

# Beam Alignment Tool for Fluorescence-Guided Electrophysiology System

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## Introduction

The ability to examine neuronal signal propagation deep inside the brain is crucial in elucidating mechanisms of neurological function, including perception, attention, and memory [1]. Current devices for high resolution neuronal recordings face limitations when attempting to record at increased depths due to light attenuation in tissue [2,3]. The Barbara Smith Lab developed the Fluorescence-Guided Electrophysiology (FGE) system to overcome this barrier through the use of fluorescent detection [4].

Due to the utilization of various optics in the FGE system, there remains a **periodic need for realignment** of the system due to mechanical perturbations which lead to low signal-to-noise ratios which affect the system's detection capabilities.

## Hardware Setup

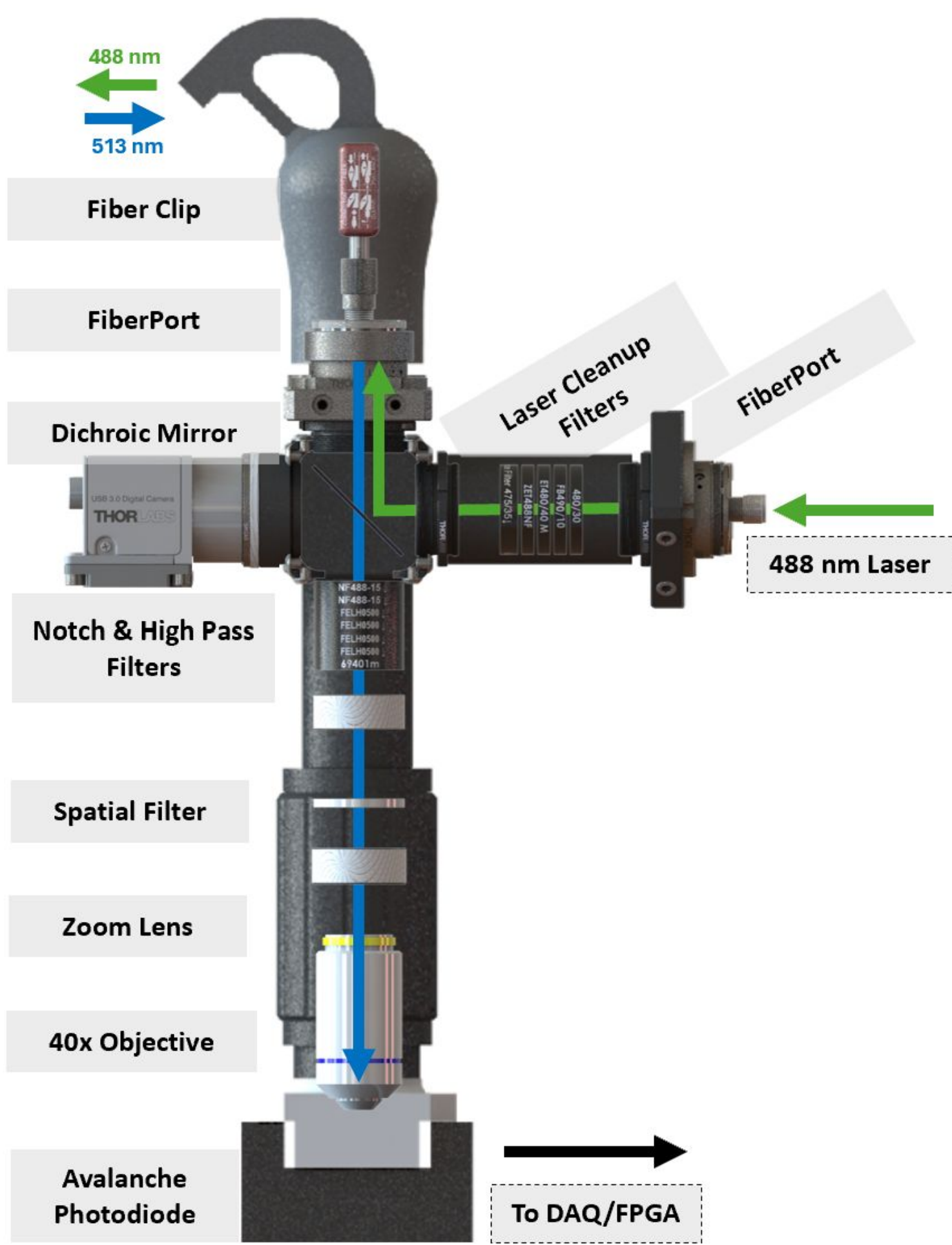


Figure 1: FGE System Architecture. Excitation light from a 488 nm laser is cleaned up via spectral filters and coupled to the optical fiber.

