



MetaXpress[®] 6 Software Guide

Acquiring Images Using Digital Confocal

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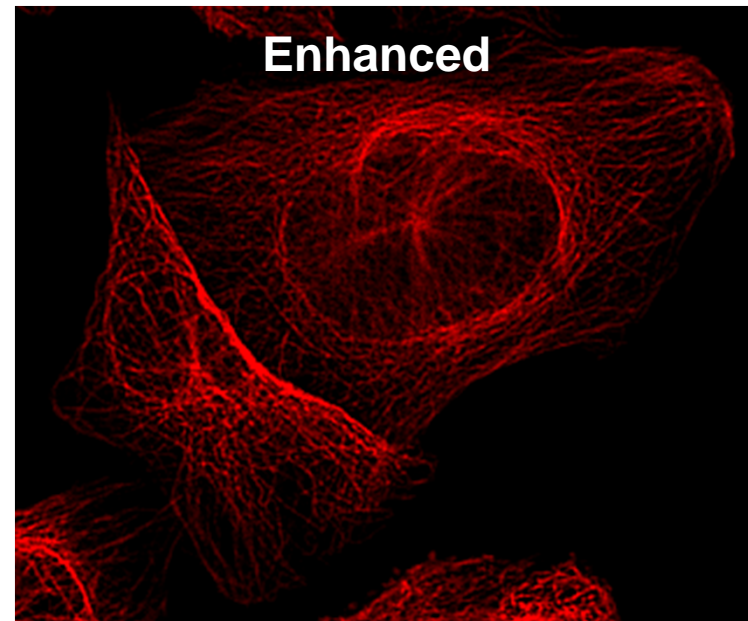
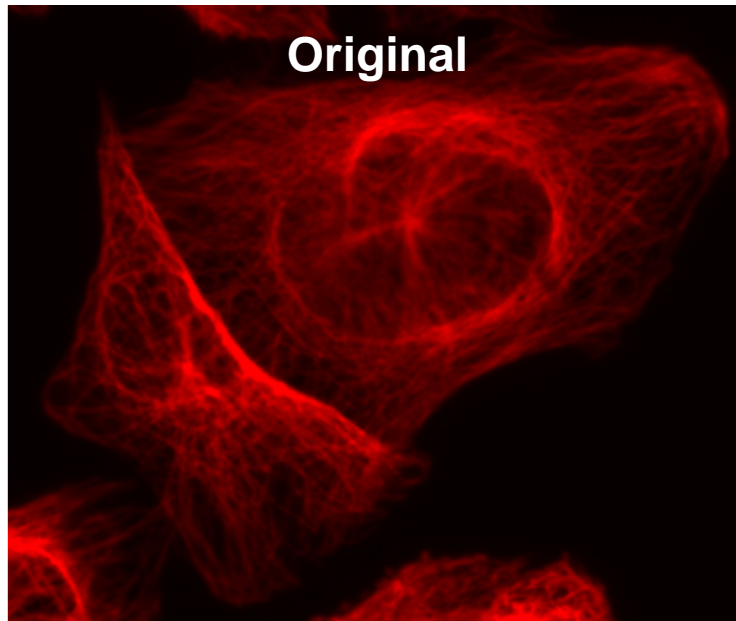
Chapter Purpose

The purpose of this chapter is to guide the user through configuring the **Digital Confocal** feature available on the **W** tabs (wavelength) of **Plate Acquisition Setup**. This feature is an on-the-fly 2-dimensional deconvolution routine that enhances image contrast, resolution, and sharpness thereby improving image analysis results.

