Everest System / Slidebook Operating Procedures

NOTICE: This guide is meant to supplement training, not replace it. All users must be trained first hand by a core employee. Training of others in your lab is not allowed and will result in revocation of your privileges.

Startup:

- 1. <u>Sign In</u> the log book.
 - a. ***we bill you for lamp hours, not for the time you spend taking pictures. Therefore, log your time at the time you turn on the lamp and the time when you turn off the lamp.
- 2. Turn on Xenon Lamp and write the number on the meter in the sign in book.
- 3. Turn on the scope
 - a. To do this, turn on the surge protector behind the scope.
- 4. Turn on the computer and enter your sign in information.

MAKE SURE THAT YOUR SLIDES ARE COMPLETELY DRY AND FREE FROM EXCESS MOUNTING MEDIA OR NAIL POLISH! THESE MATERIALS CAN DAMAGE THE OBJECTIVES IF THEY CONTACT THEM.

Basic Imaging:

- 1. Open the Slidebook program
- 2. Load Specimen on the stage
 - a. Use the stage control buttons on the bottom left side of the scope to change the stage height between the load and work positions.
 Do not touch the focus knob until you have raised the stage to the work position. If you

do, you will have to manually move the stage back to the work position.

- 3. Open the Focus Window:
 - a. Click the FOCUS button (red arrow points to it below)

	SlideBook - Slide2
h	For Help, press F1
b. F	This window will open:

- c. Click OPEN BRIGHT or OPEN FLUOR to turn on either the brightfield (white) light or the fluorescent light. Click them again to turn them off.
- d. Use the controls in this box to change the light to go to the camera (computer) or to the scope (to focus your specimen)
- e. Use these buttons to change between objectives
 - i. Be careful not to get oil on the dry objectives. BE SURE to CLEAN any oil off of objectives after use!
- f. Use these buttons to change to the correct filter cubes
 - i. When using brightfield, usually select DIC cube.
- g. Focus on your area of interest

- h. Turn off the lights using the buttons by arrow C when not taking pictures or focusing to avoid bleaching your samples.
- i. You can take a quick picture here by clicking the SNAP button. Adjust Exposure slider to avoid saturated pixels.

Basic Image Capture:

1. After you have focused and found your area of interest, click the Camera button:

🔌 SlideBook - Slide2			
<u>File E</u> dit Image <u>Vi</u> ew <u>M</u> ask <u>Windo</u>	w <u>H</u> elp		
	i 🗃 💡		
🐏 Slide2			
Image	Comments	Capture Date	
		>	
For Help, press F1			NUM

2. This window will open:

Capiture Settings	Extent, Offset and Brinn	ů (bixeje)		the second se		
Default 💌	F Image Bin Fact	Dr: Width: 13	94 • Height: 1040	· Ustare		
Current:	C Autorogan [tws]	X Offsett	Y Offset: [n	- NOR		
Default	White Balance	1		THOP		
[Advenced]	Timelapse Capture		Available: 1.78 GS	Required: 2.77 MB		
Capture Type	# of Time Points:		- 3D Capture	name And I		
T 30	Estimated Ouration:	1 -	R penton	Kange (Jm): 1		
F stopens	Interval:		Constant and a second s	# Planes:		
Timelapse	Daplact IT :	THE PARTY OF ALL	C Opertup and Sb	ep 52e (um): [1.67		
C Sedarein.	At danke Will acation Cambo		Tanton cornina	Offset (um):		
T Stereology	G Currenttocation C :	California Manageria	1 Cargo a series and	COLOR DE LA COL		
			1. Martin Company	AND ADDRESS OF ADDRESS		
Ther Set: Fixed			Advert Excess on	ND: E		B B
Iter Set: Fixed	2	Fanose:	Adjust Exposure	ND:		B
Her Set: Fixed	2	Expose:	Adjust Exposure	ND:		В
Her Set: Fixed	-	Expose:	Adjust Exposure	ND:		B
Pher Set: Fixed		Expose:	Adjust Exposure	ND: 10		B
Texas Red		Expose: 100 Correct:	Adjust Exposure	ND: 10		■ B
Ther Set: Fixed CY3 Texas Red OC OPEN PGold DAPT Vetual RG8		Expose:	Adjust Exposure	ND: 00 00 Control 00 00 Control 00 00 Control 00 C	A	- B
Her Set: Fixed		Expose:	Adjust Exposure	ND: 0	A	- B
Her Set: Fixed		Expose:	Adjust Exposure	ND: UN A	A	В
Her Set: Fixed CY3 Trexas Red DIC OPEN PGold DAPI Virtual RG8 Cy 5.5 CY5 Move Up N	eve Down	Expose: 100 Terret: Carret:	Adjust Exposure	ND: 0 Alls ND: 0 MR ND: 0 MR Construction interactive Construction Office: -	A	В
Her Set: Fixed CY3 Texas Red DIC OPEN PRold DAPI Virtual RGB Cy 5.5 CY5 Move Up N	love Down	Expose: 100 Correct: 0	Adjust Exposure	ND: III Auto ND: III Mitter ND: III Mitter ND: III Mitter ND: III Offset: -	A	В
Her Set: Fixed Fix	tove Down	Expose: 100 Correct: 0 Information (Optional ismet:	Adjust Exposure	ND: III Auto ND: III Mitter ND: III Mitter ND: III Mitter ND: III Officet: - Start -	A	В
Her Set: Fixed Field Field Field Field Field Field Field DAPI Pield	tove Down	Expose: 100 Test Carrect: 0 Information (Optional same:	Adjust Exposure	ND: III Autr ND: III ND: - ND: -	A	В

3. Pick one of the Fluorophores that you want to image (such as FITC).

- a. Click the TEST button and look at the peak. The peak should cover about half of the graph window with a maximum value of approximately 3000.
- b. Adjust Exposure:
 - i. Hit FIND BEST and wait or use the ONCE button. You will see the exposure time change.
 - ii. Or, you may adjust the exposure manually by typing how many milliseconds you want the shutter open.
- c. Repeat this for each fluorophore you want to image, one at a time.
- 4. You may name your image at this point if you desire.
- 5. Click START to take the image.

