

(a)

SeqAutoCollect.gui

### ROI Selection Tool

0 cell(s) selected from this coverslip  
(hover mouse over a region to view number of cells selected in that region)

1,1	1,2	1,3	1,4	1,5	1,6	1,7	1,8	1,9	1,10
2,1	2,2	2,3	2,4	2,5	2,6	2,7	2,8	2,9	2,10
3,1	3,2	3,3	3,4	3,5	3,6	3,7	3,8	3,9	3,10
4,1	4,2	4,3	4,4	4,5	4,6	4,7	4,8	4,9	4,10
5,1	5,2	5,3	5,4	5,5	5,6	5,7	5,8	5,9	5,10
6,1	6,2	6,3	6,4	6,5	6,6	6,7	6,8	6,9	6,10
7,1	7,2	7,3	7,4	7,5	7,6	7,7	7,8	7,9	7,10
8,1	8,2	8,3	8,4	8,5	8,6	8,7	8,8	8,9	8,10
9,1	9,2	9,3	9,4	9,5	9,6	9,7	9,8	9,9	9,10
10,1	10,2	10,3	10,4	10,5	10,6	10,7	10,8	10,9	10,10

### Hamamatsu sCMOS

Exposure Time (seconds): 0.02  
Lamp focus: 0.02  
Laser focus: 0.2  
Laser sequence: 0.01

Find Coverslip  
Open sCMOS GUI  
Reset sCMOS

### Light Sources

647nm Laser  
On for focus: ☐ Power (mW): 50  
On for sequence: ☐ Power (mW): 50  
Open 647nm Laser GUI

405nm Laser  
On for focus: ☐ Power (mW): 11.84  
On for sequence: ☐ Power (mW): 11.84  
Open 405nm Laser GUI

LED Lamp  
Lamp Power (% of max): 35  
Open LED Lamp GUI

### Sample Stage

(use mouse wheel to control z piezo)

+Z +X  
+Z -Y -y +x +y +Y  
-Z -X

Stepper Position (mm)  
X: 1.000  
Y: 1.000  
Z: 1.000

Piezo Position (um)  
X: 10.000  
Y: 10.000  
Z: 10.000

Load Sample Open Stepper GUI Reset Piezos  
Unload Sample Open Piezo GUI Reconnect Piezos

### Directory/File Options

Save Directory:   
Coverslip Name:   
Label Number: 1  
Filename Tag:

Status: Setting up main sCMOS...

### Alignment/Registration

Use Active Registration ☐  
Use Periodic Registration after every 1 sequence(s) ☒  
Find Coverslip Offset

### Acquisition

Number of Sequences: 7  
Number of Frames: 6000  
Pre-Activate Fluorophores for 10 second(s) ☒

Indicate Photobleaching Round: ☐  
Automatically Publish Results: ☐  
Start Autocollect  
Abort Acquisition

(b)

SRcollect.gui

### FILE

Save Directory: Y:\SRuser\18-01-31\   
Base FileName: Cell1   
File type: .mat

### CAMERA

Camera Gain: Low (Alexa647)  
Camera ROI: Left Bottom  
Exp. Time Focus: 0.01 Actual:   
Exp. Time Seq.: 0.01 Actual: 0.03203  
Num Frames: 2000 Zoom: 400...

### REGISTRATION

Exp. Time Reg.: 0.01 Take Current Center Stage  
Load Reference   
Align Show Reference Take Reference Save Reference  
☒ No Registration  
☐ Align to Self (Takes/Saves Reference Image)  
☐ Align to Reference

### LIGHT SOURCE

	405 nm	488 nm	561 nm	642 nm	Lamp
On during Focus	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
On during Acquisition	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Low Power	1	1	1	1	50
High Power	4	10	10	80	

[MinPower, MaxPower] [0.25, 8.5] [0, 100] [0.125, 100] [0, 80] [0, 100]  
(mW) (mW) (mW) (mW) (Percent)

### CONTROL

Number of Sequences: 20  
Focus Lamp Focus Laser (Low)  
Lamp Focus Laser (High)  
START ABORT

(c)