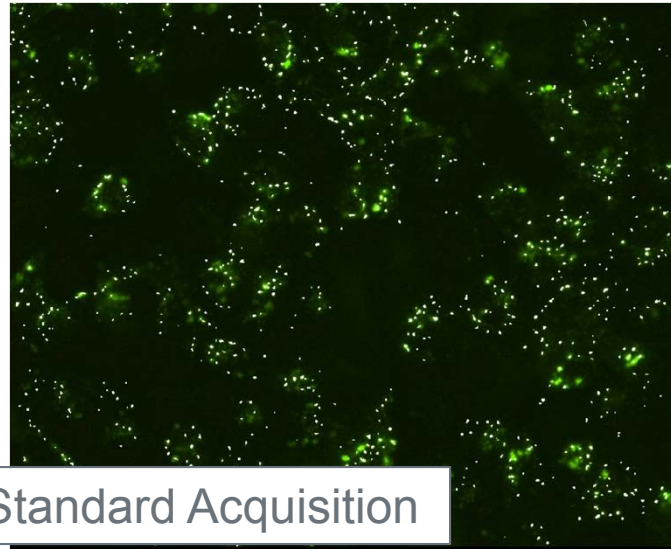
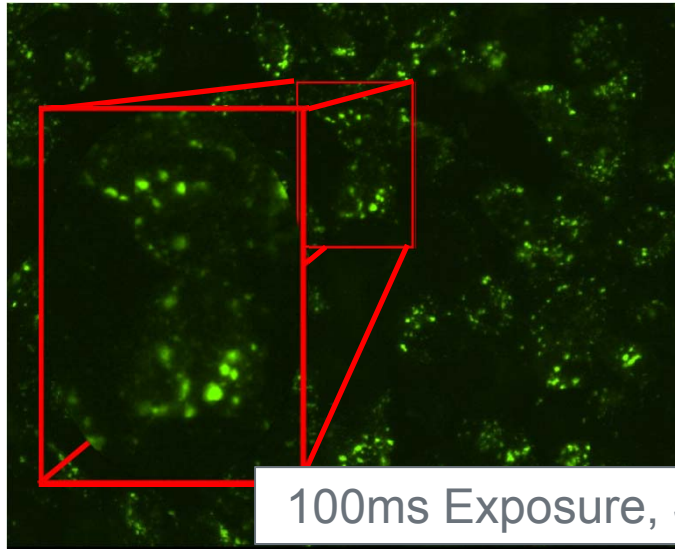


What is Digital Confocal?

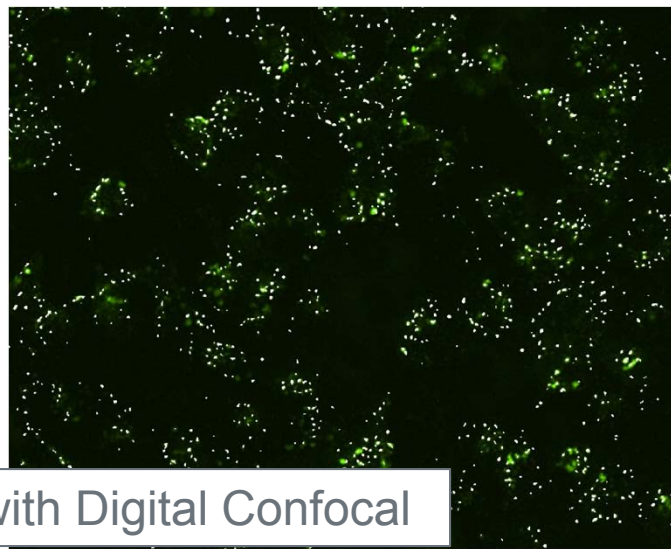
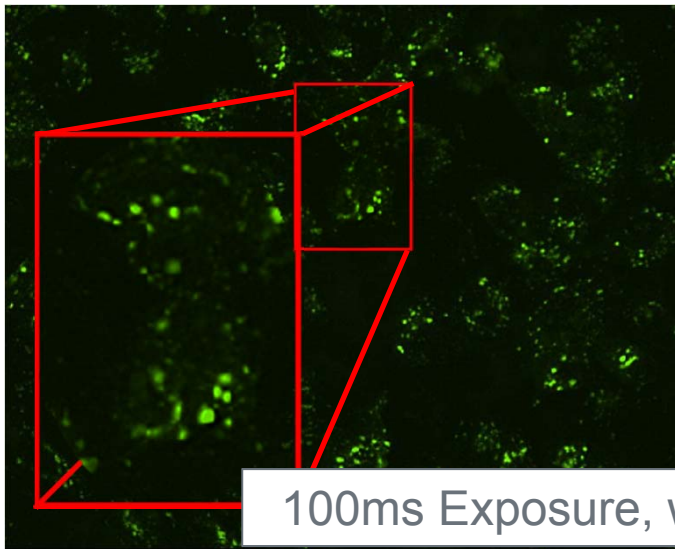
- On-the-fly 2D deconvolution
- Uses objective theoretical point spread function and image acquisition properties to enhance image quality
 - Enhances contrast, improves resolution, and sharpens the image
 - Can reduce exposure times
 - Can be applied independently to each wavelength



