MetaXpress® 6 Software Guide

Acquiring Images Using Digital Confocal

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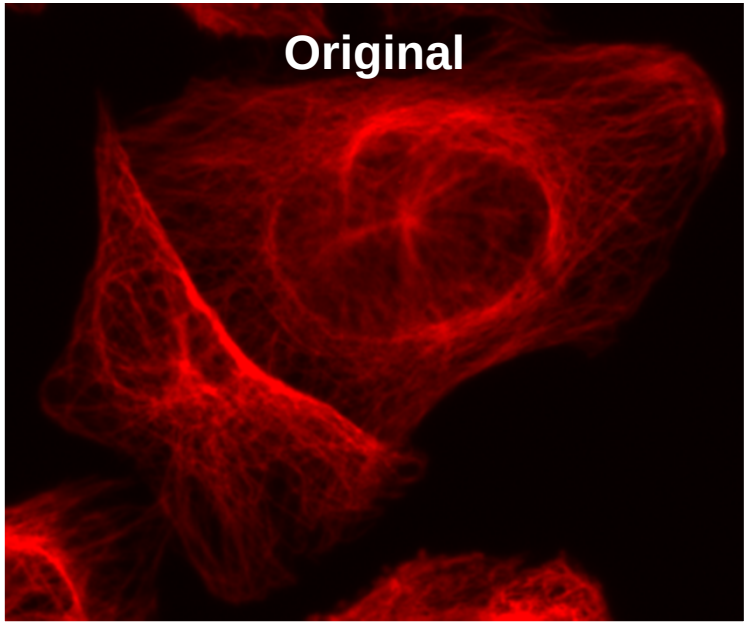
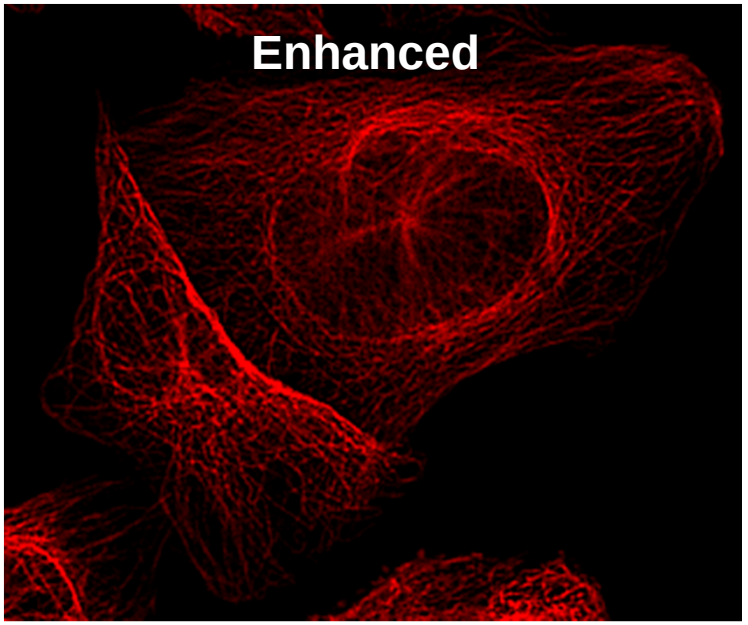
Chapter Purpose

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.