ExampleCamera.gui(obj): obj

Display Camera Parameters

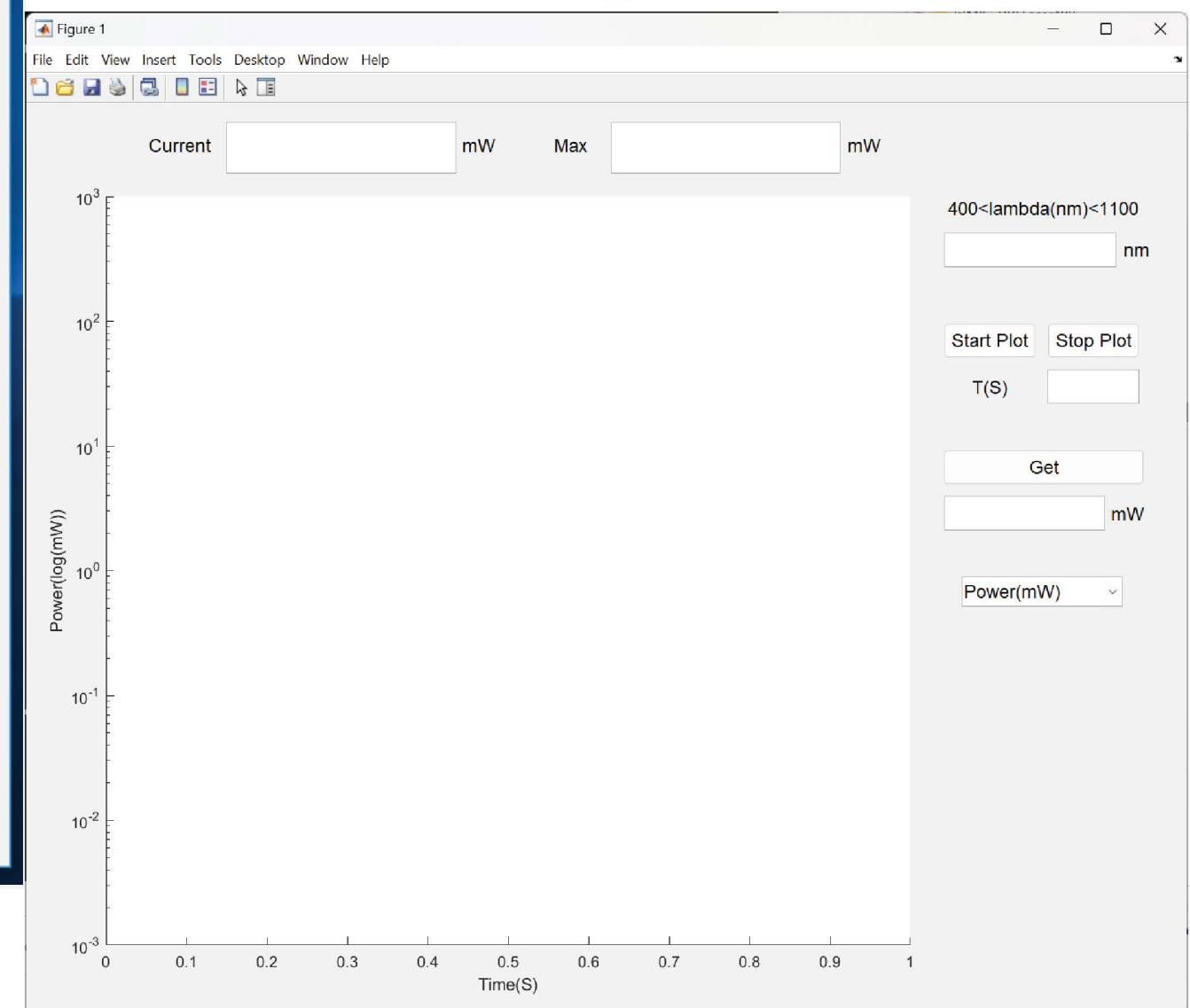
### Action

Exposure Times (s)		Output Variable
0.01	Focus	focus
0.02	Capture	capture
0.05	Sequence	sequence

Sequence Length: 10 Zoom: 100%  
Sequence Period: 0.1

22 Abort Exit

(g)



(d)

TCubePiezo X

0 um 20 um

< F C F C >

Fine step size 0.2 um  
Coarse step size 2 um

Mouse wheel fine/coarse Fine

Set Position 0 um

(e)

NanoMax Stage Control

UP  
up  
down  
DOWN

X- X- X- X-  
Y+ y+ y- Y-  
X+ X+ X+ X+  
Y+ y+ y- Y-  
X+ X+ X+ X+

(f)

TIRFLaser488

Min Power 0 mW Max Power 100 mW

Set Power 0 mW

Off