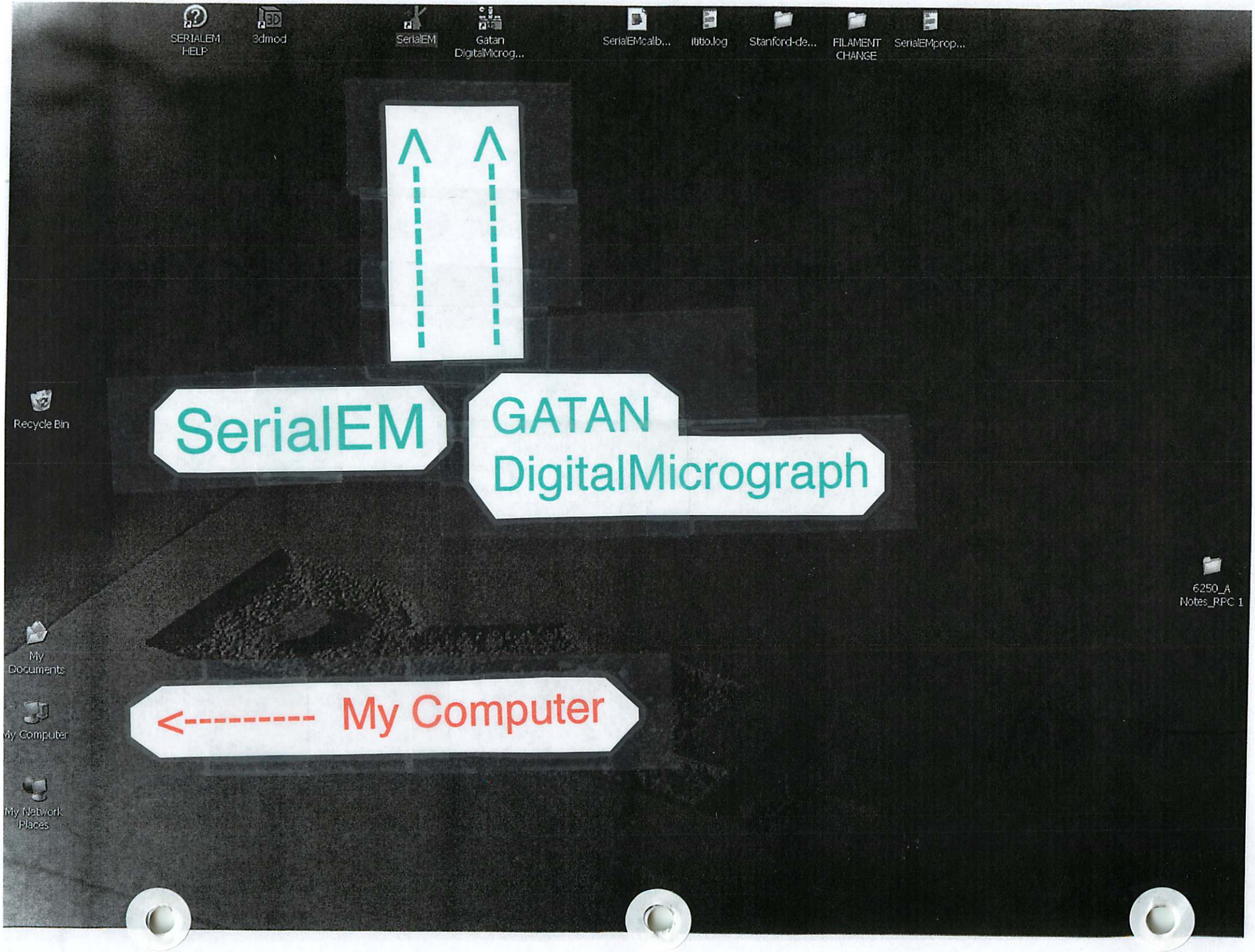
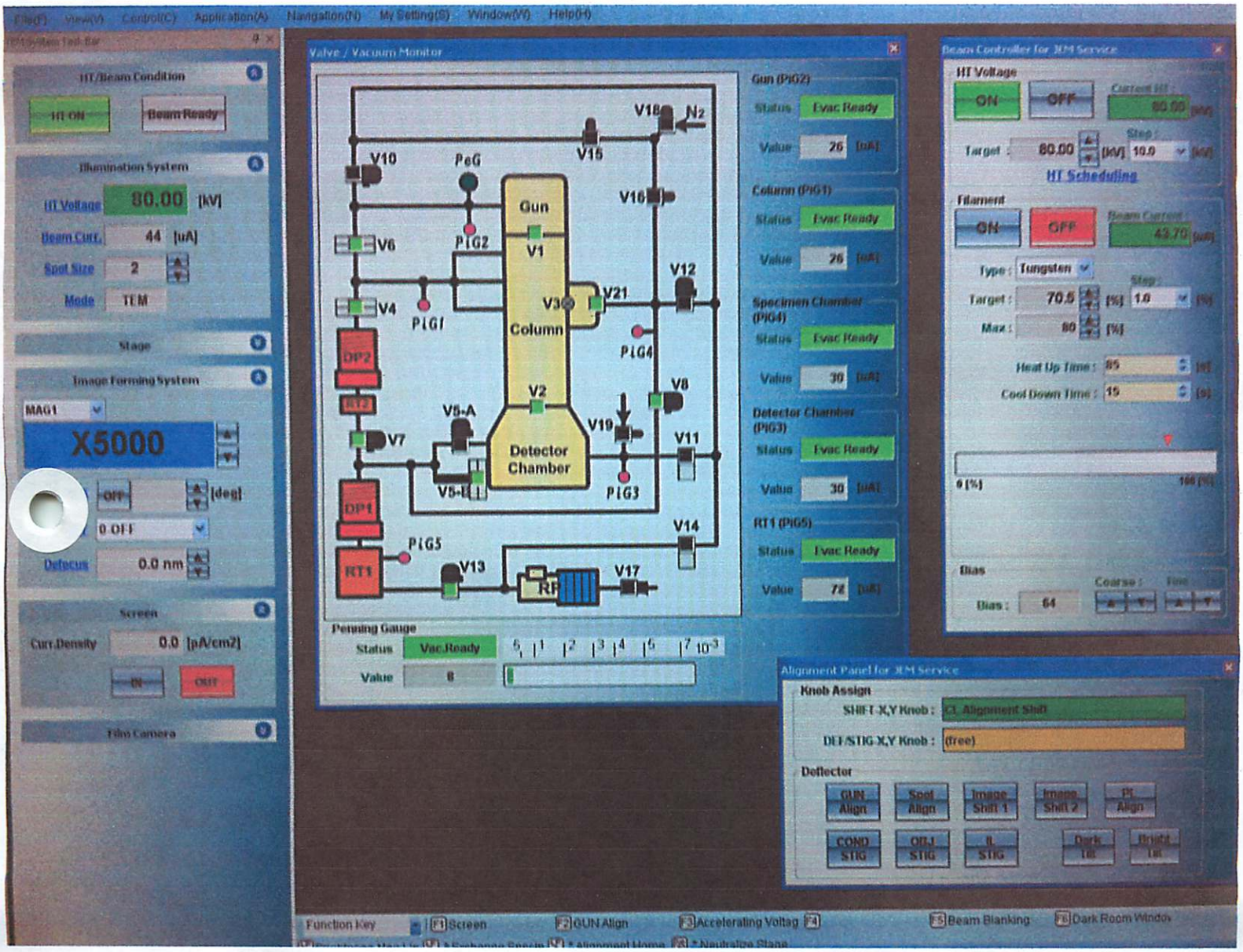
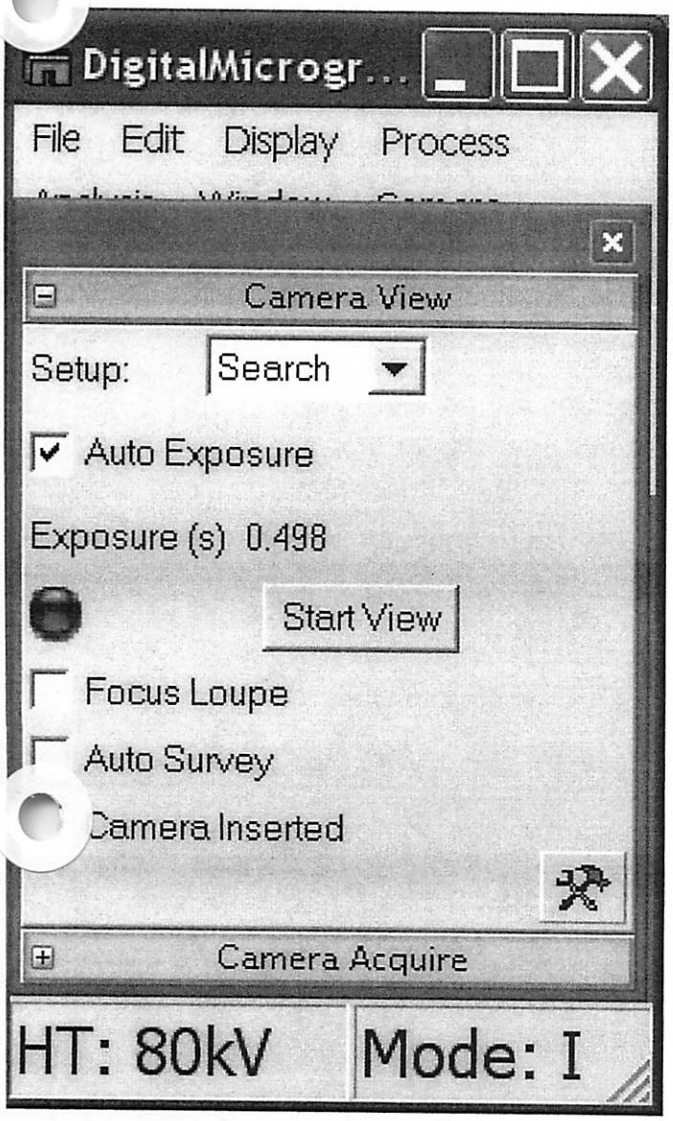