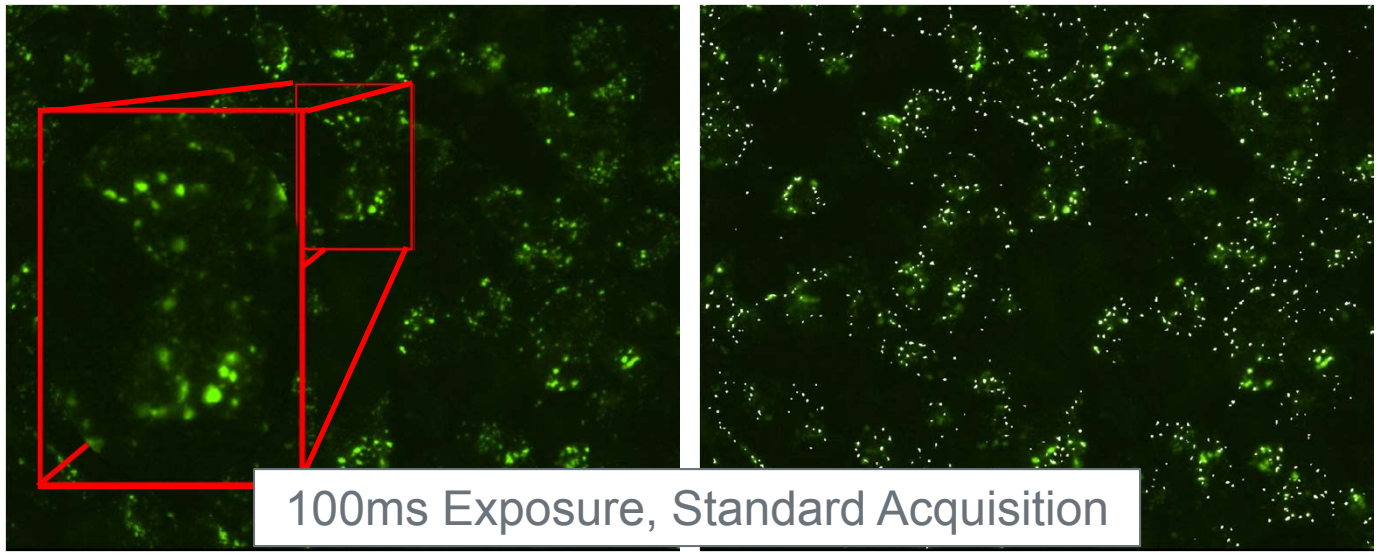
What is Digital Confocal?

