

ImageStream^x™ Spatial Offset Procedure



May, 2009



ImageStreamXTM Spatial Offset Procedure

Rev. X1 5/27/09

SPATIAL OFFSET PROCEDURE



Part Number: 782-02330-01
Current Revision: X3

Revision History:

Rev	Revision Description	Author	Date	ECO	Initials
X1	Initial Release	M. Thullbery	5/27/09		
X2	Reformatted	D.Meissner	01/11/09		
X3	Filter stack screw locations corrected	J. Clynes	07/2011		
X4	Added Illumination Selection for Channel 3 and 7	T. Lam	11/17/11		

Initial Release Approval Signatures:

Engineer _____

VP R&D _____

COO

1. Purpose

This document defines a method of adjustment for Spatial Offset Calibration in ASSIST page. This procedure is to be used in the event the spatial offset calibration fails. This procedure is for the ImageStreamX™ instruments and is developed for internal use by Amnis engineers and technicians to ensure optimum performance of the ImageStreamX™ instruments.

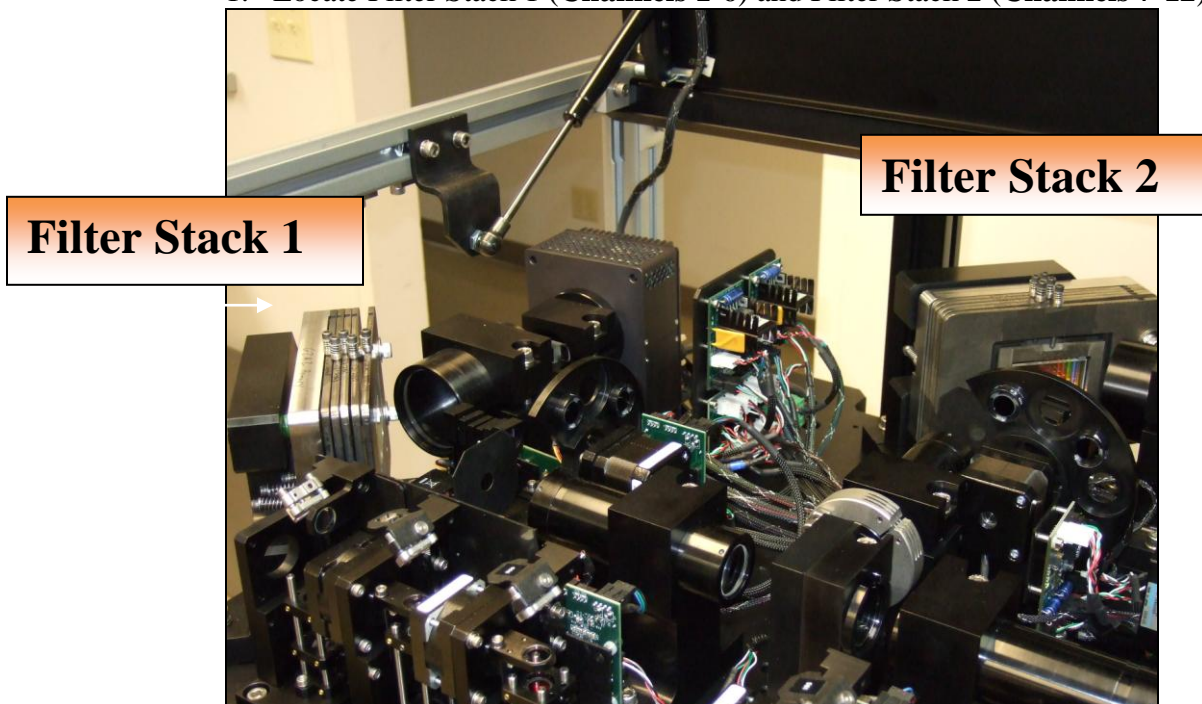
2. Overview

The Spatial Offset Procedure will involve the following steps:

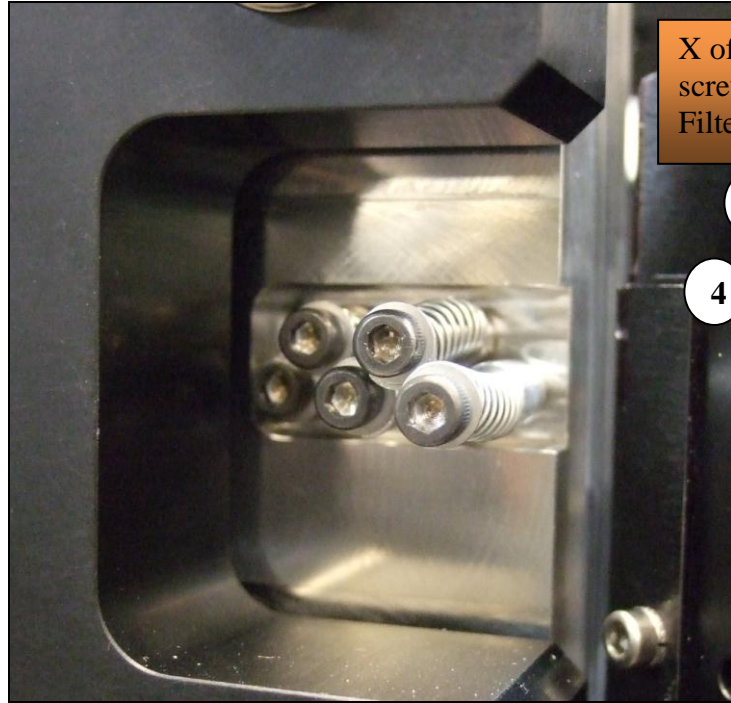
- A. Locate X and Y offset alignment screws on Filter Stack 1 (Channels 1-6) and Filter Stack 2 (Channels 7-12) (If Installed)**
- B. Run Spatial Offset Calibration from ASSIST tab in INSPIRE**
- C. Adjust offset alignment screws.**

A. Locate X and Y offset alignment screws on Filter Stack 1 and Filter Stack 2 (if installed)

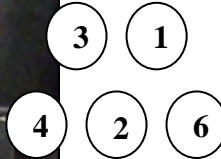
1. Locate Filter Stack 1 (Channels 1-6) and Filter Stack 2 (Channels 7-12)



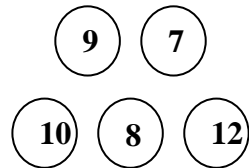
Locate X offset alignment screws and note unique position of 6 & 12



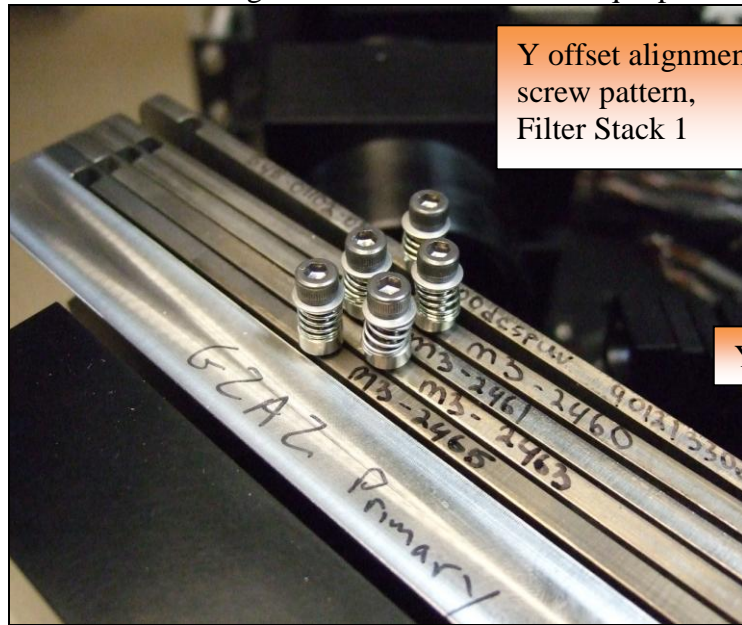
X offset alignment
screw pattern,
Filter Stack 1



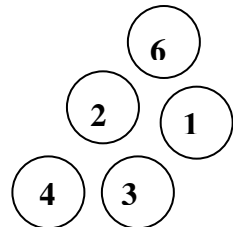
X offset alignment
screw pattern,
Filter Stack 2



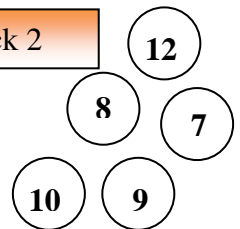
Locate Y offset alignment screws and note unique positions of 6 & 12