SERIALEM HELP
3dmod
SerialEM
Gatan DigitalMicrog...
SerialEMcalib...
ibtilo.log
Stanford-de...
FILAMENT CHANGE
SerialEMprop...
Recycle Bin
SerialEM
GATAN DigitalMicrograph
6250_A Notes_RPC 1
My Documents
<--------- My Computer
My Computer
My Network Places

TEM Center for JEM-1400 window

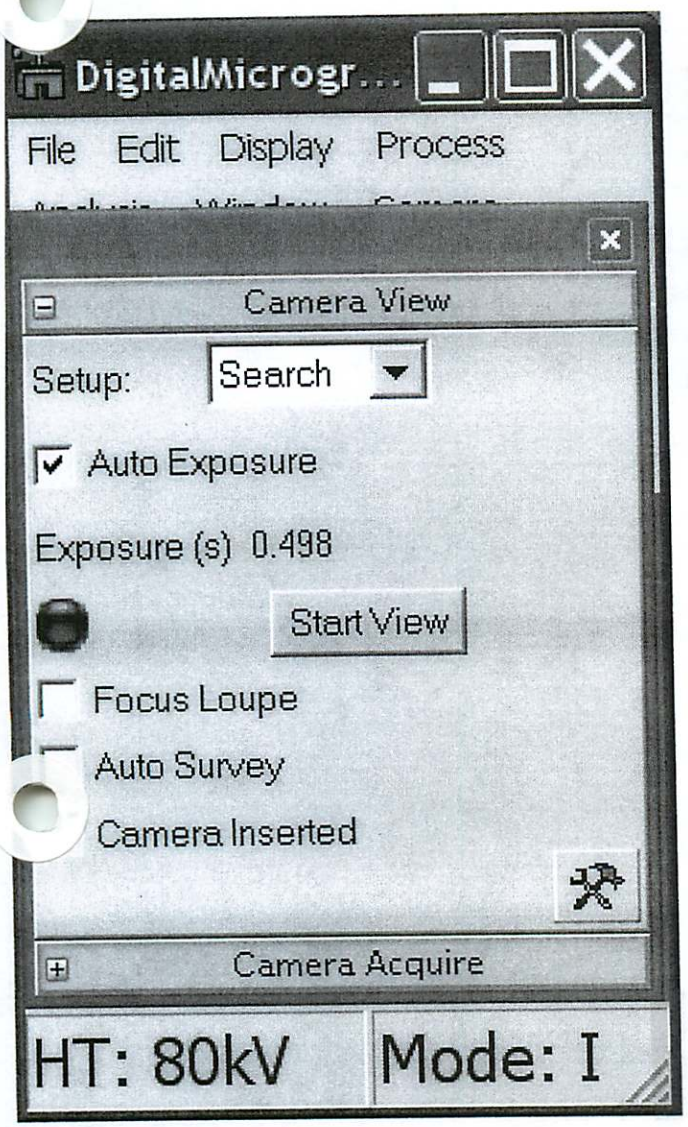


(see following page)

Decided to close and open the TEM Center for JEM-1400 window each day. To open the small TEM Center Domain Controller window in desktop, click the TEM Center Domain icon. Next, click the Start TEM Center button. This opens the TEM Center for JEM-1400 window. The Screen window in the left-hand panel will require opening. The TEMCON icon in desktop opens the Controller for JEM-1400 window. This is the panel for engineers/service, but it can be used to verify information in the TEM Center for JEM-1400 window. For example, use it verify if the HT is off (blue) before changing filament. If it is on, HT is highlighted in green. Also, use this window to re-select the full range of magnifications if the TEM Center for JEM-1400 window crashes ..... the only mag that will be available is 10K. From the menu > Options > Mag Select > recheck the full range of magnifications in both low and high mag. Go back to the TEM Center for JEM-1400 window to verify ..... Image Forming System window in left-hand panel.