Digital Confocal Improves Assay Quality



Z' for Pit
Area Per Cell



Digital Confocal Increases Assay Speed

- Decrease exposure time >2x
- Decrease total acquisition time

To Get Z' of 0.6 for Pit Area Per Cell	GFP Exposure Time	384 Well Plate Acquisition Time (mm:ss.ms)	Speed Increase
Without Digital Confocal	500ms	20:27.152	12%
With Digital Confocal	100ms	17:54.100	



Enabling Digital Confocal

1. Open Plate Acquisition Setup

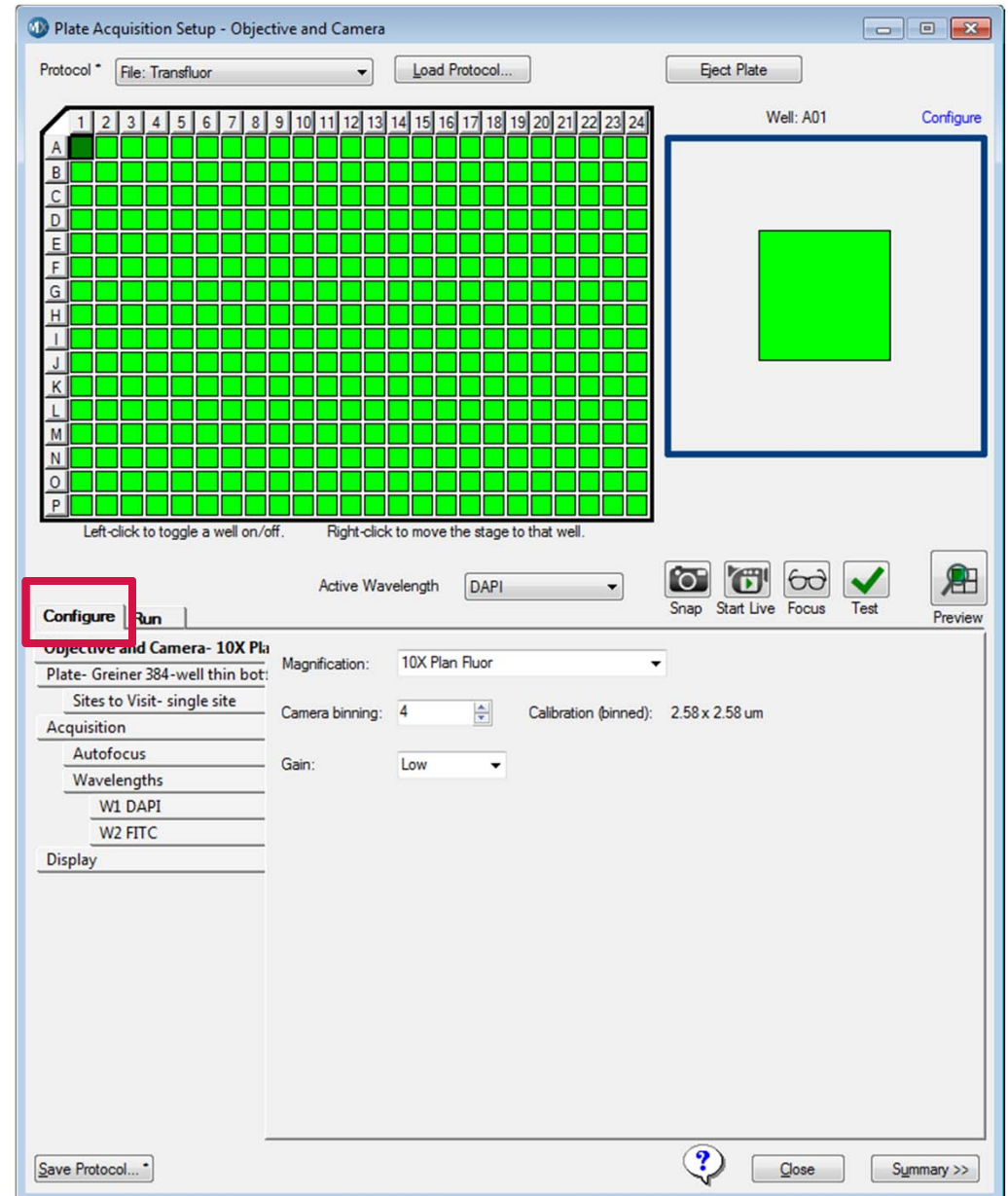
- In the main toolbar click on



OR

- Under the **Screening** menu, select **Plate Acquisition Setup**

2. Select the **Configure** tab



Enabling Digital Confocal

3. Select the appropriate **W** tab (wavelength)
4. Configure the wavelength settings as you would for acquisition
 - i. Select desired **Illumination Setting** from the drop-down menu
 - ii. Calculate **Focus offset**
 - iii. Determine **Exposure time**
5. Enable **Digital Confocal**

Objective and Camera- 10X Plat
Plate- Greiner 384-well thin bot:
Sites to Visit- multi-site
Acquisition
Autofocus
Wavelengths
W1 DAPI
W2 FITC
Display

Illumination setting: DAPI
Exposure (ms): 50 Auto Expose Target max intensity: 33000
Autofocus options
Post-laser offset (um)
Laser with z-offset 12.36
Calculate Offset < Use Z stack Custom Range Range (um) Step (um)
138.89 5.56
Acquisition Options
 Digital Confocal (info) << Increase sharpness Reduce noise >> 0.0159
Shading Correction: Off



What is Digital Confocal K Value?