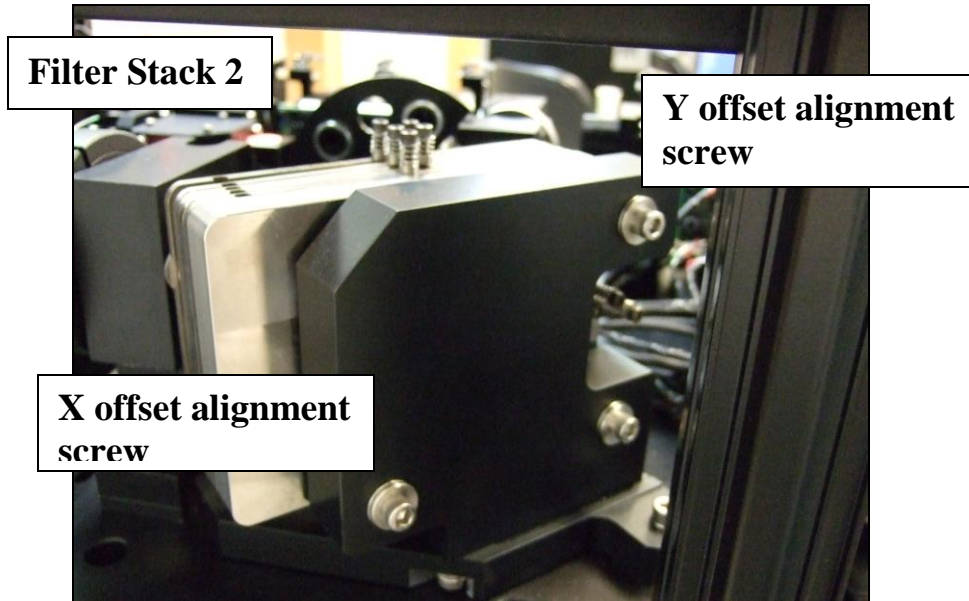
Y offset alignment
screw pattern,
Filter Stack 1



Y, Filter Stack 2



Locate the X & Y offset alignment screws for **channel 11** on Filter Stack 2
(note: channel 5 is fixed).



B. Run Spatial Offset Calibration from ASSIST tab in INSPIRE

From ASSIST tab within INSPIRE, click the Spatial Offsets Calibration

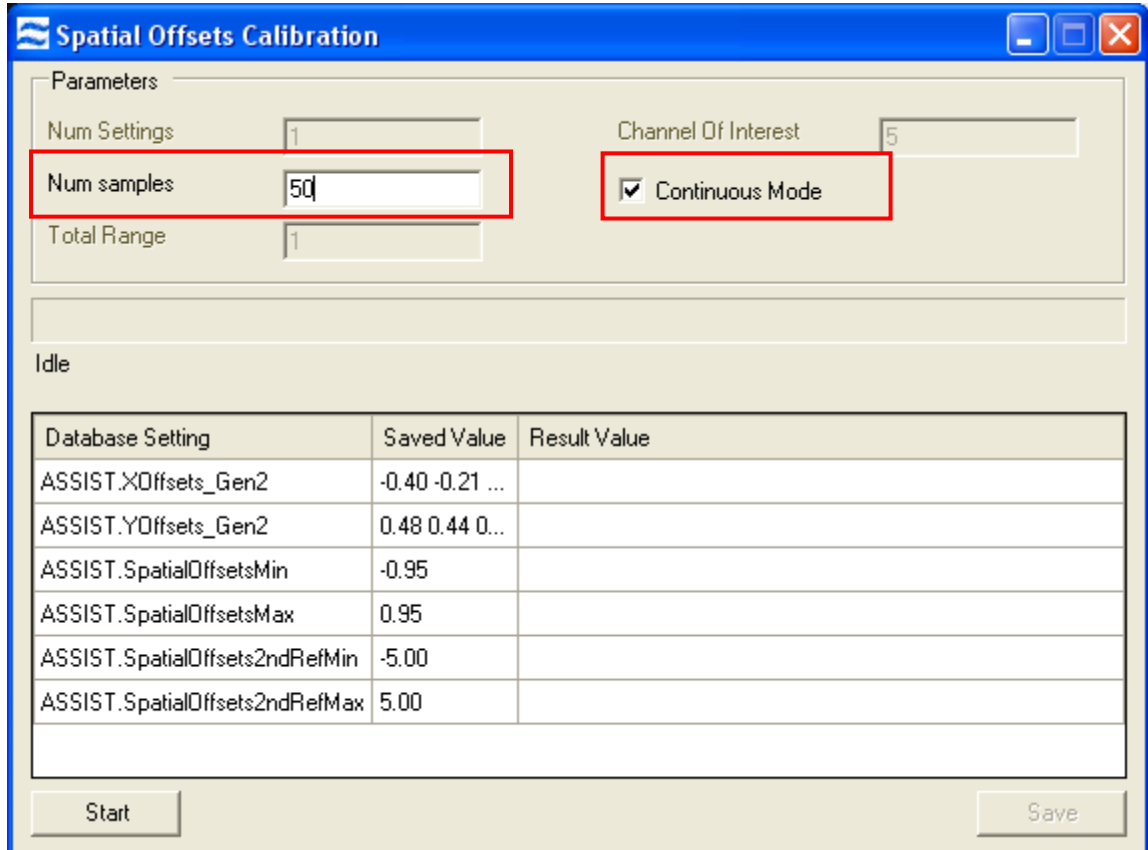
The screenshot shows the INSPIRE software interface with the ASSIST tab selected. The 'Spatial Offsets Calibration' dialog box is open, displaying parameters for the calibration. The 'Spatial Offsets Calibration' entry in the list on the right is highlighted with a red box.