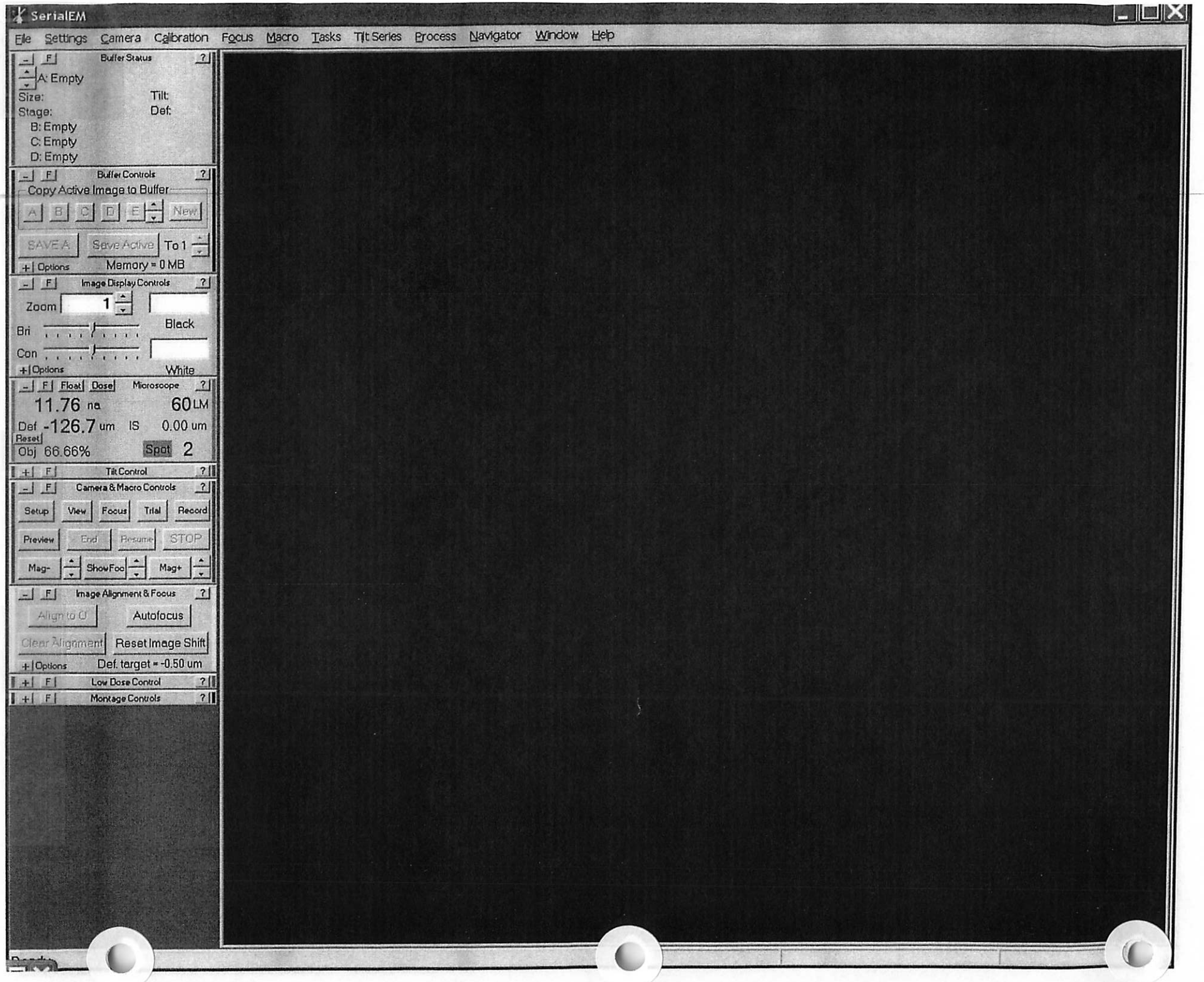


To open double-click GATAN DigitalMicrograph icon (see previous page). Select (✓) Camera Inserted.

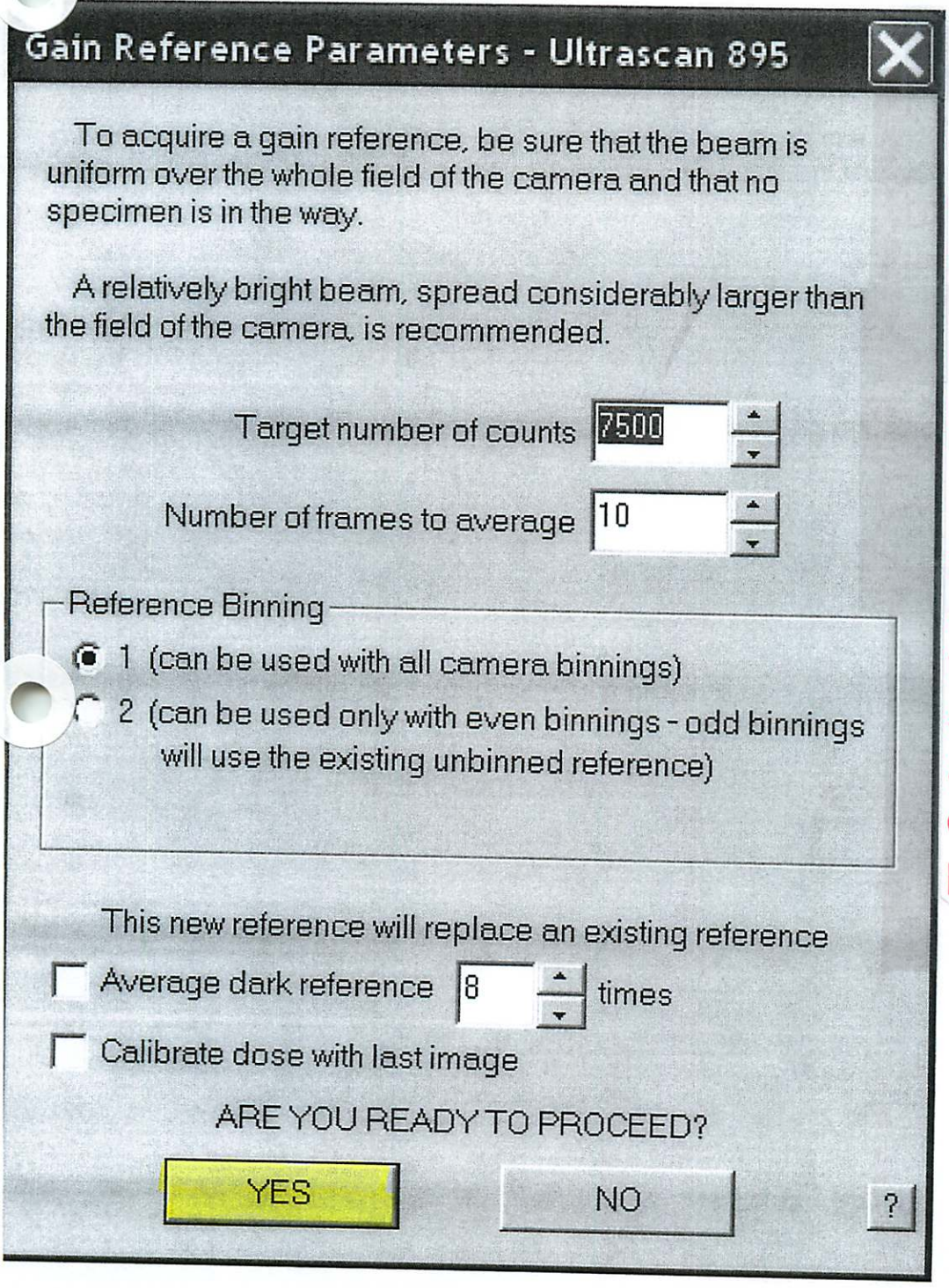


Each day de-select Camera Inserted, close DigitalMicrograph window, open again, and select (✓) Camera Inserted. This refreshes program so that it will not malfunction during the capture.

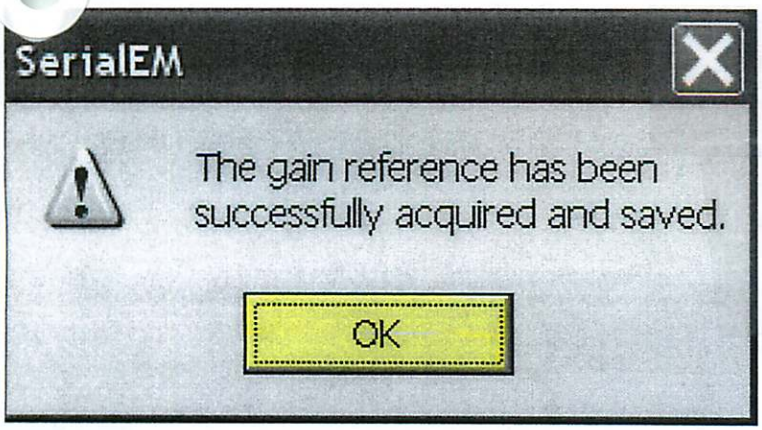
<-------- Check the HT: reading at bottom of window. It should be the same as high tension (80KV) set for scope. If it reads (0KV), simply close and open the window again.



Also, each day perform Prepare Gain Reference. ---v



If specimen/rod is inserted for capture the night before, pull out rod to the first hold position (pull out until it stops and turn counter clockwise until resistance is met). Turn on beam (1 and 1/2 minutes), center it, and spread a couple of partial turns outside the edge of the screen. All values in adjacent window are default. Click YES to proceed.

