit image, use the following procedure:

Step	Action
1	From the Display menu, choose Scale Image. The Scale Image dialog box opens.
2	Select the desired 16-bit image using the <i>Image</i> selector.
3	If you want to specify gray levels that are outside of the default range of the images lowest and highest gray levels (<i>Image Min/Max</i>), select <i>8-Bits</i> , <i>10-Bits</i> , <i>12-Bits</i> , <i>14-Bits</i> , or <i>16-Bits</i> from the <i>Range</i> list. This will change the range to include all of the gray values available in the specified image depth type.

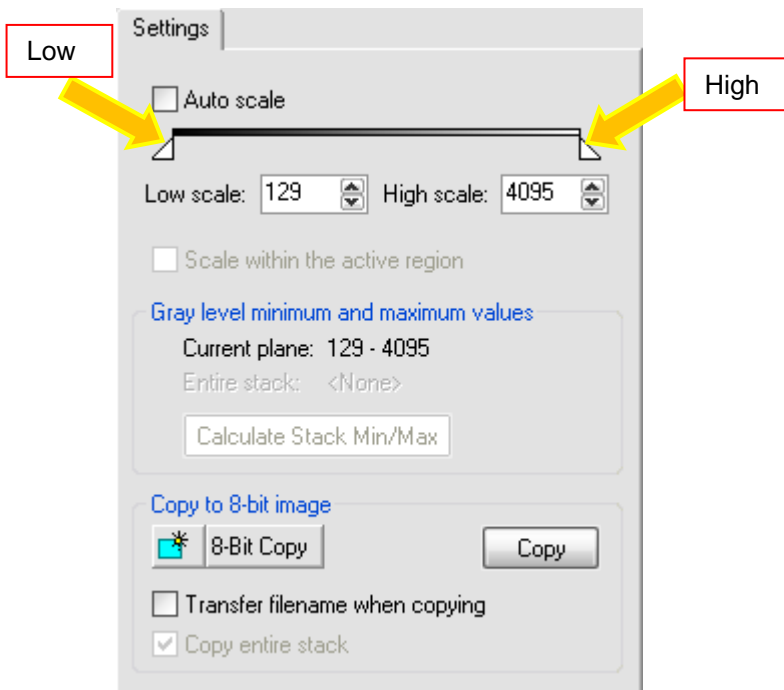
- 4 To specify the high and low scale values manually, click and drag the Low and High Scale Sliders on the Scale Bar, or use the *Low Scale* and *High Scale* edit boxes.
OR
If you want to scale the image based on a percent of pixels to exclude, select *Auto Scale*. If desired, specify a percentage of pixels (not gray values) to be excluded from the lower and upper ends of the scaling ranges with the *Low %* and *High %* edit boxes.
- 5 To apply the maximum and minimum intensity values of an active region of interest to the entire image, select *Scale within the active region*.
NOTE: This option is only available when *Auto Scale* is enabled.
- 6 To copy the altered image to an 8-bit image, click the destination icon , choose to overwrite, add to, or create a new file for the resulting image, then click *Copy*.
Note: If *New* is selected as the destination, the default file name of the new image will be in the format *8-Bit Copy of Original File Name*. If you want to select a different name for the 8-bit copy, Click *8-Bit Copy* and select *Specified* to open the Specify Image name dialog box.
OR
To copy all planes in a stack to an 8-bit stack, select *Copy entire stack*. Click the destination icon, choose to overwrite, add to, or create a new file for the resulting image, then click *Copy*.
- 7 Choose *Close* when you have finished.

Alternately, the image can be scaled using the same set of scaling commands found on the sidebar of the image itself.



The two wedges on the top and the bottom of the scale image histogram correspond to the wedges in the scale image dialog box. Use the High Wedge to set the White and use the Low Wedge to set the Black.

NOTE: Pixels equal and above the High intensity value are White and pixels equal and below the low intensity value are Black



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