

## FLIM and Confocal Image with ASI Stage

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## FLIM and Confocal Image with ASI Stage

### 1. Introduction and Background

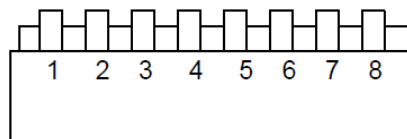
The ASI stage is good for multi-well FCS measurement. It is now also good for taking Confocal image and FLIM(Fluorescent Lifetime Image).

### 2. Procedure of Installing the ASI stage controller

- A. RS232 Baud rate check.

#### Dip-Switches

The Dip-Switches allow the user to modify the configuration of the MS-2000's input and output devices. Switches 1-3 select the LCD screen options. Switches 4 and 5 set up the serial baud rate or USB mode. Switches 6-8 adjust the deflection of the input devices such as the joystick.



## FLIM and Confocal Image with ASI Stage

### Dip Switch Settings

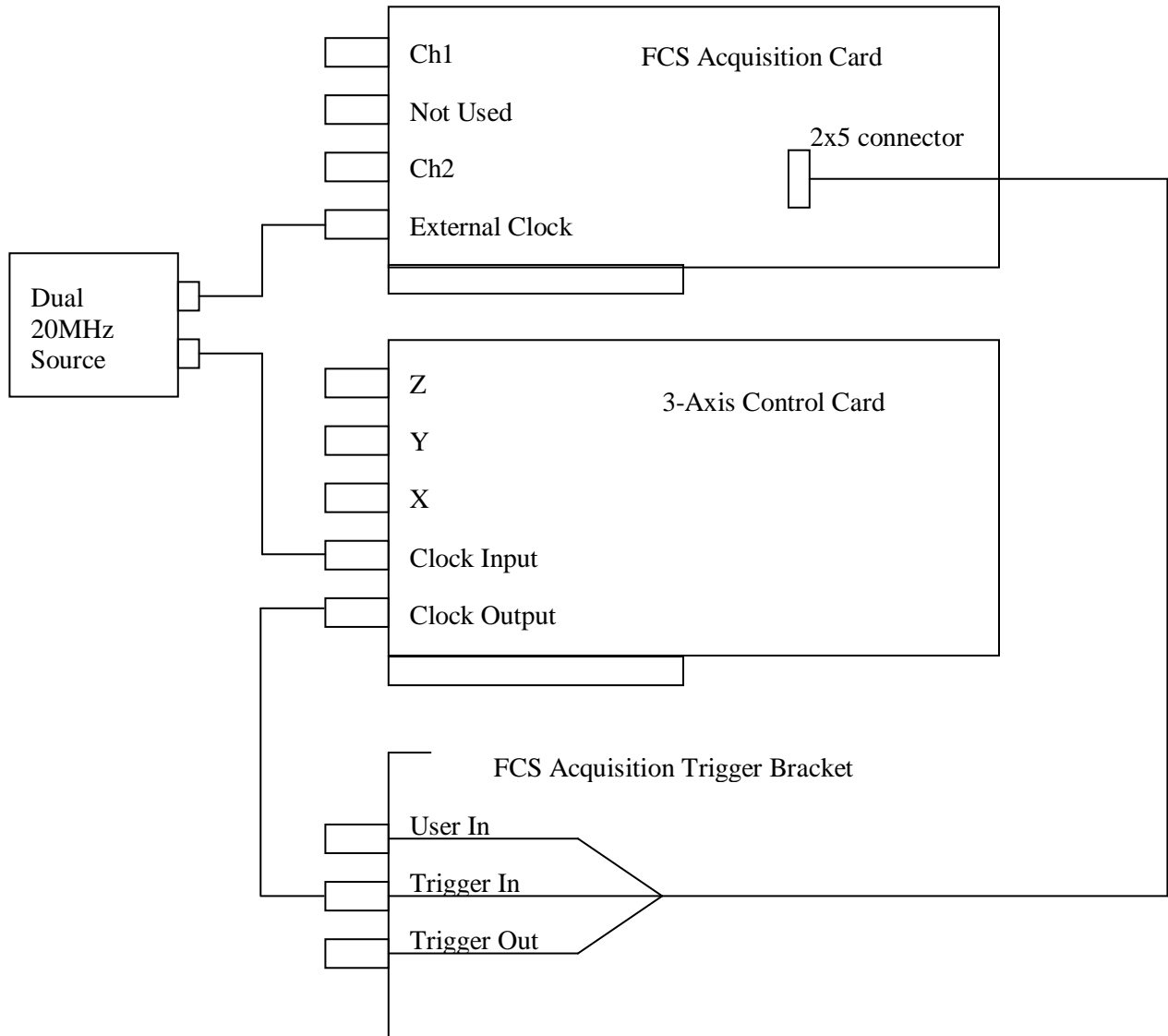
1. LCD: DOWN = User Mode, UP = Calibration Mode
2. LCD: Bottom Line Information Switch
3. Encoder Type for XY: UP = Rotary, DOWN = Linear
4. See Chart below.
5. See Chart below.
6. Command Encoder Knob: if UP, clockwise = down; if DOWN, clockwise = up
7. Joystick Y deflect: if UP, normal Cartesian plane; if DOWN, reverse Cartesian plane
8. Joystick X deflect: if UP, normal Cartesian plane; if DOWN, reverse Cartesian plane

Switch 4	Switch 5	Baud Rate
UP	UP	9600
UP	DOWN	19200
DOWN	UP	28800
DOWN	DOWN	USB Mode