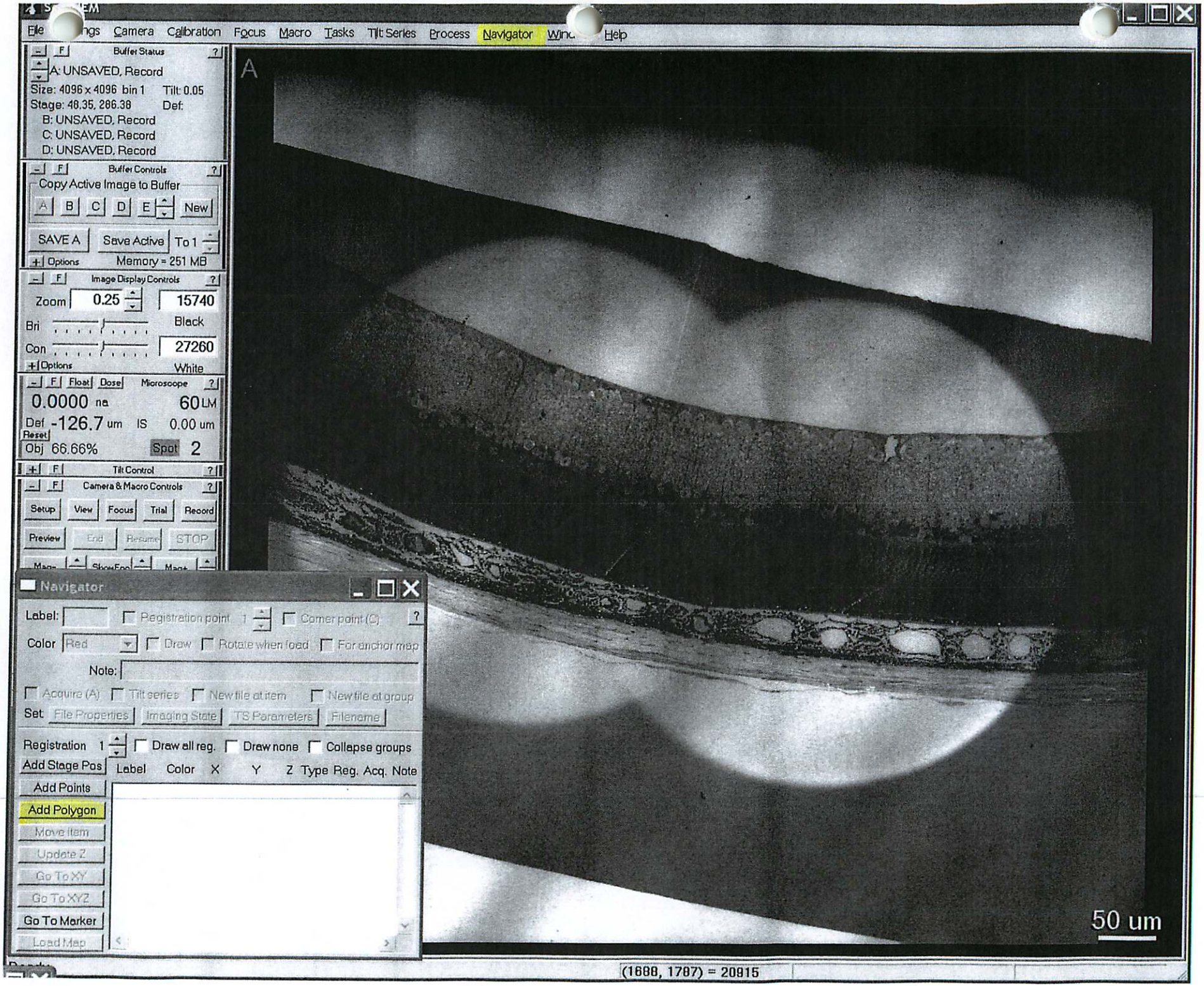


Click OK and you are ready to proceed.

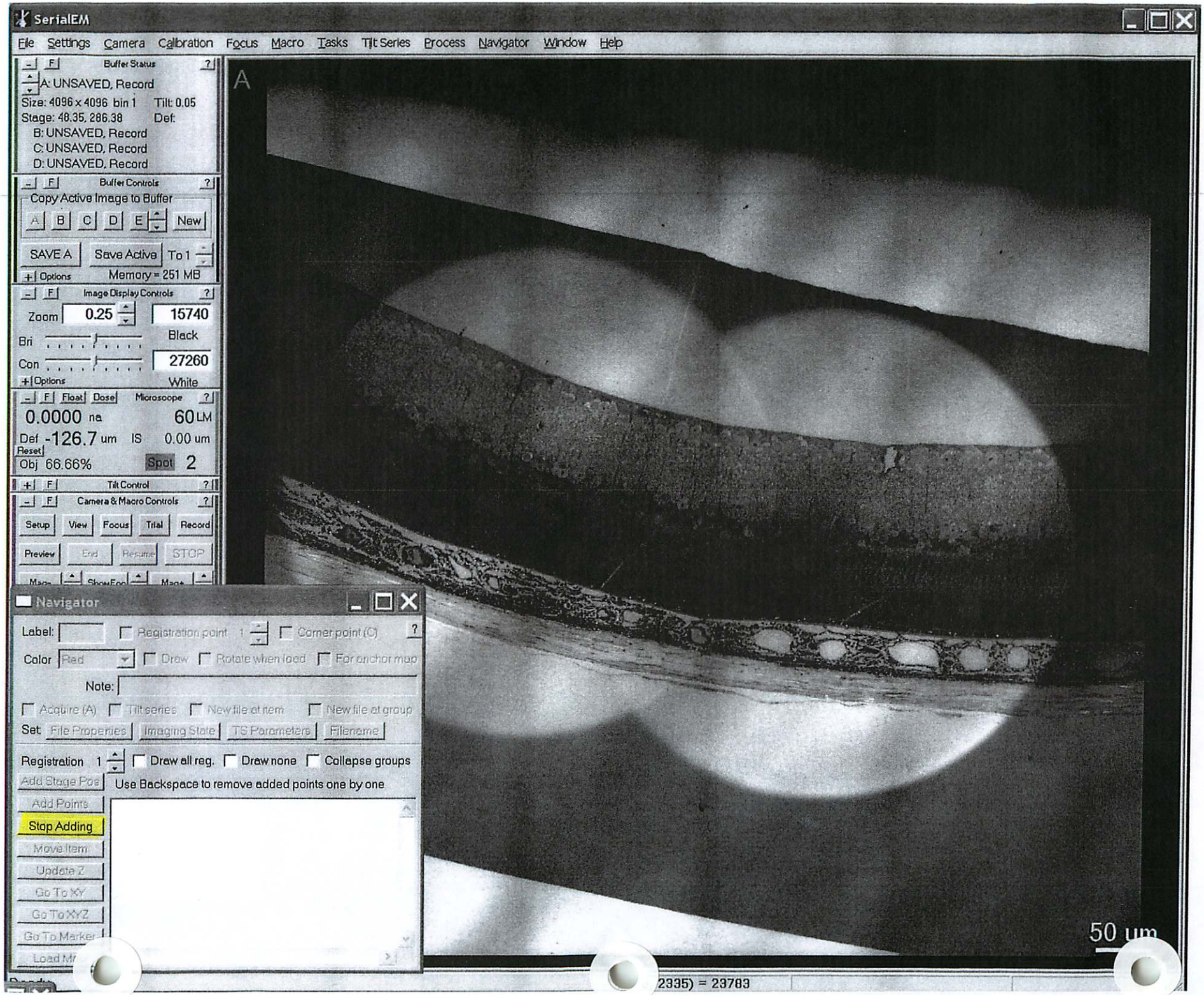
After refreshing Digital Micrograph and performing Prepare Gain Reference, remove specimen rod and make exchange to next section to be captured.* Once in scope, turn on beam and set up at 150X (low mag). Select spot size 1 for strongest/brightest beam and cook (4 to 5 minutes) the region of section to be catpured. Then switch to 60X and note that the image rotates 90 degrees. Spread beam and click Record from left-side menu of SerialEM to capture image. Open Navigator window (Navigator>Open).