## Software Setup

A MATLAB app was created to equip the laser operator with the knowledge and means of correcting beam misalignments.

### Calibrated Image Collection:

1. Calibrate system optimizing photon count reaching APD.
2. Take calibrated images every time a change is made to the system.
  - Set up system per test setup.
  - Take first image at the 0 mm extension length of the zoom lens.
  - Take next image at the 5 mm extension length of the zoom lens.

### Calibrated Laser Characterization:

1. Run calibration portion of software to characterize calibrated beam.

### Experimental Image Collection:

1. Take experimental images before testing. Repeat previous image collection procedure.

### Misalignment Correction:

1. Run experimental portion of software to characterize experimental beam and determine next steps.

## Realignment Reference Equations

### X/Y Realignment:

Software determines how far and in which direction the image 1 experimental data is in comparison to the image 1 calibrated data. This information is utilized to decide which screw rotations are necessary to reach realignment.

Screw Rotation	X Movement	Y Movement
0.25 X	-653.20	-163.08
-0.25 X	+336.75	+111.70
0.25 Y	-224.33	+195.36
-0.25 Y	+150.80	-323.15

### Tilt Analysis:

Software determines difference between calibrated image 1 and 2 distance and experimental image 1 and 2 distance. If difference is greater than 50 pixels, Z screws must be tightened.

$$\text{tilt} = \sqrt{(\text{Image 2 Centerpoint X} - \text{Image 1 Centerpoint X})^2 + (\text{Image 2 Centerpoint Y} - \text{Image 1 Centerpoint Y})^2}$$

## Methods

The calibration step characterizes the calibrated beam by finding its center points. The experimental step characterizes the experimental beam by finding its center points and compares them to the calibrated center points. This comparison is quantified and used in the determination of a realignment procedure and tilt analysis. The output directs the operator in coarse system realignment by rotating X, Y, and Z screws.