Saving Images:

- 1. Make sure you save the image and the slide. We recommend doing this after every 2-3 pictures, to avoid losing your work.
- 2. Save Picture:
 - a. On the slide, double click the picture you want to save.
 - i. For SINGLE 8-bit images:
 - 1. View > Export > Tiff OR
 - 2. View > Export > Default Views of all Images as TIFFs
 - ii. For 3D images in 8-bit format:
 - 1. View > Export > Tiff Series
 - iii. For 16-bit TIFF images to be analyzed in other software:
 - 1. Image > Export > 16-Bit Tiff
- 3. SAVE YOUR SLIDE!
 - a. File > Save Slide As
 - Update your slide regularly, usually each time you save a single picture by using File >Save Slide

3-D Imaging:

1. In the Focus Window, click the "Z" tab:

	Top Set Top Set Top	
	Not Sel Number of Planes: NA Center Total Travel (unt): NA Networks Step size (unt): NA Desi All Step size (unt): T87 @ 10X as	
3	Botom	4
	Camera Test Canera 0	
	Cameras Test Camera 0 Exposure 100 me Ben: [1x1 Zoom: + Matter Automatic Color Step Smep] Fiber Sets Fixed ODC OPEN Ficed [7] Color Export	
	Ceneral Test Canera 0 Exposure 100 ms	

- 2. While viewing your image live (with 100% light to camera and the bright or fluor on), use the focus knob to defocus your image in one direction. Turn the focus knob until you can barely see your specimen and click SET TOP.
- 3. Repeat step 2, turning the knob the other direction and click SET BOTTOM.
- 4. Determine what step size you want to use and then enter that into the box.

3-D Image Capture:

- 1. Do steps 1-4 from Basic Image Capture section if you haven't done so already.
- 2. Select "3D" under capture type

0	efault • P Image wrent: efault • White Ba	Bin Factor: Width: 139- Itx1 Y Offset: 0	Y Offset: 0	
	Advenced Timelapee Ca sphure Type 30 F And Carried Timelapee Conformer Stereology F CurrentL F F CurrentL F CurrentL F CurrentL F F F CurrentL F F F F F F F F F F F F F F F F F F F	pture Pante: 1 5 2 seterc 1 5 2 setercal: 1000 5 2 setercal: 1000 5 2 sector Capture sector: C 2 corr cat/Moncage	Available: 1.78 GB 30 Capture Unt current Constrain	Required: 2.77 MB re (um): Planes: re (um): 1.01 re (um): 0.01
Pite	r Set: Fixed CY3 CY3 Texas Red OIC OPEN Poold DAPI Virtual RGB Cy 5.5 CY3	Expose: 100 Correct: 1	Adjust Exposure Adjust Exposure Adjust Exposure Adjust Exposure Adjust Exposure Adjust	Or Date of Construction of Con
	Move Up Move Down	0	65535	
- Or	xics	Image Information (Optional)		

- 3. Select "Use Top and Bottom Positions"
- 4. Click START to take the stack.
- 5. Once the 3D stack has been collected, you may scroll through the different planes of the stack using the middle mouse wheel.

Montaging/ImageStitching:

- 1. Do the steps in the Basic Imaging Section and steps 1-4 in the Basic Image Capture Section.
- 2. Click the "XY" tab in the Focus Window.
- 3. While looking at the image live (with 100% light to camera and the bright or fluor on), find the top left corner of your area of interest using the joystick. (The button on top of the joystick will make the stage move slower after it is clicked.)
- 4. Click the top left arrow button in the "Montage Extent" area (4).
- 5. Find the bottom right side of your area of interest and click the bottom right button.

Sel Post Hurtage Edent
Vaz Paret A
New Friel Z
Reset ABZ and and
Clear Point Hore.
Ces At Cater Strengt
2000 +
Domer 100 ms
Domary 100 ms
Domare 100 ms Brc [21 Image: Brc [21 <t< td=""></t<>

6. In the Camera window, select "Entire List/Montage"

Capiture Settings Default <u></u> Current: Default	Batent, Offset and Brinnig (positi) Proge Bin Factor: Weth: 1294 Vetrage Bin Factor: Weth: 1294 Vetrage Bin Factor: Vetrage Bin
Capture Type Capture Type T 30 This common Treelappe T Sterrelogy Piter Set	Threspace Capture a of Time Points: a of Time Points:
Cr3 Cr3 Crexxs Red OfC OPEN Poold DAPF Cr 5.5 Cr 5.5	Expose: Internet interadive Correct: IT Decision IF Red Field Office:
Cr3 Texas Red OfC OPEN Codel Dury S.S Cr3 Move Up	tove Down 0 65535
Cr3 Texts Red OfC OPEN Poold DAFE Virtual RGB Cr3.5 Cr3.5 Cr5.5 Move Up Move Up Move Up Move Up	tove Down 0 0.5535

- 7. As before, you may name your picture here, and then click start to begin the image capture.
- 8. When working with large montages, we recommend that you don't put more than 1-2 montages on a slide, but save each slide separately and open a new one for a new montage.