------>

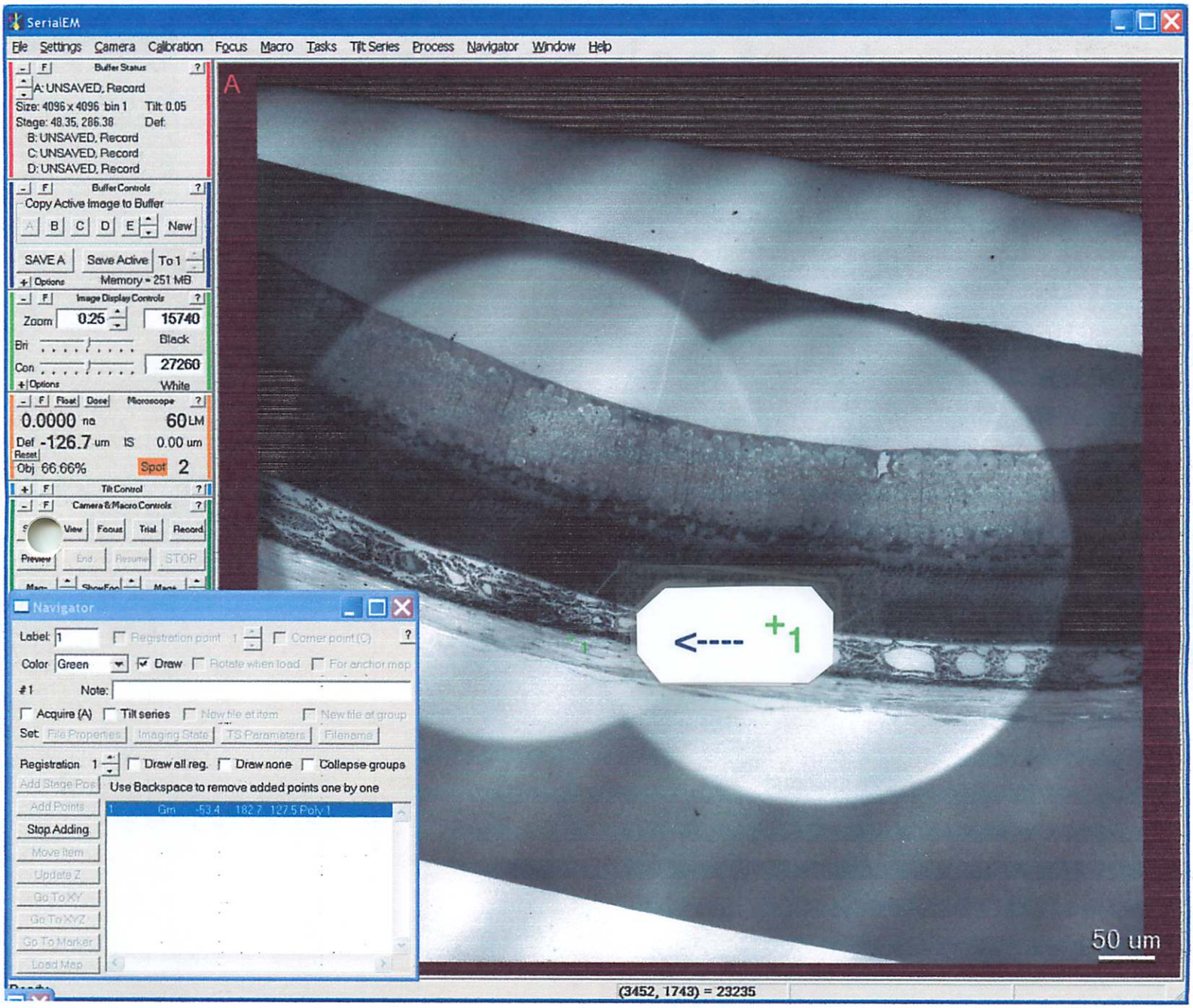
* It is important to position the grid so that the long axis of the sample to be captured is parallel to the long axis of the specimen rod. Positioned as such, the long axis of the sample is in the vertical plane at 150X, the magnification chosen for pre-exposure to the beam. When the magnification is changed to 60X (for capture) the sample will rotate 90 degrees counterclockwise, and place the long axis of the sample in the horizontal plane. As such, the vertical columns of capture in SerialEM will be at their minimum. This produces less stress on the software used to montage individual image tiles.


File
Camera
Calibration
Focus
Macro
Tasks
Tilt Series
Process
Navigator
Help
Buffer Status
A: UNSAVED, Record
Size: 4096 x 4096 bin 1
Tilt: 0.05
Stage: 46.35, 286.38
Def:
B: UNSAVED, Record
C: UNSAVED, Record
D: UNSAVED, Record
Buffer Controls
Copy Active Image to Buffer
A
B
C
D
E
New
SAVE A
Save Active
To 1
Options
Memory = 251 MB
Image Display Controls
Zoom
0.25
15740
Bri
Black
Con
27260
Options
White
Float
Dose
Microscope
0.0000 na
60 LM
Def -126.7 um
IS
0.00 um
Reset
Obj 66.66%
Spot
2
Tilt Control
Camera & Macro Controls
Setup
View
Focus
Trial
Record
Preview
End
Resume
STOP
Navigator
Label:
Registration point
Corner point (C)
Color
Red
Draw
Rotate when load
For anchor map
Note:
Acquire (A)
Tilt series
New file at item
New file at group
Set
File Properties
Imaging State
TS Parameters
Filename
Registration
Draw all reg.
Draw none
Collapse groups
Add Stage Pos
Label
Color
X
Y
Z
Type
Reg.
Acq.
Note
Add Points
Add Polygon
Move Item
Update Z
Go To XY
Go To XYZ
Go To Marker
Load Map
A
50 um
(1688, 1787) = 20915