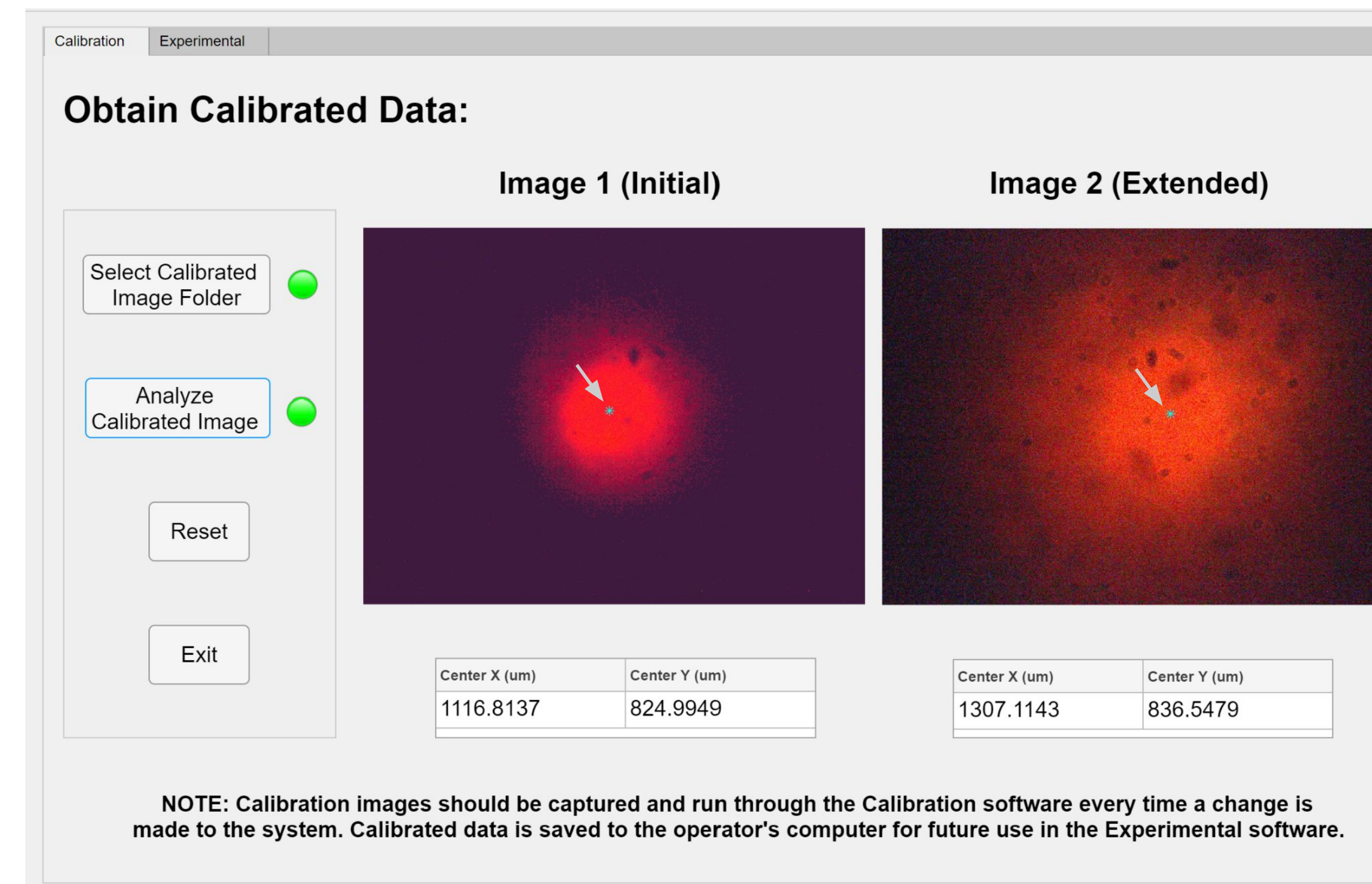


Figure 2: Calibration portion of software, utilized when system changes.