## FLIM and Confocal Image with ASI Stage

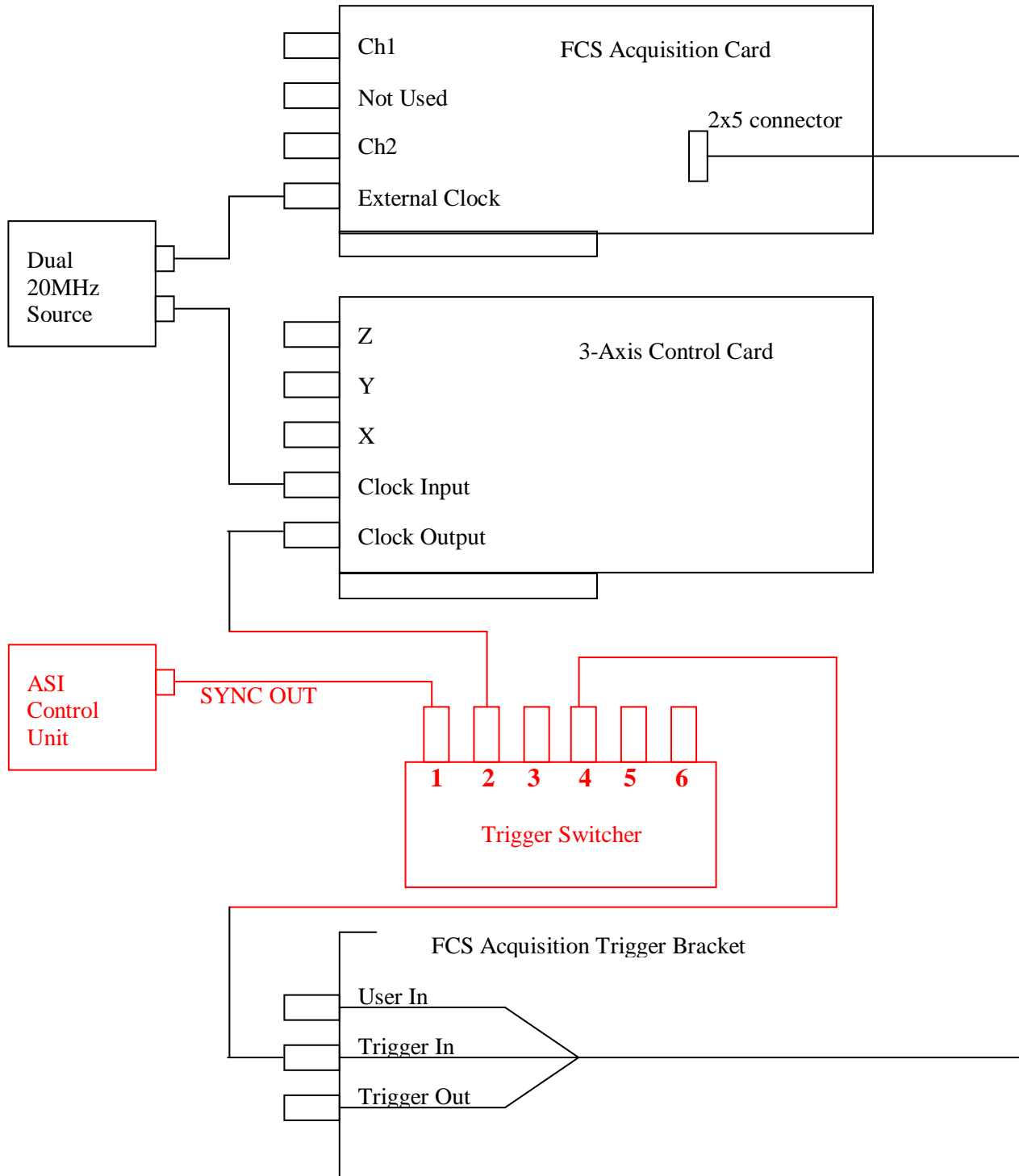
B. Connection from 3-axis trigger and ASI “SYNC OUT” to ISS switcher box.

Original Connection:



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The ASI Control Unit and Trigger Switcher is added as follow:



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### 3. Procedure of Operating the ASI stage in the software

- A. Open the software by double click the icon as follow:

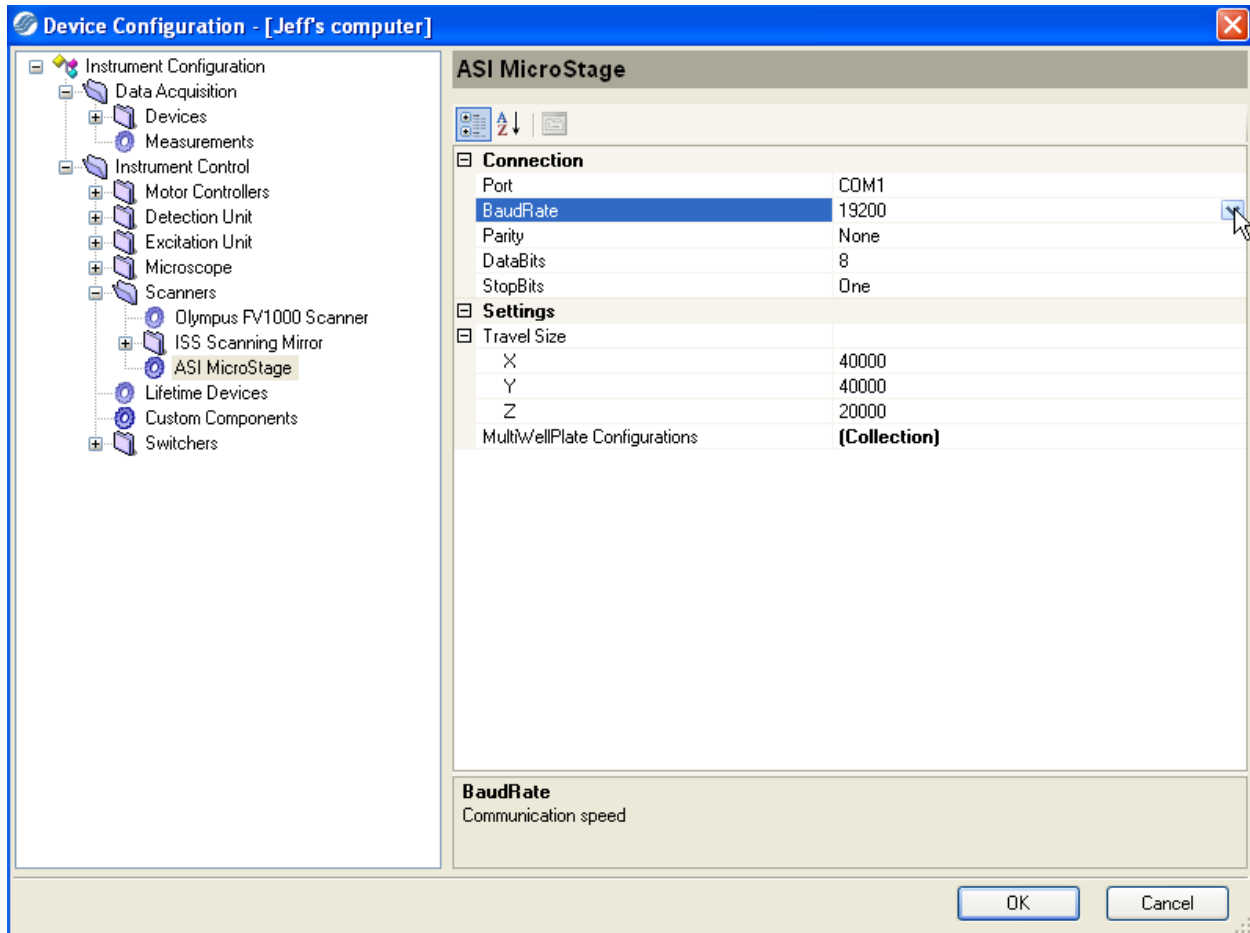


- B. Click "Edit".



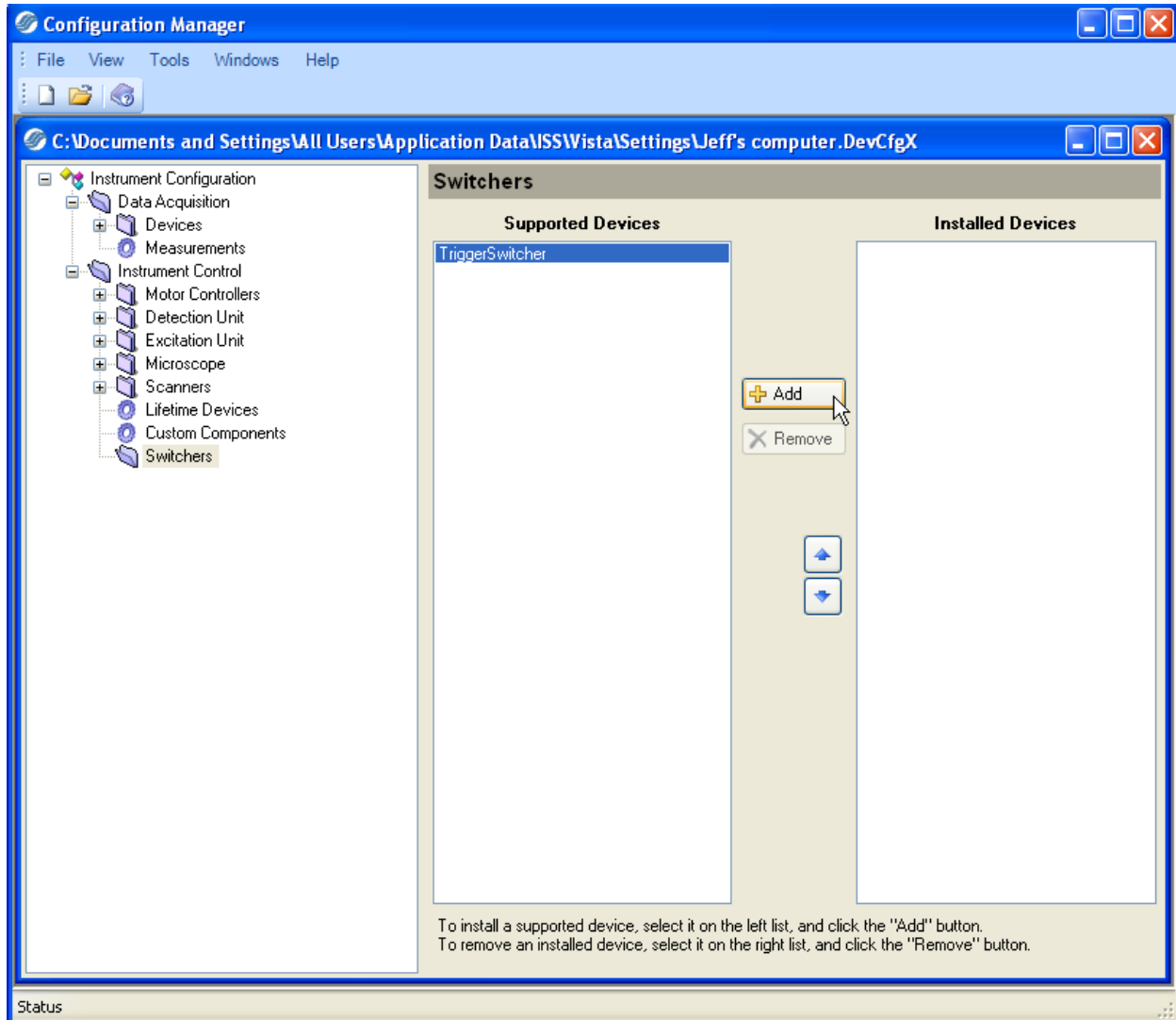
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- C. A window pop up, click on “ASI MicroStage”, check the proper “BaudRate”.