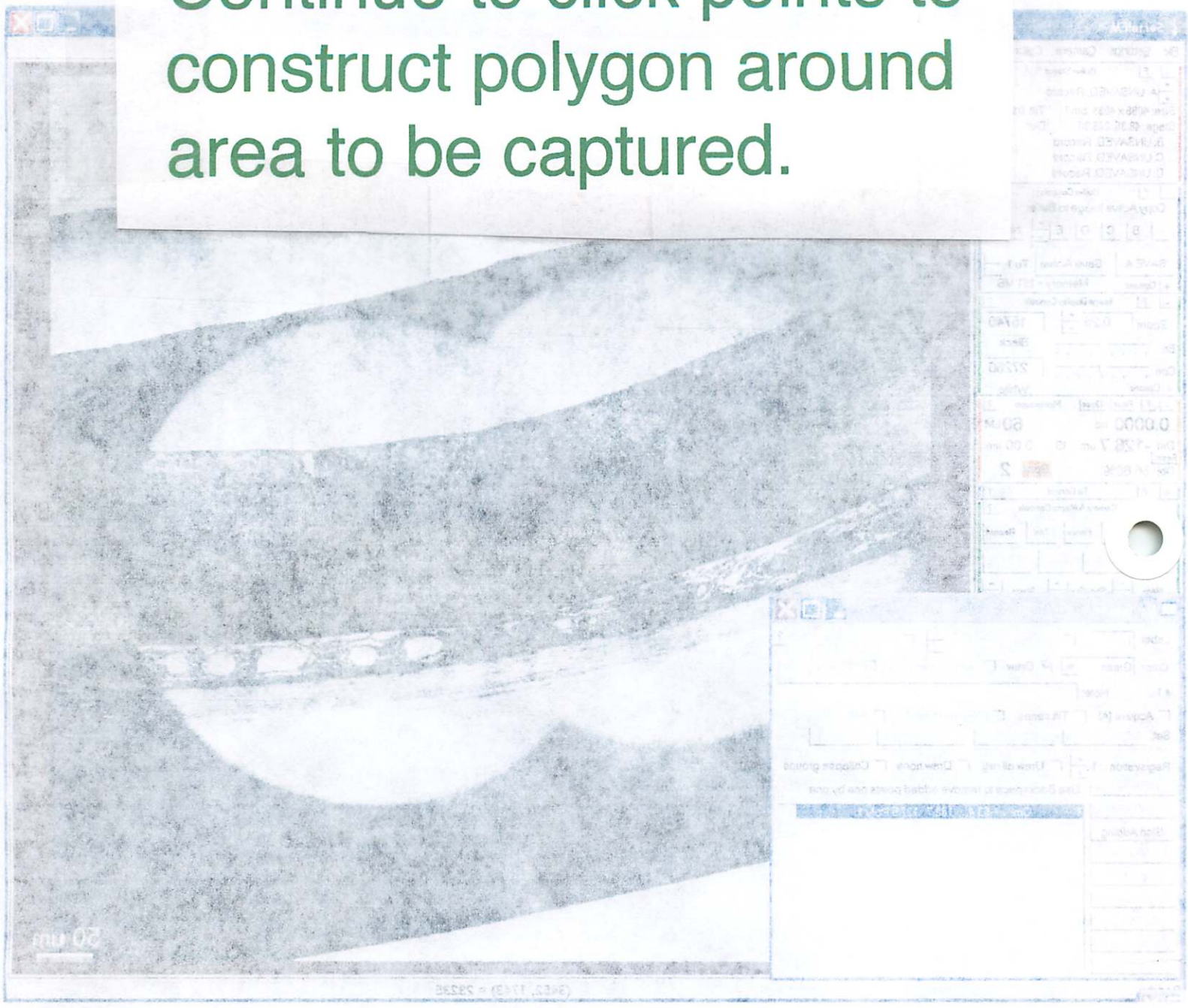
Select Add Polygon

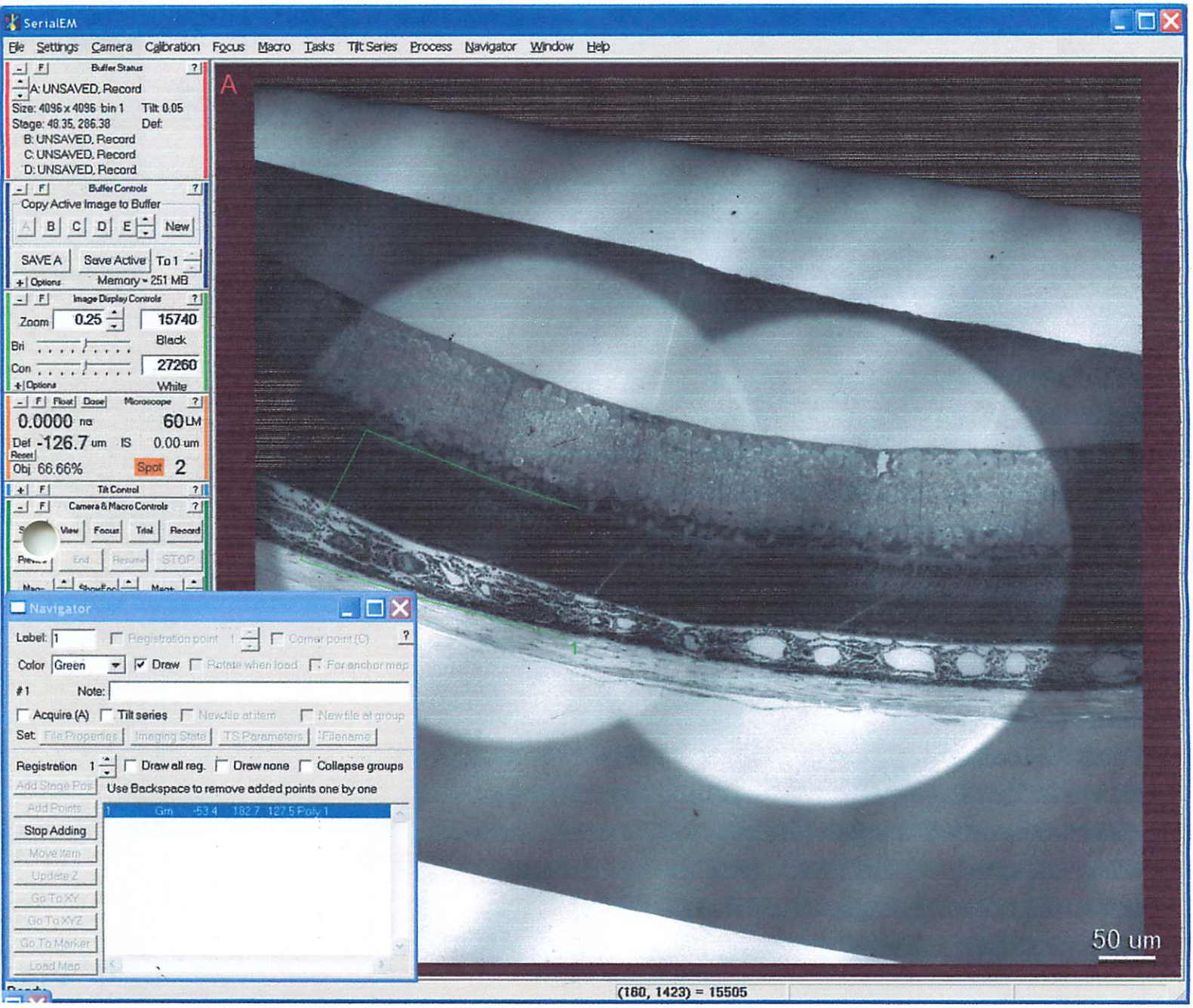

SerialEM
File Settings Camera Calibration Focus Macro Tasks Tilt Series Process Navigator Window Help
Buffer Status
A: UNSAVED, Record
Size: 4096 x 4096 bin 1 Tilt: 0.05
Stage: 46.35, 286.38 Def:
B: UNSAVED, Record
C: UNSAVED, Record
D: UNSAVED, Record
Buffer Controls
Copy Active Image to Buffer
A B C D E New
SAVE A Save Active To 1
Options Memory = 251 MB
Image Display Controls
Zoom 0.25 15740
Bri Black
Con 27260
Options White
Float Dose Microscope
0.0000 na 60 LM
Def -126.7 um IS 0.00 um
Reset
Obj 66.66% Spot 2
Tilt Control
Camera & Macro Controls
Setup View Focus Trial Record
Preview End Resume STOP
Navigator
Label: Registration point 1 Corner point (C)
Color Red Draw Rotate when load For anchor map
Note:
Acquire (A) Tilt series New file at item New file at group
Set: File Properties Imaging State TS Parameters Filename
Registration 1 Draw all reg. Draw none Collapse groups
Add Stage Pos Use Backspace to remove added points one by one
Add Points
Stop Adding
Move Item
Update Z
Go To XY
Go To XYZ
Go To Marker
A
50 um
2335) = 23783

Select first point for drawing polygon around area to be captured.