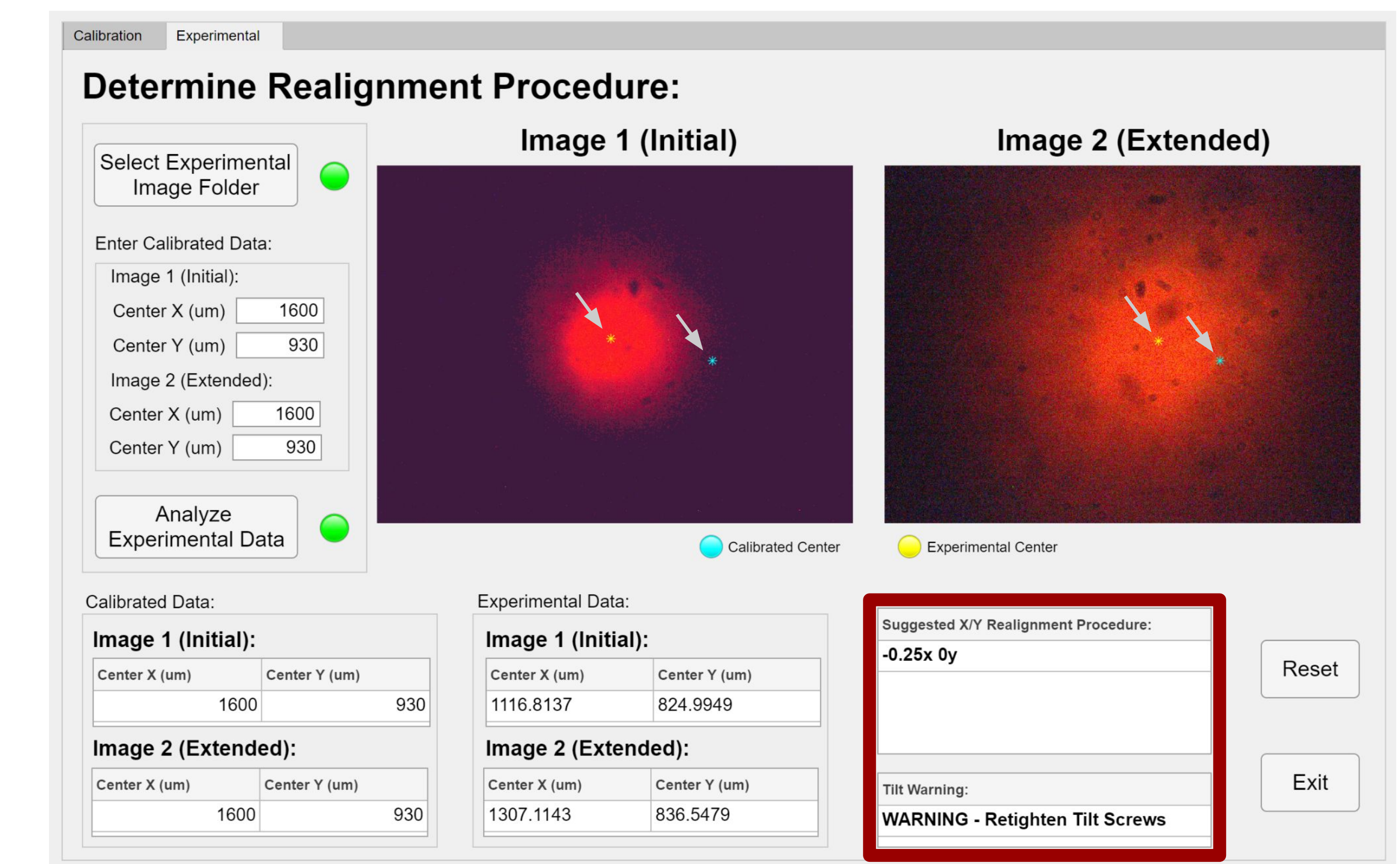


Figure 3: Experimental portion of software, utilized before testing.

## Results

### Center Point Detection Functionality & Accuracy

Image pairs were artificially created to test the software's ability to identify objects of various shapes, colors, and sizes (images not shown). Additionally, image pairs with squares of known center values were created to test the software's ability to accurately determine center point locations. Software passed.

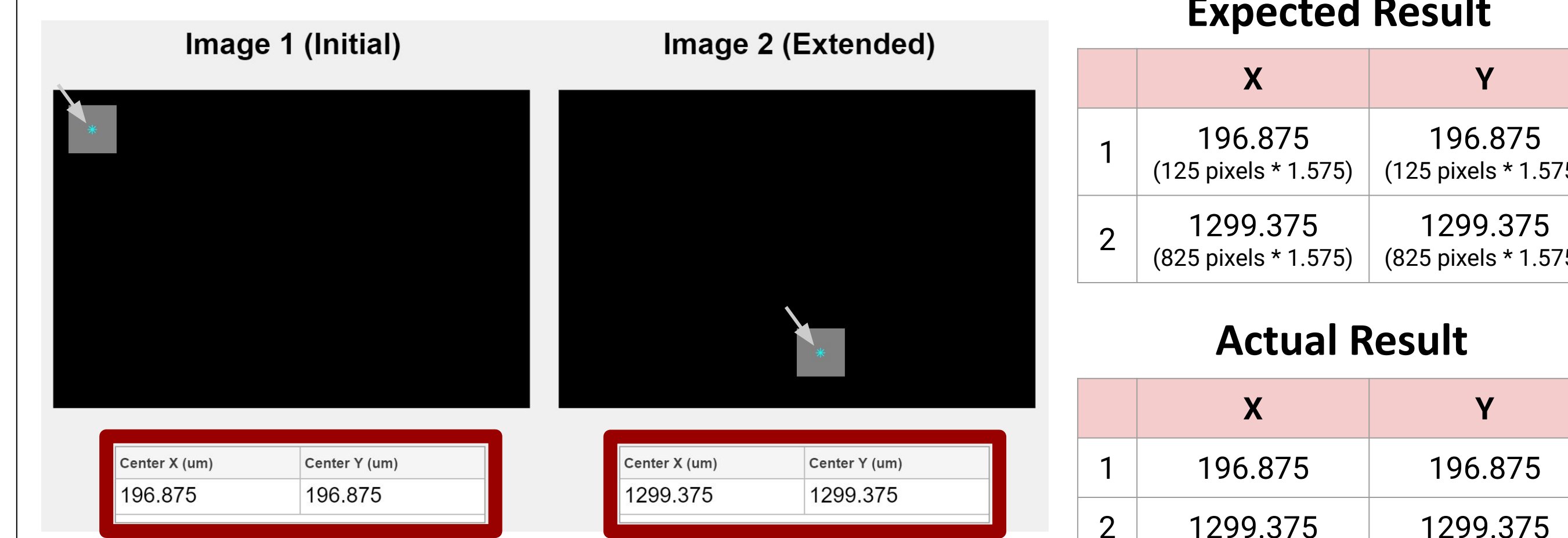


Figure 4: The actual center determined by the software matched the known center, proving the accuracy of the center point detection function.

### X/Y Realignment Procedure & Tilt Warning Accuracy

Image pairs were artificially created with squares of known center values and used as experimental data. Fake data points with known realignment steps and tilt were chosen as calibrated data. This tested the software's ability to accurately determine realignment procedures & tilt warnings. Software passed.