- 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

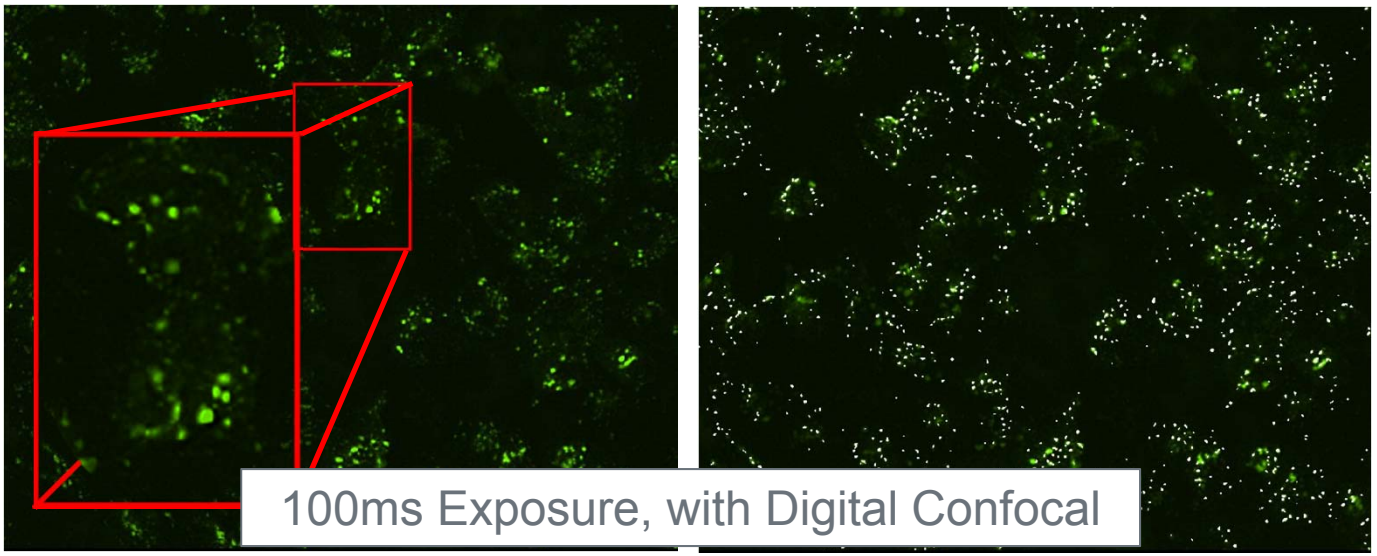




Digital Confocal Improves Assay Quality



100ms Exposure, Standard Acquisition



100ms Exposure, with Digital Confocal

Z' for Pit Area Per Cell

0.48

0.66

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

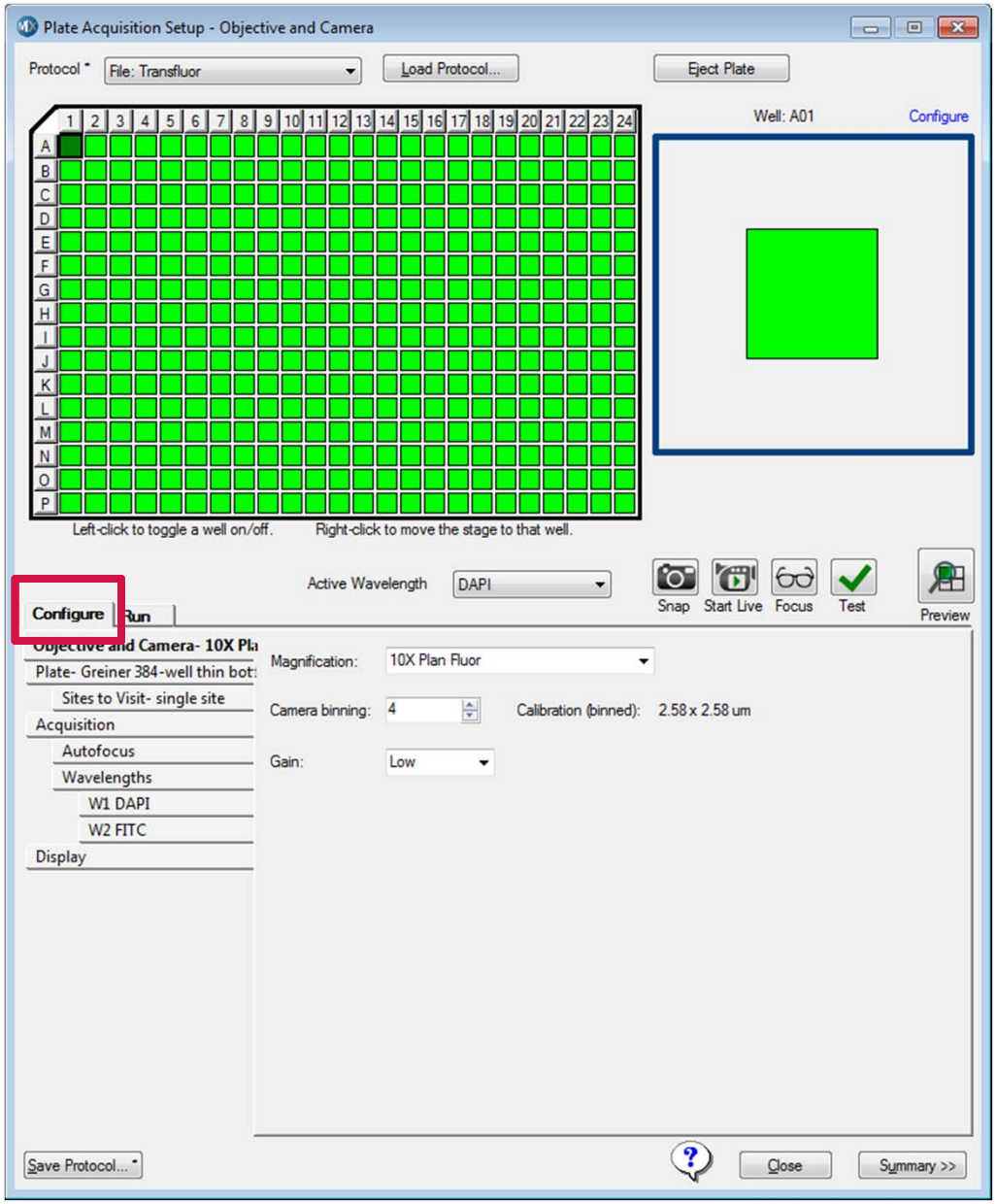
1. Open **Plate Acquisition Setup**

   - In the main toolbar click on