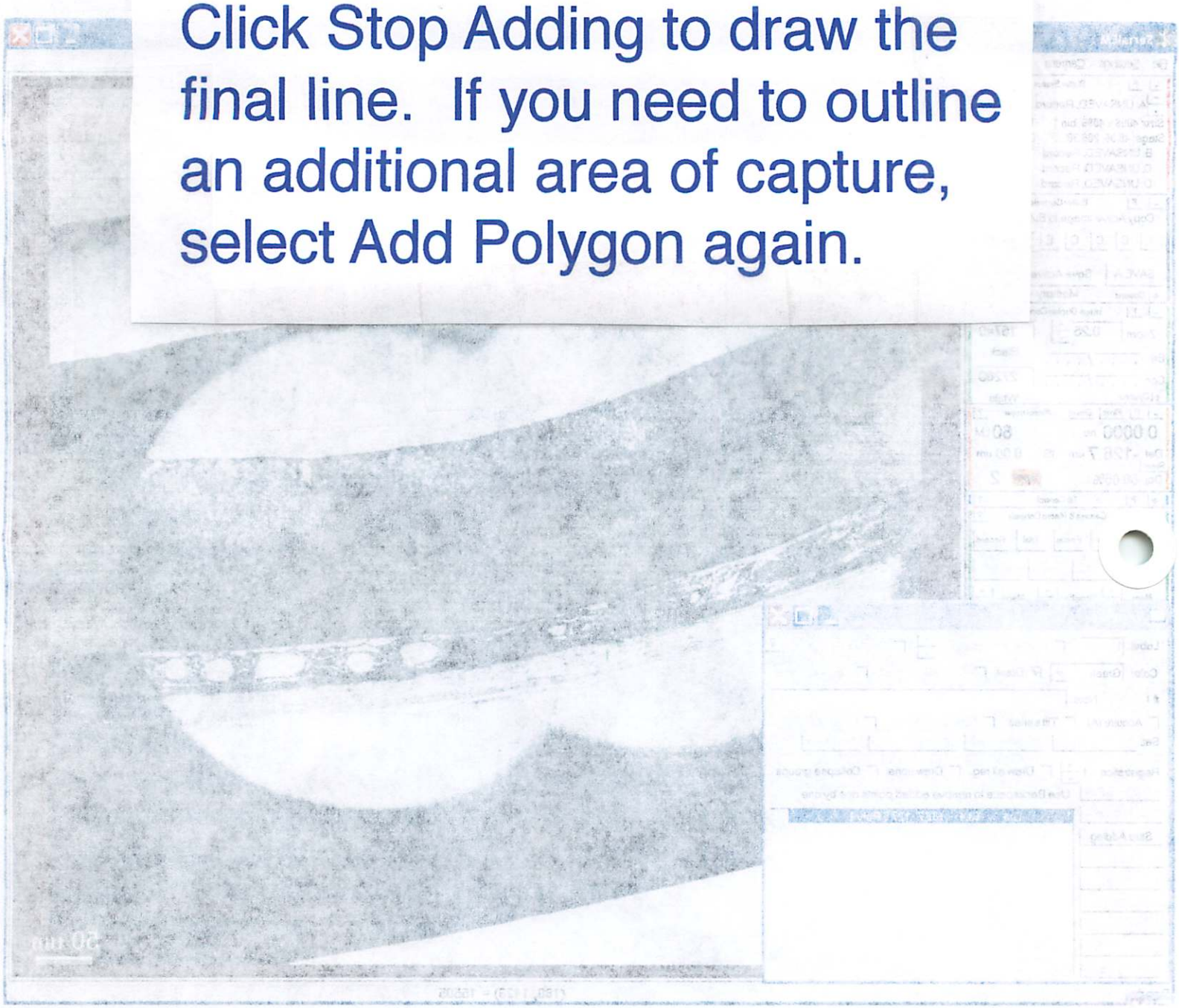

SerialEM
File Settings Camera Calibration Focus Macro Tasks Tilt Series Process Navigator Window Help
Buffer Status
A: UNSAVED, Record
Size: 4096 x 4096 bin 1 Tilt: 0.05
Stage: 48.35, 286.38 Def:
B: UNSAVED, Record
C: UNSAVED, Record
D: UNSAVED, Record
Buffer Controls
Copy Active Image to Buffer
A B C D E New
SAVE A Save Active To 1
Options Memory = 251 MB
Image Display Controls
Zoom 0.25 15740
Bri Black
Con 27260
Options White
Float Dose Microscope
0.0000 na 60 LM
Def -126.7 um IS 0.00 um
Reset
Obj 66.66% Spot 2
Tilt Control
Camera & Macro Controls
View Focus Trial Record
Preview End Resume STOP
Navigator
Label: 1 Registration point 1 Corner point (C)
Color Green Draw Rotate when load For anchor map
#1 Note:
Acquire (A) Tilt series New file at item New file at group
Set: File Properties Imaging State TS Parameters Filename
Registration 1 Draw all reg. Draw none Collapse groups
Add Stage Pos Use Backspace to remove added points one by one
Add Points
1 Grn -53.4 182.7 127.5 Poly 1
Stop Adding
Move Item
Update Z
Go To XY
Go To XYZ
Go To Marker
Load Map
A
<---- +1
50 um
(3452, 1743) = 23235

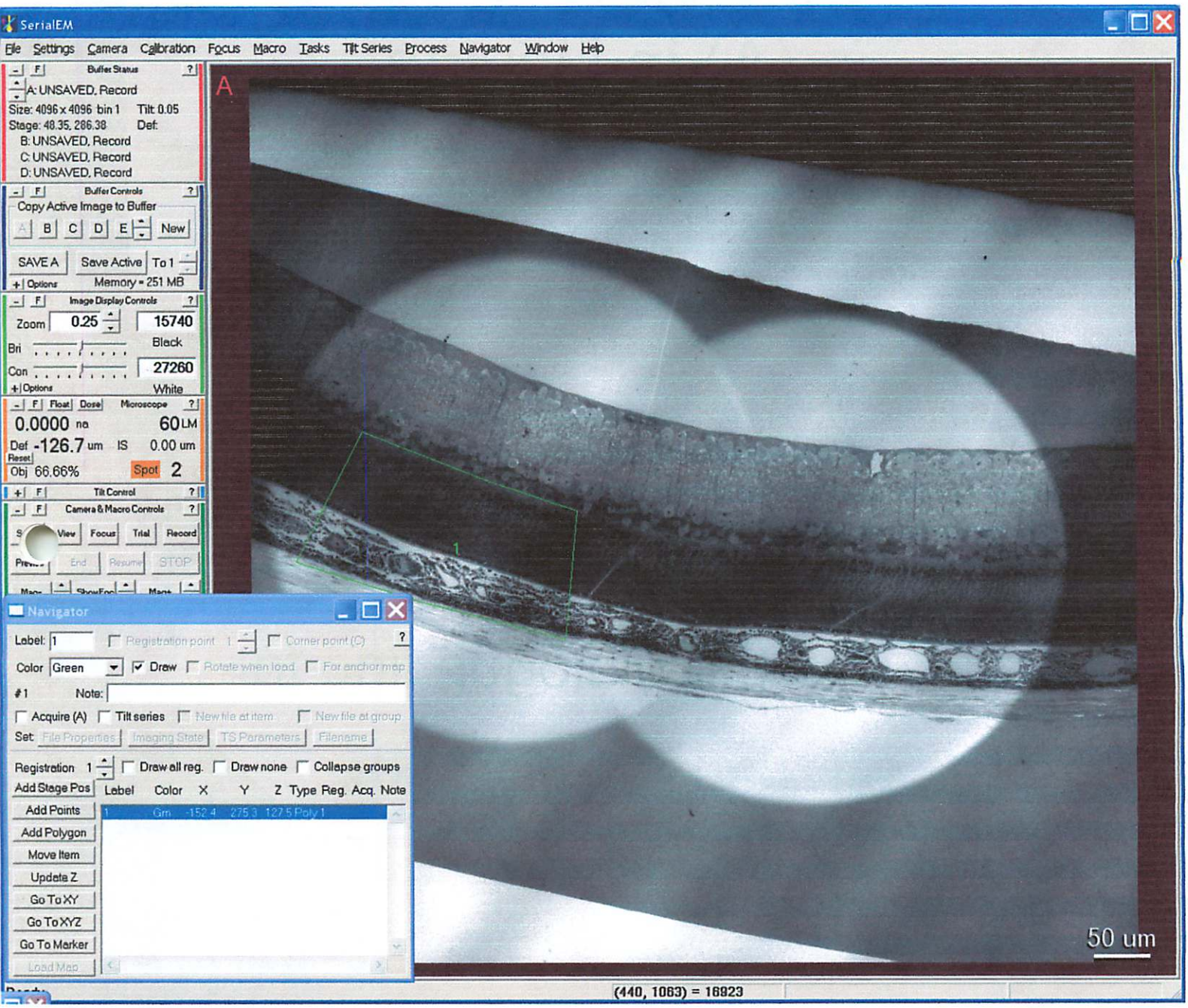
Continue to click points to construct polygon around area to be captured.