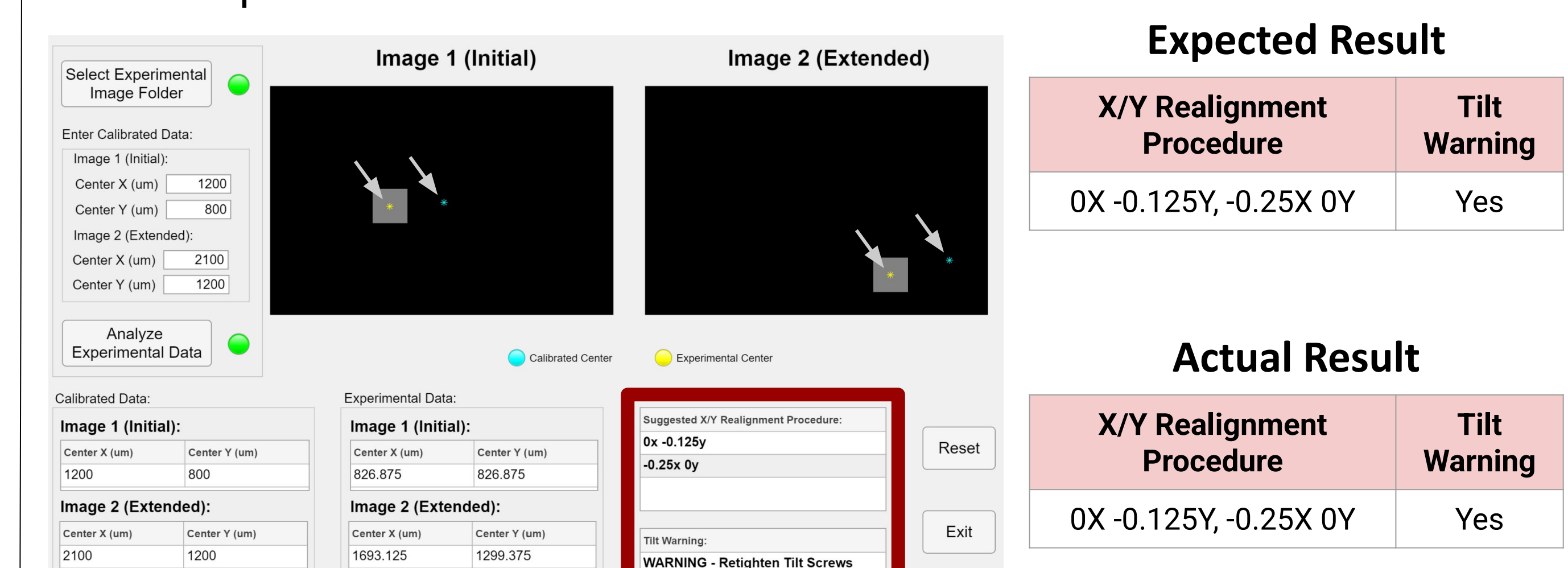


Figure 5: The actual realignment procedure and tilt analysis results matched the known values, proving the accuracy of these functions.

## Conclusion

The optical alignment tool successfully compares experimental and calibrated data to support the realignment of the FGE system. This provides value by allowing for quicker optical fiber replacements, higher consistency in test results, and higher overall throughput of the imaging system.

## References

- [1] Chu, A. (2025, March 11). *Introduction to patch-clamp electrophysiology*. Precisionary. <https://precisionary.com/introduction-to-patch-clamp-electrophysiology/?srsltid=AfmB0orYsCJHzFH0-8BCGGUYbW0WG-FWkATVVZeaEu63dNEsc-62mVly>
- [2] Steinmetz, I. (2009, March 17). *New standard in electrophysiology and deep tissue imaging*. Learn & Share | Leica Microsystems. <https://www.leica-microsystems.com/science-lab/life-science/new-standard-in-electrophysiology-and-deep-tissue-imaging/>
- [3] *Multiphoton microscopy*. Nikon's MicroscopyU. (n.d.). <https://www.microscopyu.com/techniques/multi-photon/multiphoton-microscopy>
- [4] Miranda C; Howell MR; Lusk JF; Marshall E; Eshima J; Anderson T; Smith BS; (n.d.). *Automated Microscope-independent fluorescence-guided micropipette*. Biomedical optics express. <https://pubmed.ncbi.nlm.nih.gov/34513218/>

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