   OR

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

2. Select the **Configure** tab

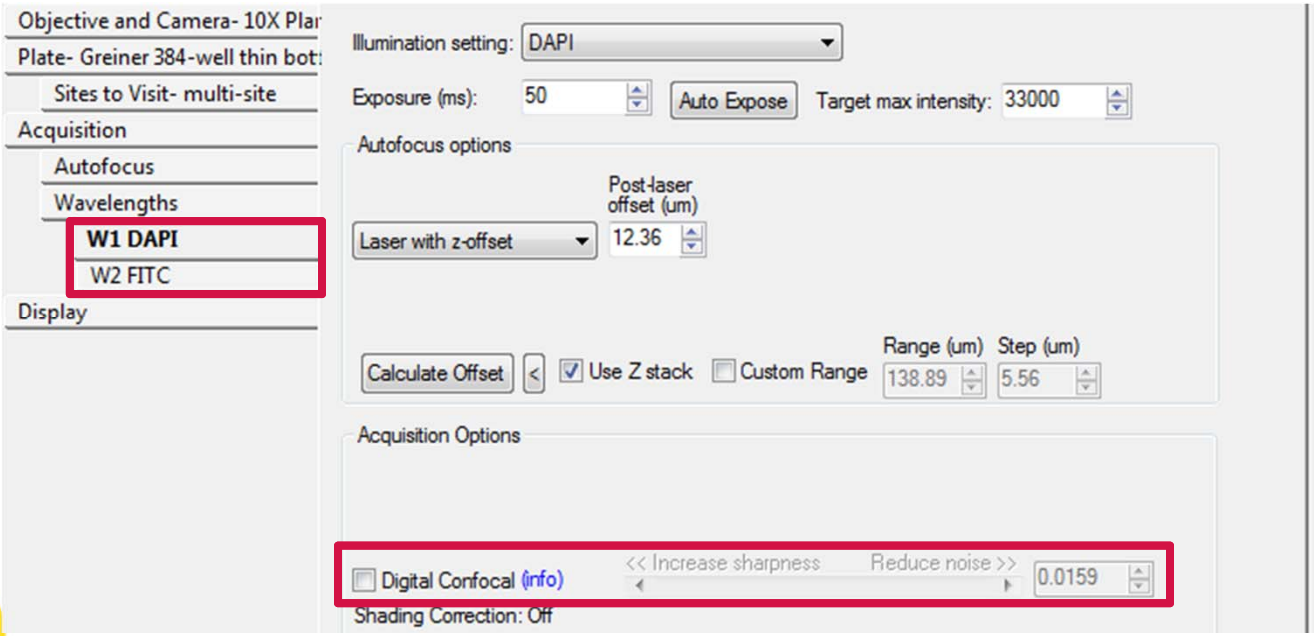


Enabling Digital Confocal

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**



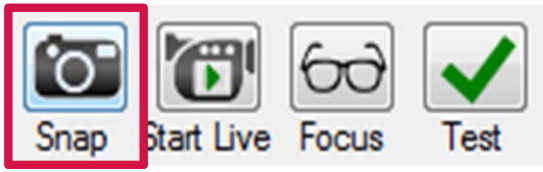
What is Digital Confocal K Value?

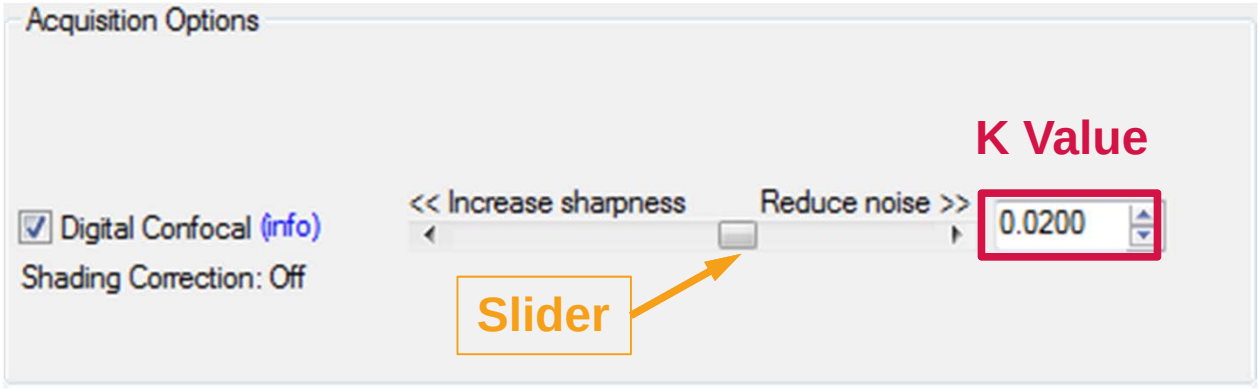
Image enhancement is optimized by adjusting the **K Value**