Calibration	Last Run Time
Camera Synchronization Calibration	5/22/2009 11:56:57 AM
Spatial Offsets Calibration	5/22/2009 12:17:52 PM
Dark Current Calibration	5/22/2009 11:59:37 AM
Brightfield Gain Calibration	5/22/2009 11:55:35 AM
Autofocus Z Curve Calibration	5/22/2009 12:16:13 PM
405 Horizontal Laser Calibration	5/22/2009 12:08:39 PM
488 Horizontal Laser Calibration	5/22/2009 2:21:03 PM
561 Horizontal Laser Calibration	5/22/2009 12:03:59 PM
594 Horizontal Laser Calibration	5/22/2009 12:03:01 PM
658 Horizontal Laser Calibration	5/22/2009 12:06:35 PM
765 Horizontal Laser Calibration	5/22/2009 12:07:25 PM
Side Scatter Calibration	5/22/2009 12:07:59 PM

Test	Last Run Time
405nm Laser Power Test	5/22/2009 12:08:22 PM
488nm Laser Power Test	5/22/2009 12:08:08 PM
561nm Laser Power Test	5/22/2009 12:08:40 PM
594nm Laser Power Test	5/22/2009 12:10:11 PM
658nm Laser Power Test	5/22/2009 12:10:42 PM
765nm Laser Power Test	5/22/2009 12:11:14 PM
Brightfield Intensity Selection Test	5/22/2009 12:12:38 PM
Brightfield Uniformity Test	5/22/2009 12:13:04 PM
Flow Core Axial Stability Test	5/22/2009 12:13:38 PM
Flow Core Lateral Stability Test	5/22/2009 12:13:56 PM
Flow Core Position Test	5/22/2009 12:14:15 PM
Focus Offset Test	5/22/2009 12:15:38 PM
Image Quality Encouped Energy Test	5/22/2009 12:15:35 PM

Utilities	Last Run Time
Brightfield Calibration Utility	5/21/2009 2:33:31 PM
EDF Excitation Utility	
Focus Plan Utility	
PMT Focus Utility	5/18/2009 11:48:53 AM
SCurve Peaks Utility	

Make sure settings in Spatial Offsets Calibration are as follows: “Continuous Mode” box checked, and “Num samples” set to 50.