## FLIM and Confocal Image with ASI Stage

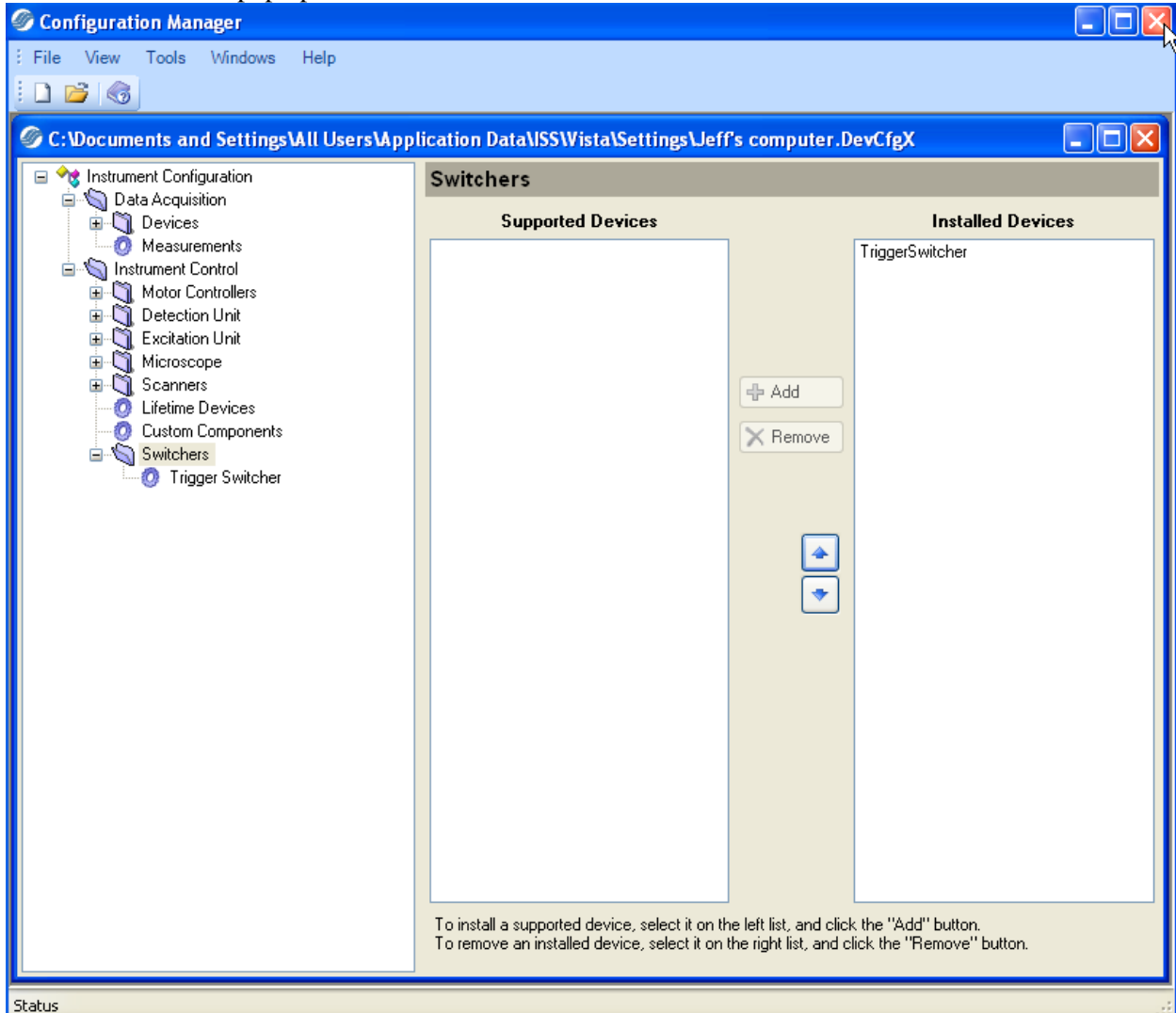
- D. Click on the last “Switchers” in the end of the left side panel. In the right side panel, select “TriggerSwitcher” and click “Add” as follow:



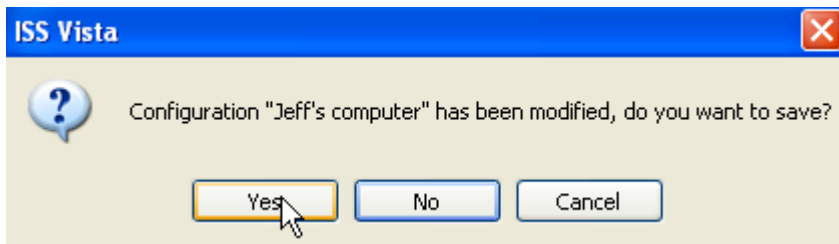
## SUPPORT NOTES

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E. Close the pop up window.