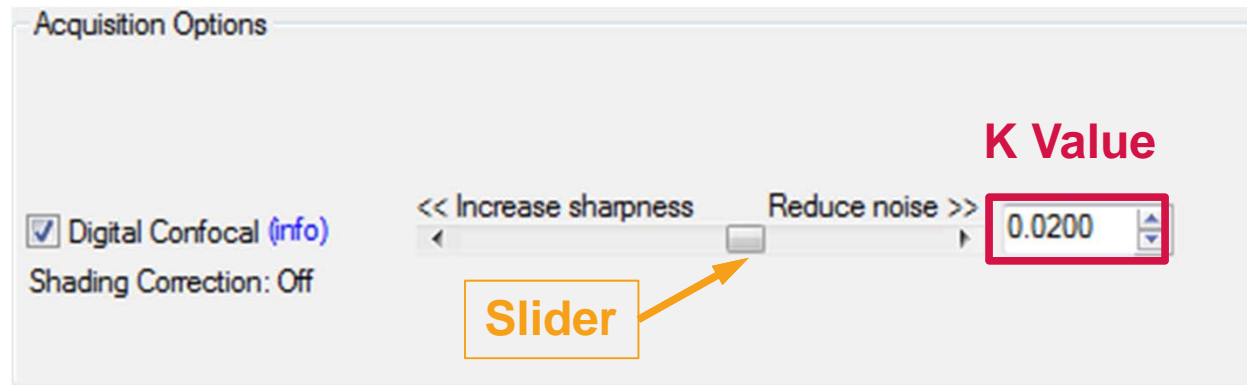


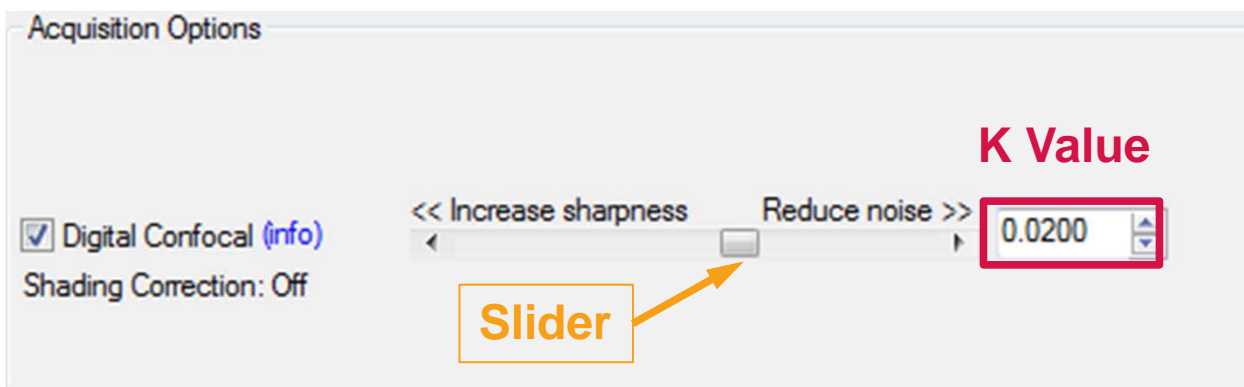
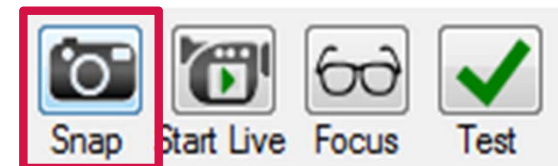
Image enhancement is optimized by adjusting the **K Value**

- K Value is adjusted using the slider or entering a number
- Moving the slider to the right (higher K value) reduces noise in the image but reduces image sharpness
- Moving the slider to the left (lower K value) increases image sharpness but also increases noise



Method 1: Determine K Value Using Image Snap

6. Click on the **Snap** button to take an image (assumes your sample is in focus)