Spatial Offsets Calibration

Parameters

Num Settings: 1

Channel Of Interest: 5

Num samples: 50

Continuous Mode:

Total Range: 1

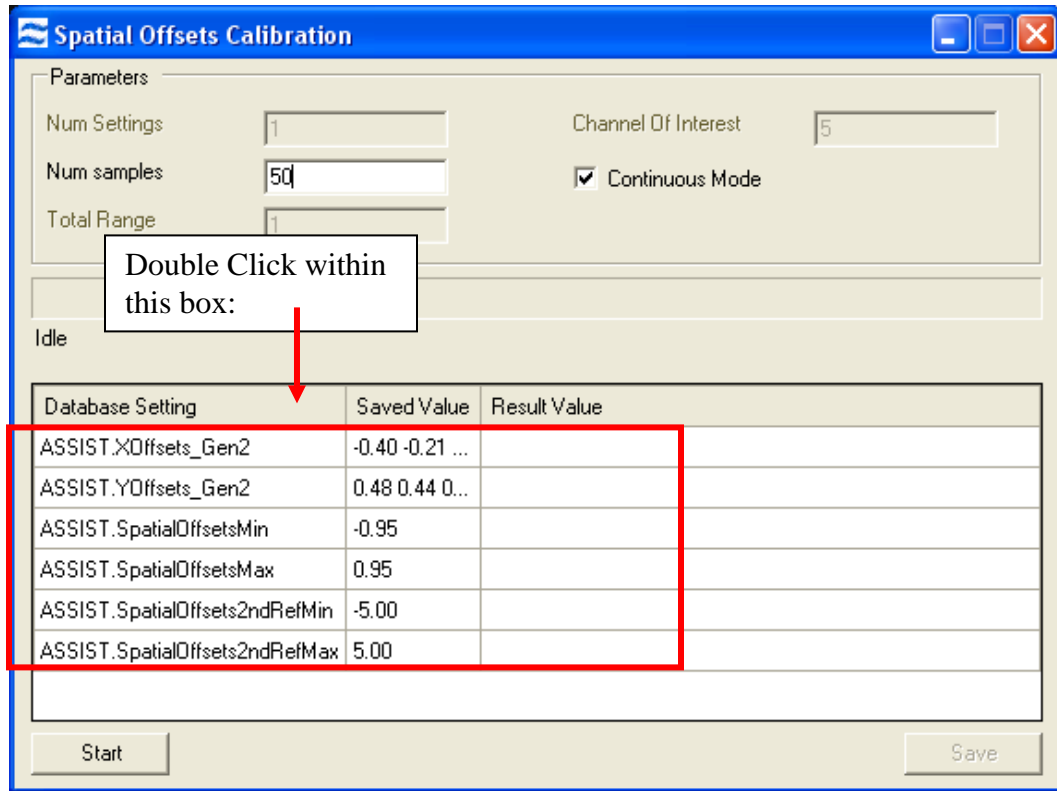
Idle

Database Setting	Saved Value	Result Value
ASSIST.XOffsets_Gen2	-0.40 -0.21 ...	
ASSIST.YOffsets_Gen2	0.48 0.44 0...	
ASSIST.SpatialOffsetsMin	-0.95	
ASSIST.SpatialOffsetsMax	0.95	
ASSIST.SpatialOffsets2ndRefMin	-5.00	
ASSIST.SpatialOffsets2ndRefMax	5.00	

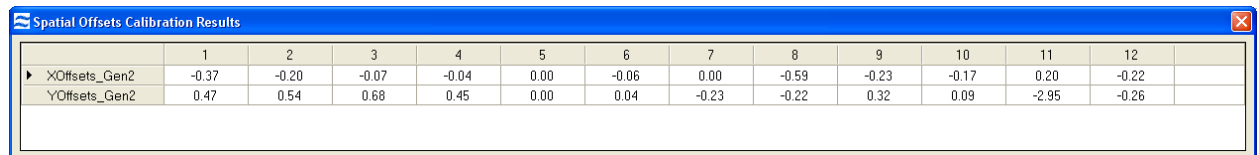
Start Save

Click “Start”

1. Double click on any of the Database Settings in the Spatial Offsets Calibration window to open the **Spatial Offsets Calibration Results** window.



To open this Window:



	1	2	3	4	5	6	7	8	9	10	11	12
XOffsets_Gen2	-0.37	-0.20	-0.07	-0.04	0.00	-0.06	0.00	-0.59	-0.23	-0.17	0.20	-0.22
YOffsets_Gen2	0.47	0.54	0.68	0.45	0.00	0.04	-0.23	-0.22	0.32	0.09	-2.95	-0.26

The Spatial Calibration Results window should now be updating with values in each channel and X and Y position

C. Adjust offset alignment screws

1. While monitoring window adjust X and Y spatial offset screws located in steps above (numbers on screw positions correspond with channels) to achieve values of less than 0.2. The adjustment is very sensitive so small adjustments are needed. The spec is a value of less than 0.5 (note: Channel 11 spec is less than 2).