F. Click "Yes" to save the modification.



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- G. Click “Continue”.



- H. As in the following setting:
1. Click “Data Acquisition” in the bottom tab.
  2. Under “Acquisition Settings”, in “Scanner”, select “ASI MicroStage”.
  3. Under “Acquisition Settings”, in “Sync”, select “Line”.
  4. Under “Acquisition Settings”, in “PixelTime(ms)”, select “20”.
  5. Under “Acquisition Settings”, Choose 512 x 512 pixels, range from -20,000 $\mu$ m to +20,000 $\mu$ m. Keep Z axis in the center as 50 $\mu$ m location (range from 0 to 100 $\mu$ m for Z axis).
  6. Click “Start”.

In the above setting, we can calculate the speed for the stage is:

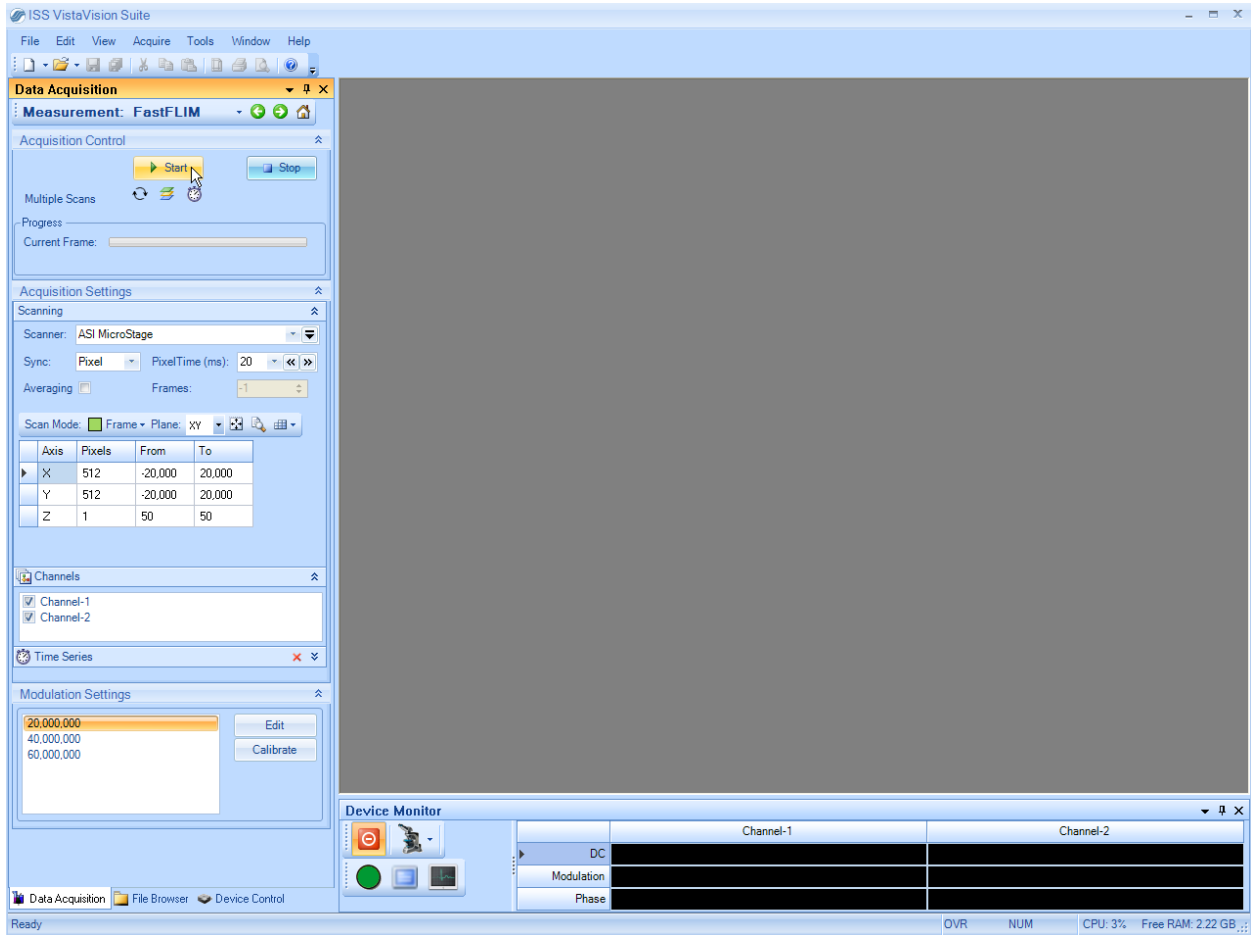
$$40\text{mm}/(512*20\text{ms})=3.91\text{mm/sec}$$

The resolution is  $40\text{mm}/512\text{pixels}=78.13\mu\text{m/pixel}$

The total time that needs to finish the image is  $512*512*20\text{ms}*3$  (for 3 different frequencies) \*2 (the speed for the stage come back is 5mm/sec), which is about 82minutes.