7. Press **Ctrl+D** on the keyboard to duplicate the image
8. Adjust **K Value** using the slider
9. Snap another image and compare to the duplicated image
10. Repeat steps 8-9 until the image looks sharp but not noisy
11. Adjust exposure time if necessary
12. Repeat for subsequent wavelengths



In practice, start with $K = 0.0200$, then adjust the slider as necessary

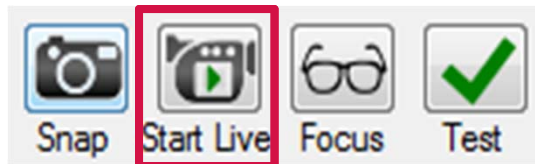
Ideally, $K \geq 1 / [\text{signal to noise ratio}]$




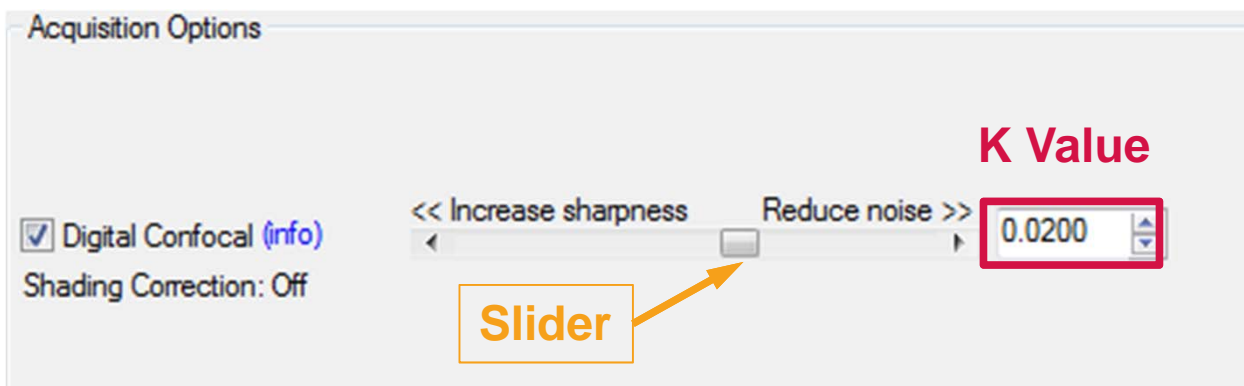
Method 2: Determine K Value Using Live Mode