The screenshot shows the 'Spatial Offsets Calibration' dialog box with the following parameters:

- Num Settings: 1
- Num samples: 50
- Total Range: 1
- Channel Of Interest: 5
- Continuous Mode:

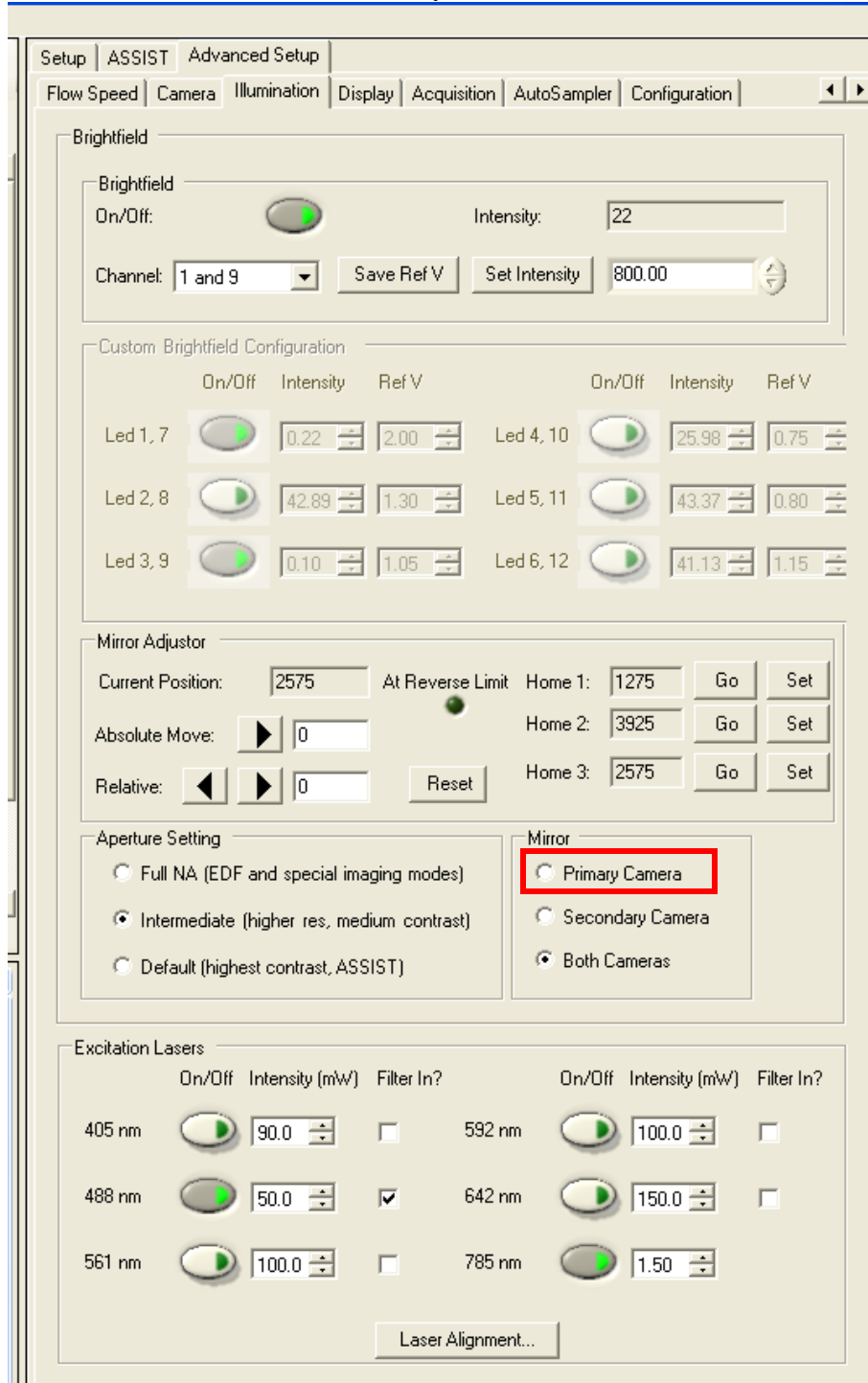
The 'Collecting Samples' progress bar is partially filled. Below the dialog, the 'Spatial Offsets Calibration Results' table is visible:

	1	2	3	4	5	6	7	8	9	10	11	12
XOffsets_Gen2	-0.37	-0.17	-0.05	-0.06	0.00	-0.11	-0.01	-0.56	-0.23	-0.15	0.23	-0.25
YOffsets_Gen2	0.44	0.48	0.61	0.39	0.00	0.01	-0.24	-0.22	0.32	0.08	-1.46	-0.32

Following adjustment of screws to within spec, click “Stop” and then “Save”.

The 'Spatial Offsets Calibration' dialog box is shown with the 'Stop' and 'Save' buttons highlighted in red boxes. The parameters are the same as in the previous screenshot.

2. With 12-channel systems, to align channel 3, go to Advanced Setup, Illumination, Mirror, select Primary Camera.



The screenshot shows the 'Advanced Setup' window with the following sections:

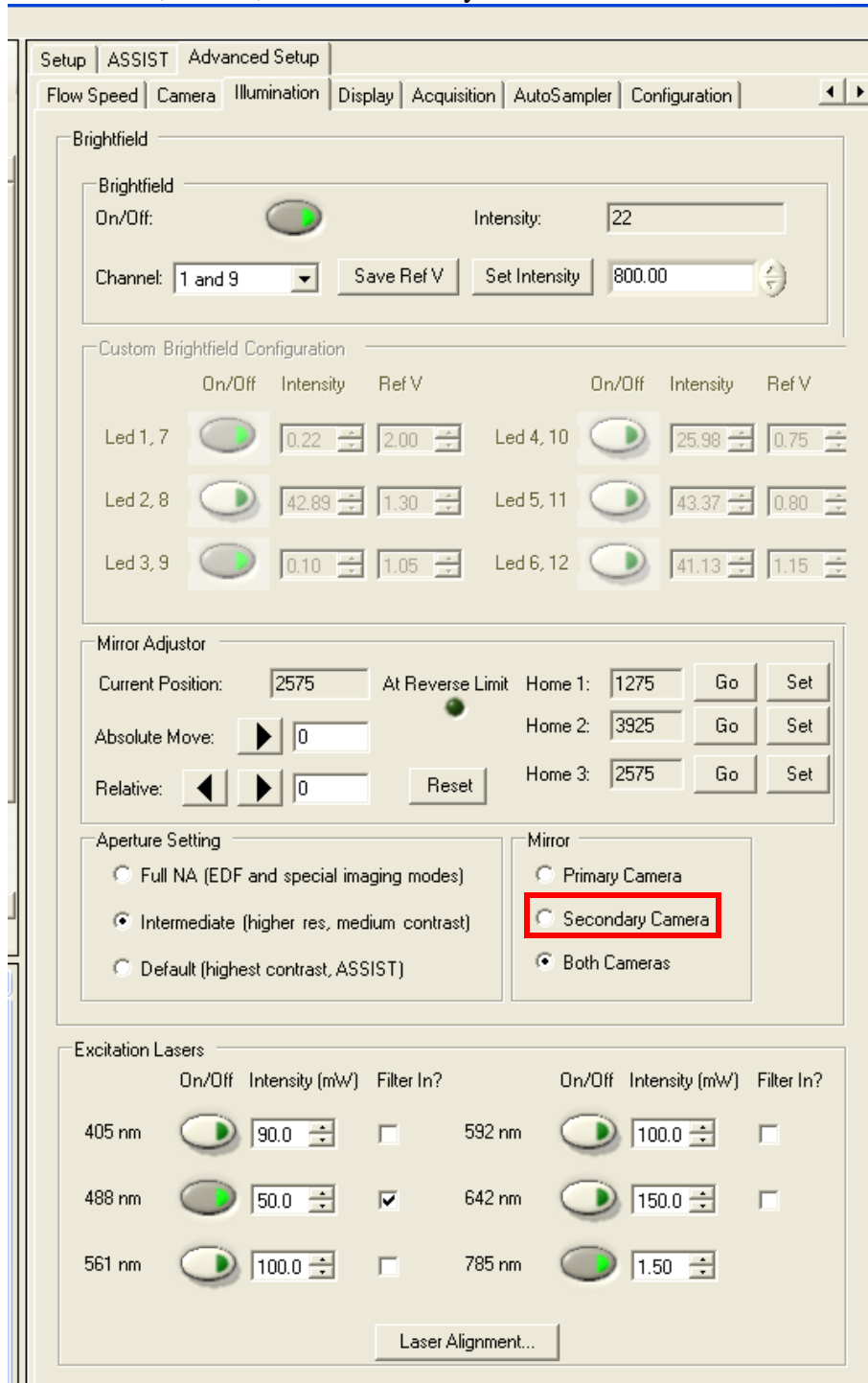
- Brightfield:** On/Off: Intensity: 22. Channel: 1 and 9. Buttons: Save Ref V, Set Intensity 800.00.
- Custom Brightfield Configuration:**