Note: The range for proper ASI to operate is between 10 $\mu$ m/sec to 5mm/sec.

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## FLIM and Confocal Image with ASI Stage

### 4. Results

The following pollen images were measured under the following setup:

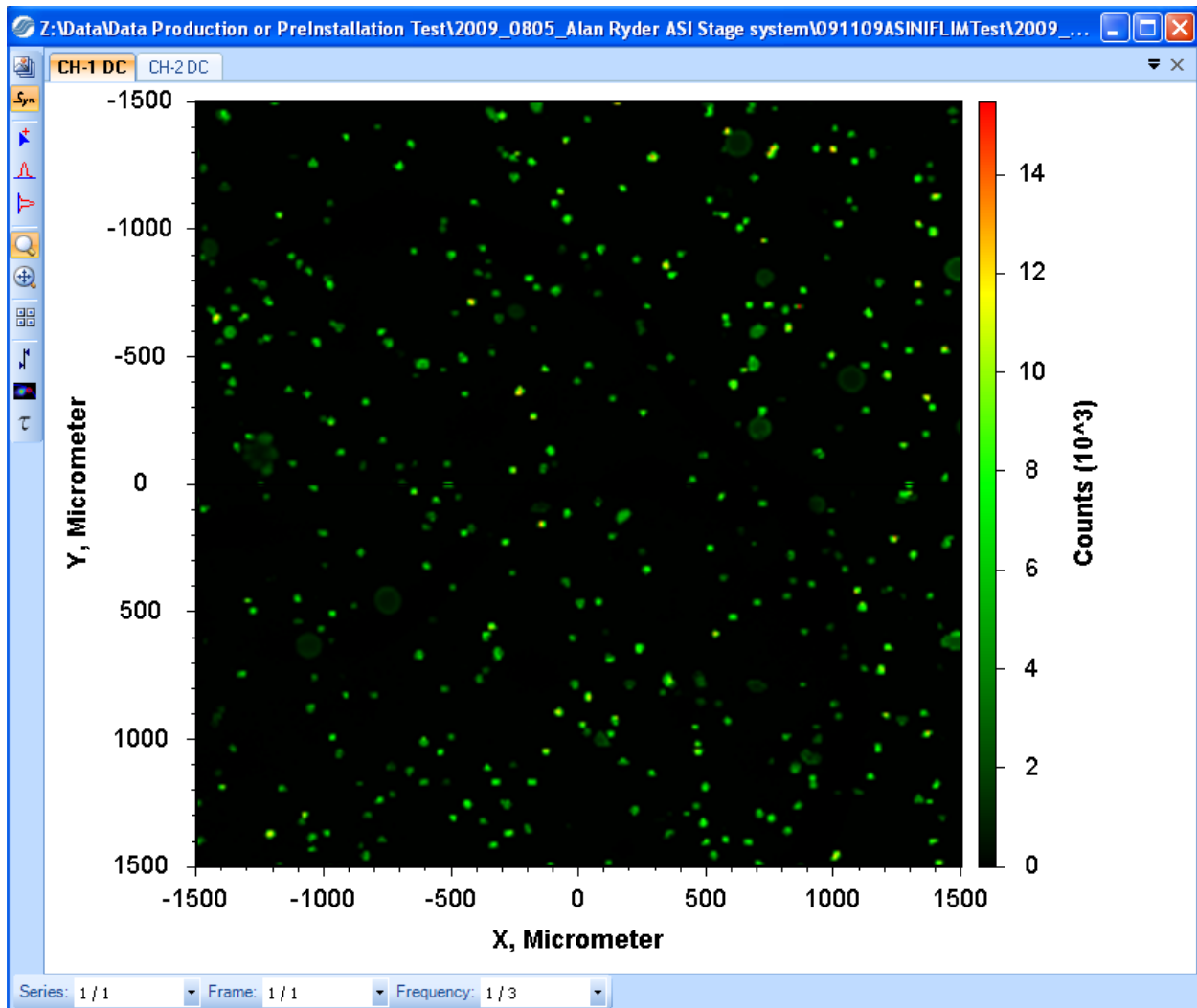
470nm laser.

60x air objective, NA 0.7.

141 $\mu$ m pinhole.