- Click on the **Start Live** button to start live mode (assumes your sample is in focus)

Note Live mode can cause photobleaching and phototoxicity of your sample



- Adjust **K Value** using the slider until the image looks sharp but not noisy
- Turn off Live Mode by pressing **F2** on the keyboard or click on the **F2:Stop** button 
- Click on the **Snap** button to view the image
- Adjust exposure time if necessary
- Repeat for subsequent wavelengths



In practice, start with $K = 0.0200$, then adjust the slider as necessary

Ideally, $K \geq 1 / [\text{signal to noise ratio}]$



Viewing K Value in Image Information

The screenshot shows the 'Image Info' dialog box with the 'Image' set to 'FITC'. The left pane contains a table of properties, and the right pane contains an 'Annotation' list. A red box highlights the 'Deconvolution Wiener Filter KValue: 0.02' in the annotation list. Another red box highlights the 'Hide Annotation <<' button at the bottom of the dialog.

Property Name	Property Value
Location on Disk	N/A
File Type	MetaSeries Single/Multi-plane TIFF
Creation Timestamp	Tue Jun 2 15:44:52.224 2015
Last Saved Timestamp	
Lookup Table Model	Set By Wavelength
Storage Requirement(Megabytes)	2.22 MB
Image Width	1080
Image Height	1080
Image Depth (bits)	16
Image X Calibration (µm/pixel)	1.29
Image Y Calibration (µm/pixel)	1.29
Number of Planes	1
Plane Stage Label	A01
Plane Stage Position X	14380
Plane Stage Position Y	11240
Plane Camera Offset X	0
Plane Camera Offset Y	
Plane Camera Horizontal Bins	2
Plane Camera Vertical Bins	2
Plane Z Distance	
Plane Z Position	2912.36
Plane Illum Setting	FITC
Plane Wavelength	536
Plane Magnification	10X Plan Fluor
Plane NA	0.3
Plane Refractive Index	1
Temperature	37
Co2 Pressure Status	OK
Camera Bit Depth	16
ImageXpress Micro Filter Cube	FITC

Annotation:

- Exposure: 100 ms
- Binning: 2 x 2
- Region: 2160 x 2160, offset at (0, 0)
- Subtract: Off
- Shading: Off
- Digitizer: Fast
- Gain: Low
- Camera Shutter: Open for Expose
- Clear Count: 1
- Clear Mode: CLEAR NEVER
- Frames to Average: 1
- Trigger Mode: Normal (TIMED)
- Temperature: 14
- Deconvolution NA: 0.3
- Deconvolution RI: 1
- Deconvolution Emissive Wavelength: 536
- Deconvolution X Image Spacing: 1.29
- Deconvolution Y Image Spacing: 1.29
- Deconvolution Spherical Aberration: 0
- Deconvolution Wiener Filter KValue: 0.02**

Plane Number: 1

Hide Annotation <<

Open Log | Configure Log... | Image Status Bar... | Print... | Close

Data Log Not Open

- The **K Value** used to acquire an image is recorded in the image metadata
- Click on the **Image Info** button  in the main toolbar to view this information
- In the **Image Info** dialog, click on the **Show Annotation>>** button
- The **K Value** is at the bottom of the annotation list



Support Resources

- F1 / HELP within MetaXpress® Software
- Support and Knowledge Base: <http://mdc.custhelp.com/>
- User Forum: <http://metamorph.moleculardevices.com/forum/>
- Request Support: <http://mdc.custhelp.com/app/ask>
- Technical Support can also be reached by telephone:
 - 1 (800) 635-5577
 - Select options for Tech Support → Cellular Imaging Products → ImageXpress Instruments





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