	On/Off	Intensity	Ref V		On/Off	Intensity	Ref V
Led 1, 7	<input checked="" type="radio"/>	0.22	2.00	Led 4, 10	<input checked="" type="radio"/>	25.98	0.75
Led 2, 8	<input checked="" type="radio"/>	42.89	1.30	Led 5, 11	<input checked="" type="radio"/>	43.37	0.80
Led 3, 9	<input checked="" type="radio"/>	0.10	1.05	Led 6, 12	<input checked="" type="radio"/>	41.13	1.15
- Mirror Adjustor:** Current Position: 2575. At Reverse Limit: Home 1: 1275 (Go, Set). Absolute Move: 0. Home 2: 3925 (Go, Set). Relative: 0. Home 3: 2575 (Go, Set). Reset button.
- Aperture Setting:**
 - Full NA (EDF and special imaging modes)
 - Intermediate (higher res, medium contrast)
 - Default (highest contrast, ASSIST)
- Mirror:**
 - Primary Camera (highlighted with a red box)
 - Secondary Camera
 - Both Cameras
- Excitation Lasers:**

	On/Off	Intensity (mW)	Filter In?		On/Off	Intensity (mW)	Filter In?
405 nm	<input checked="" type="radio"/>	90.0	<input type="checkbox"/>	592 nm	<input checked="" type="radio"/>	100.0	<input type="checkbox"/>
488 nm	<input checked="" type="radio"/>	50.0	<input checked="" type="checkbox"/>	642 nm	<input checked="" type="radio"/>	150.0	<input type="checkbox"/>
561 nm	<input checked="" type="radio"/>	100.0	<input type="checkbox"/>	785 nm	<input checked="" type="radio"/>	1.50	<input type="checkbox"/>

Buttons: Laser Alignment...

- 3.** With 12-channel systems, to align channel 7, go to Advanced Setup, Illumination, Mirror, select Secondary Camera.



The screenshot shows the 'Illumination' tab of the software interface. The 'Brightfield' section is active, showing a 'Brightfield On/Off' toggle and an 'Intensity' of 22. Below this is a 'Custom Brightfield Configuration' table with 6 columns: On/Off, Intensity, Ref V, On/Off, Intensity, Ref V. The table lists six LED channels (Leds 1, 7, 2, 8, 3, 9, 4, 10, 5, 11, 6, 12) with their respective On/Off status, Intensity, and Ref V values.

The 'Mirror Adjustor' section shows 'Current Position: 2575', 'At Reverse Limit' (checked), and three 'Home' positions: Home 1: 1275, Home 2: 3925, Home 3: 2575. There are 'Go' and 'Set' buttons for each home position, and a 'Reset' button.

The 'Aperture Setting' section has three radio buttons: 'Full NA (EDF and special imaging modes)', 'Intermediate (higher res, medium contrast)' (selected), and 'Default (highest contrast, ASSIST)'. The 'Mirror' section has three radio buttons: 'Primary Camera', 'Secondary Camera' (selected and highlighted with a red box), and 'Both Cameras'.

The 'Excitation Lasers' section has a table with 6 columns: On/Off, Intensity (mW), Filter In?, On/Off, Intensity (mW), Filter In?. The table lists six laser channels (405 nm, 488 nm, 561 nm, 592 nm, 642 nm, 785 nm) with their respective On/Off status, Intensity, and Filter In? status. A 'Laser Alignment...' button is located at the bottom of this section.