- 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

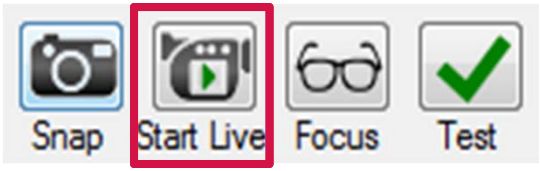




In practice, start with K = 0.0200, then adjust the slider as necessary

Ideally, K ≥ 1 / [signal to noise ratio]

Method 2: Determine K Value Using Live Mode



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

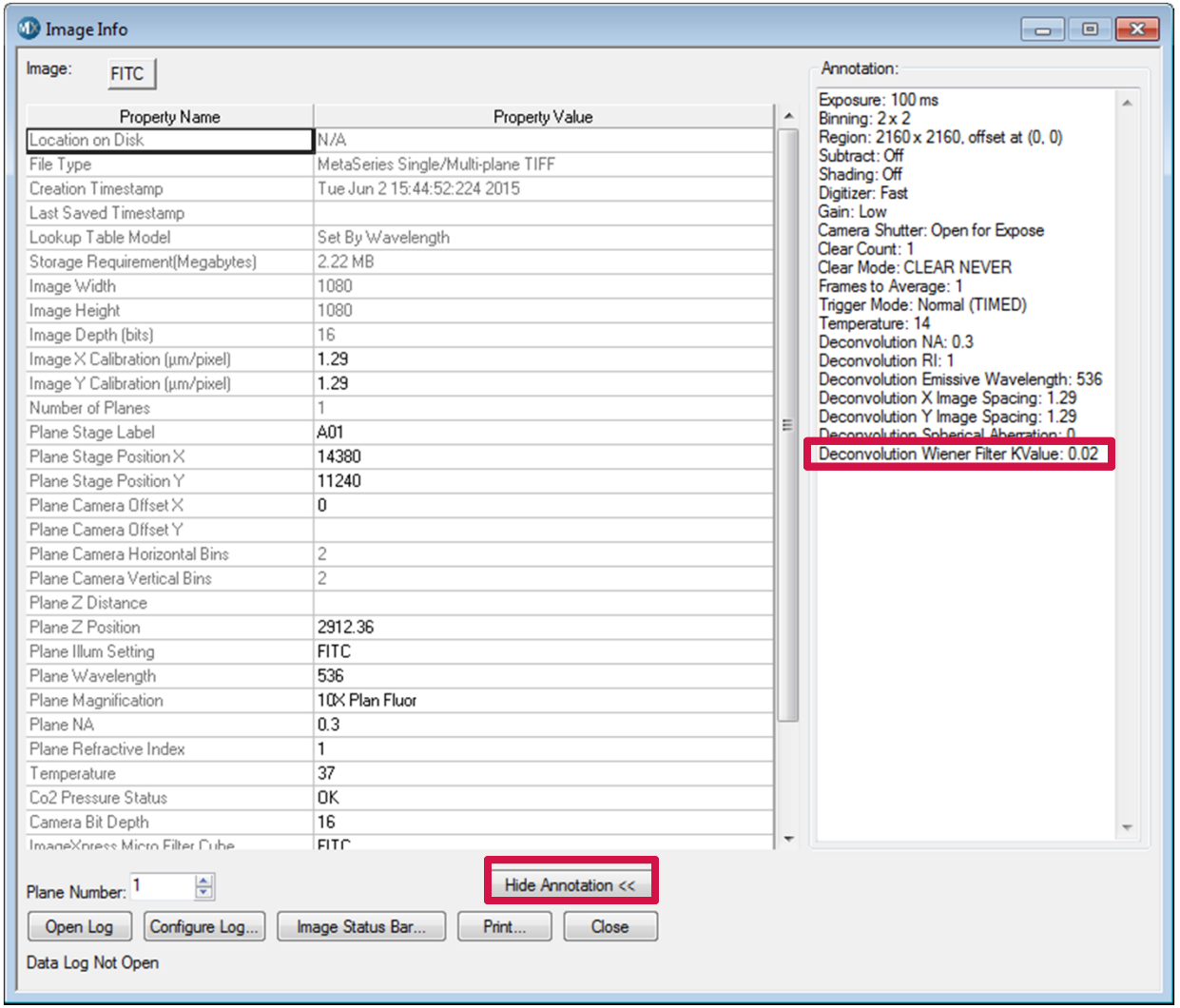
   Note Live mode can cause photobleaching and phototoxicity of your sample

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

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

Ideally, K ≥ 1 / [signal to noise ratio]

Viewing K Value in Image Information



- 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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