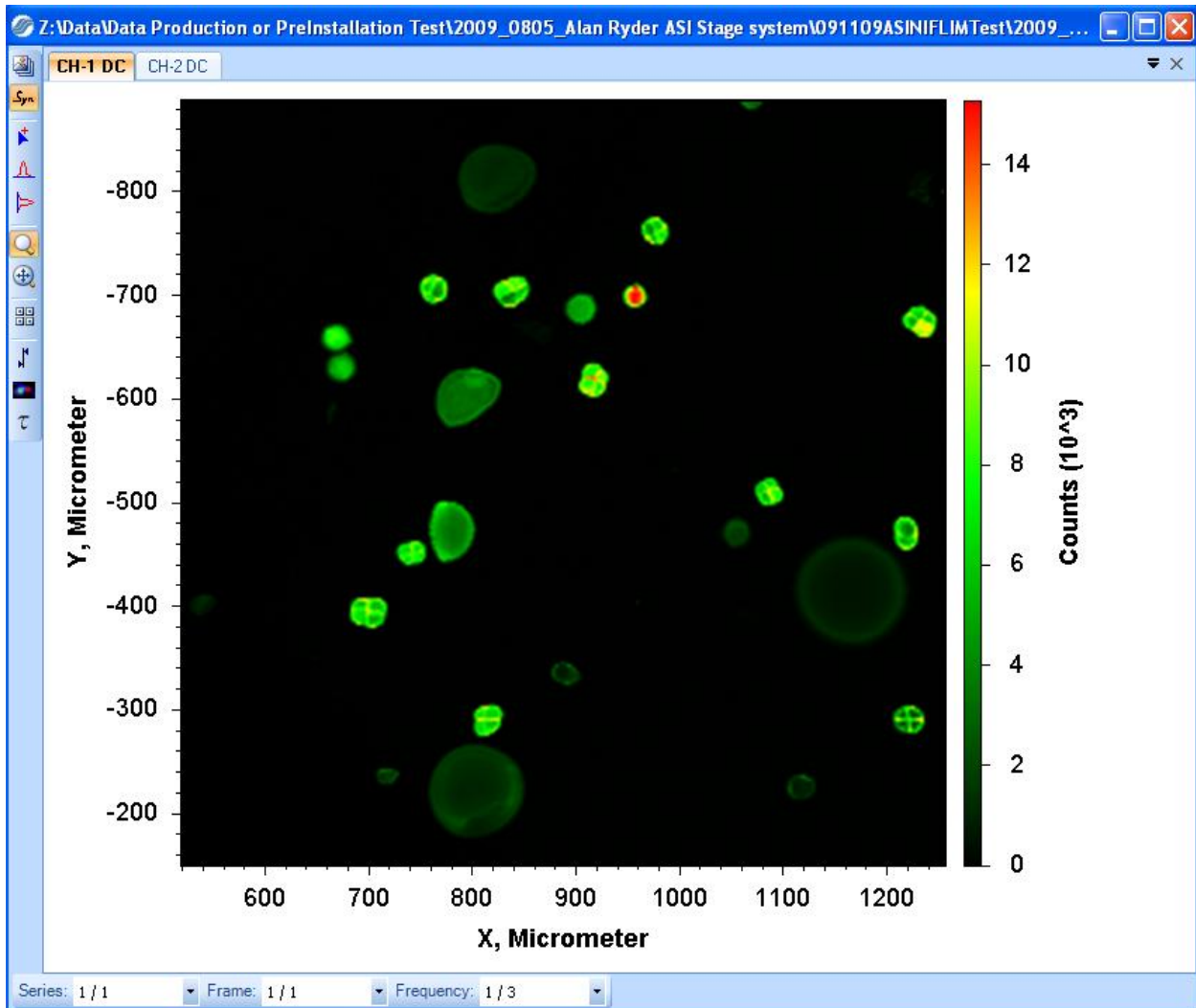
Hamamatsu R928PMT.

First, 3mm by 3mm range had been measured as follow:



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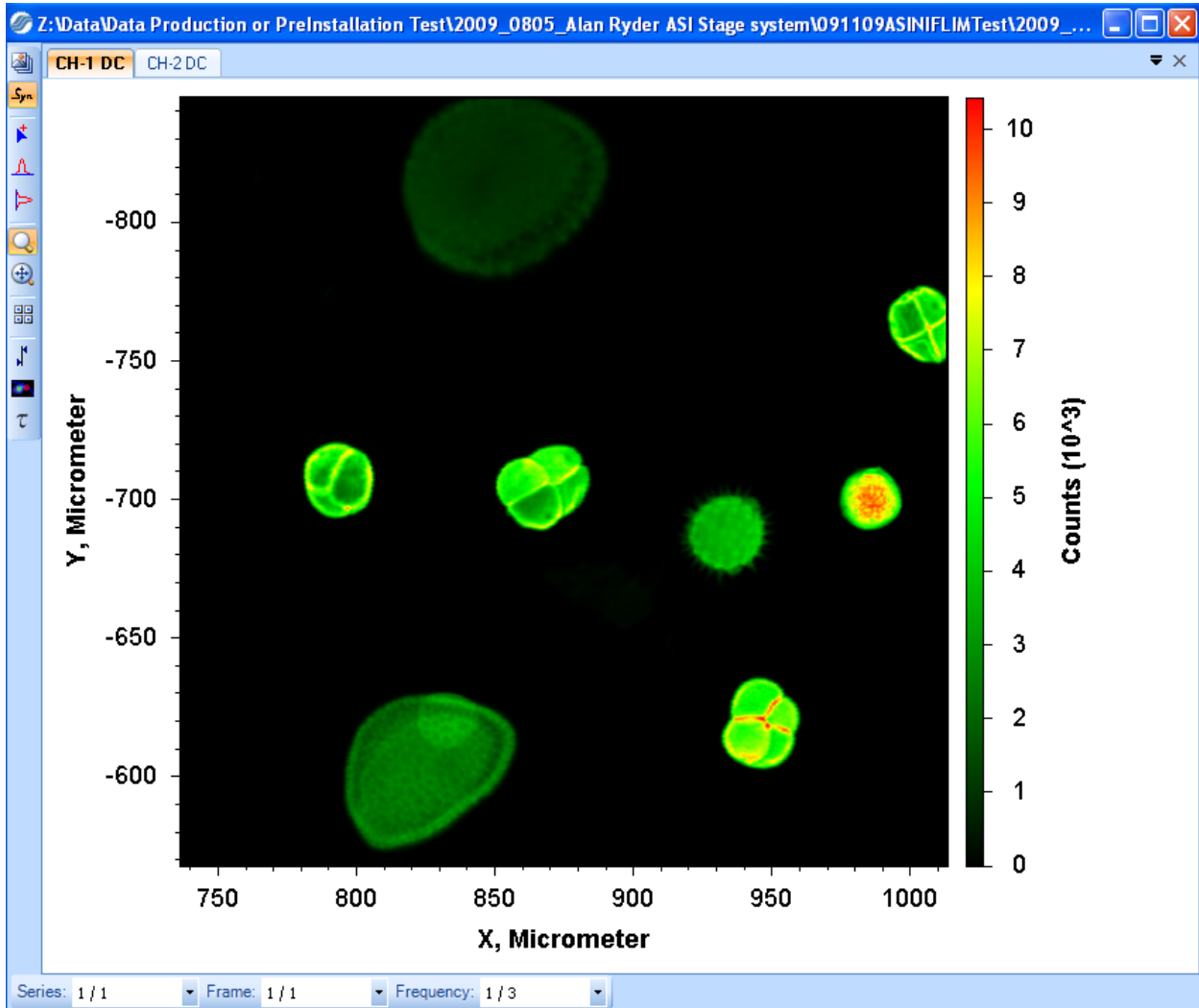
Second, 738 $\mu$ m x 738 $\mu$ m range had been measured as follow:



SUPPORT NOTES

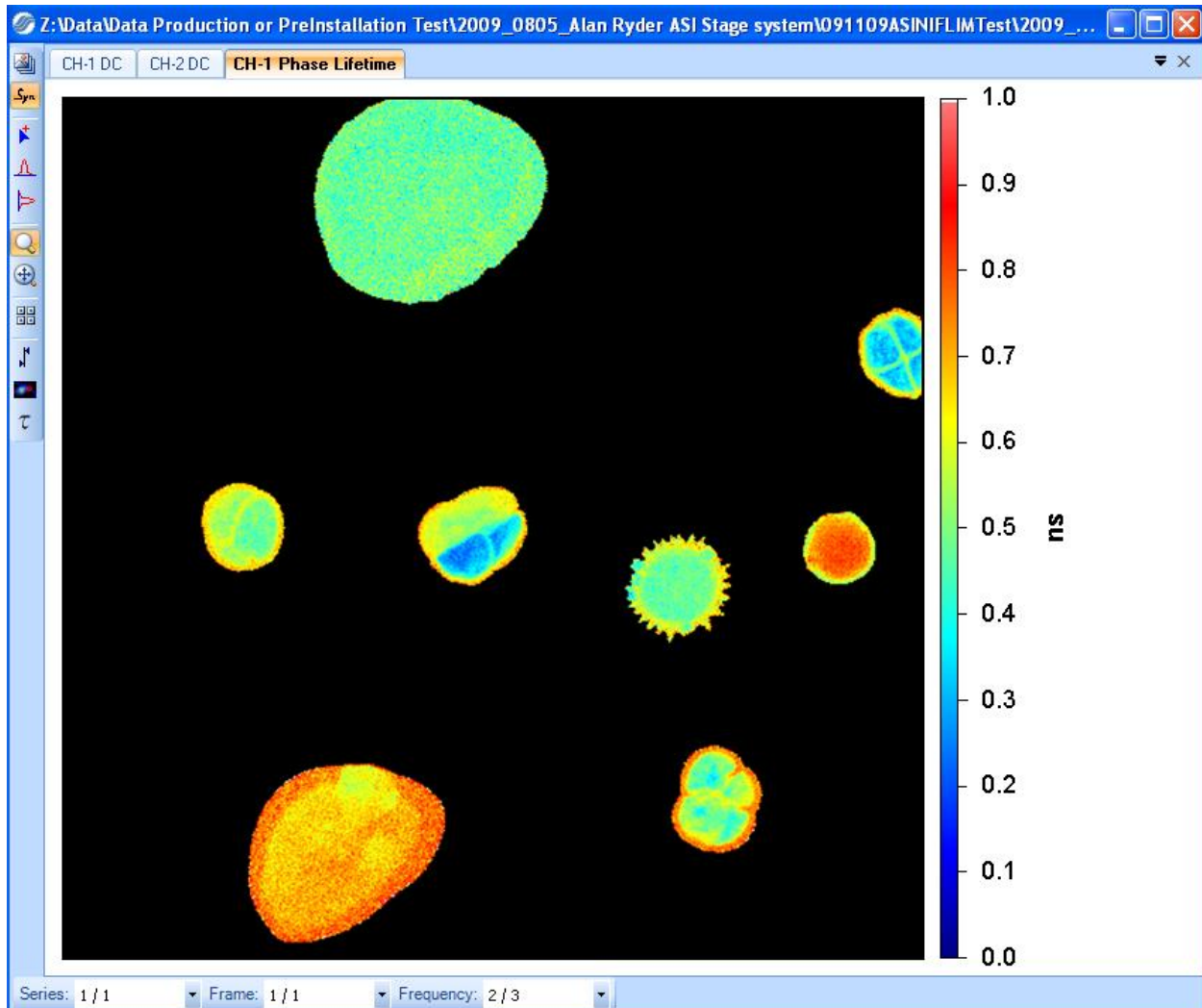
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Third, 277 $\mu$ m x 277 $\mu$ m range had been measured as follow:



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For the lifetime image of the above measurement, presenting using 85MHz as follow:



### 5. Summary and Comments

The ASI stage is capable to measure large scale image (20mm x 20mm) with very fine resolution (1 $\mu$ m).

For more information please call (217) 359-8681  
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SUPPORT NOTES

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