SerialEM
File Settings Camera Calibration Focus Macro Tasks Tilt Series Process Navigator Window Help
Buffer Status
A: UNSAVED, Record
Size: 4096 x 4096 bin 1 Tilt: 0.05
Stage: 48.35, 286.38 Def:
B: UNSAVED, Record
C: UNSAVED, Record
D: UNSAVED, Record
Buffer Controls
Copy Active Image to Buffer
A B C D E New
SAVE A Save Active To 1
Options Memory = 251 MB
Image Display Controls
Zoom 0.25 15740
Bri Black
Con 27260
Options White
Float Dose Microscope
0.0000 nm 60 LM
Def -126.7 um IS 0.00 um
Reset
Obj 66.66% Spot 2
Tilt Control
Camera & Macro Controls
View Focus Trial Record
End Resume STOP
Navigator
Label: 1 Registration point 1 Corner point (C)
Color Green Draw Rotate when loaded For anchor map
#1 Note:
Acquire (A) Tilt series New file at item New file at group
Set: File Properties Imaging State TS Parameters Filename
Registration 1 Draw all reg. Draw none Collapse groups
Add Stage Pos Use Backspace to remove added points one by one
Add Points
Stop Adding
Move Item
Update Z
Go To XY
Go To XYZ
Go To Marker
Load Map
1 Gm -53.4 182.7 127.5 Poly 1
A
1
50 um
(160, 1423) = 15505

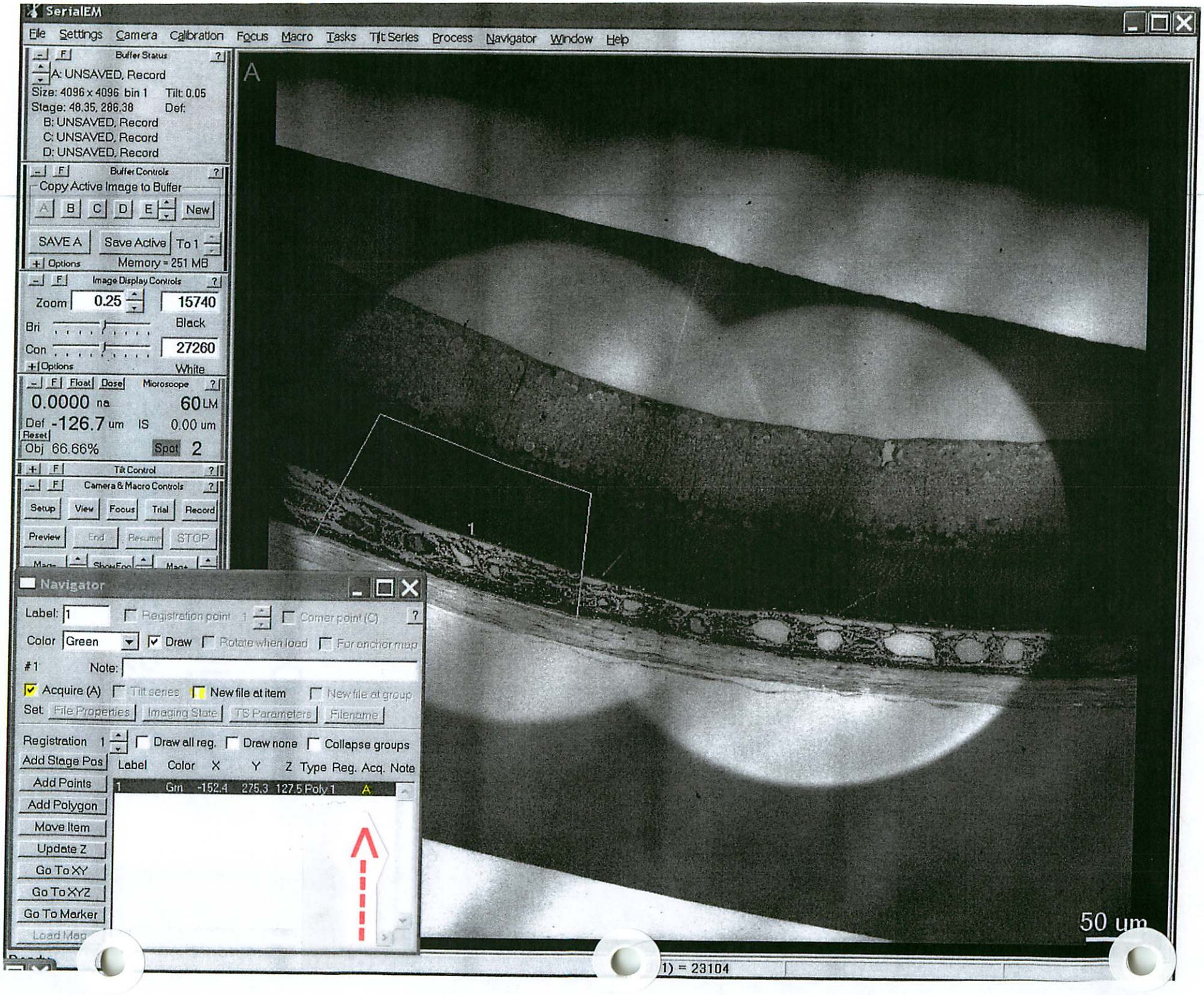
Click Stop Adding to draw the final line. If you need to outline an additional area of capture, select Add Polygon again.



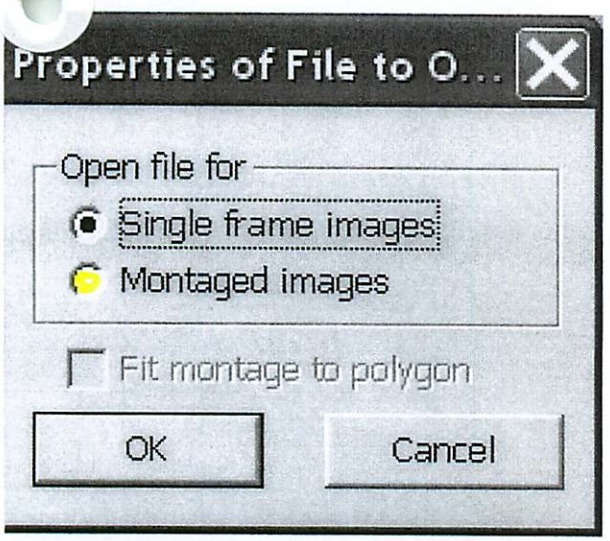

SerialEM
File Settings Camera Calibration Focus Macro Tasks Tilt Series Process Navigator Window Help
Buffer Status
A: UNSAVED, Record
Size: 4096 x 4096 bin 1 Tilt: 0.05
Stage: 48.35, 286.38 Def:
B: UNSAVED, Record
C: UNSAVED, Record
D: UNSAVED, Record
Buffer Controls
Copy Active Image to Buffer
A B C D E New
SAVE A Save Active To 1
Options Memory = 251 MB
Image Display Controls
Zoom 0.25 15740
Bri Black
Con 27260
Options White
Float Dose Microscope
0.0000 na 60 LM
Def -126.7 um IS 0.00 um
Reset
Obj 66.66% Spot 2
Tilt Control
Camera & Macro Controls
View Focus Trial Record
End Resume STOP
Navigator
Label: 1 Registration point 1 Corner point (C)
Color Green Draw Rotate when load For anchor map
#1 Note:
Acquire (A) Tilt series New file at item New file at group
Set: File Properties Imaging State TS Parameters Filename
Registration 1 Draw all reg. Draw none Collapse groups
Add Stage Pos Label Color X Y Z Type Reg. Acq. Note
Add Points
Add Polygon
Move Item
Update Z
Go To XY
Go To XYZ
Go To Marker
Load Map
1 Gm -152.4 275.3 127.5 Poly 1
A
1
50 um
(440, 1063) = 16923

The next step is to select Acquire (A) in Navigator window. Once clicked, New file at item is activated.

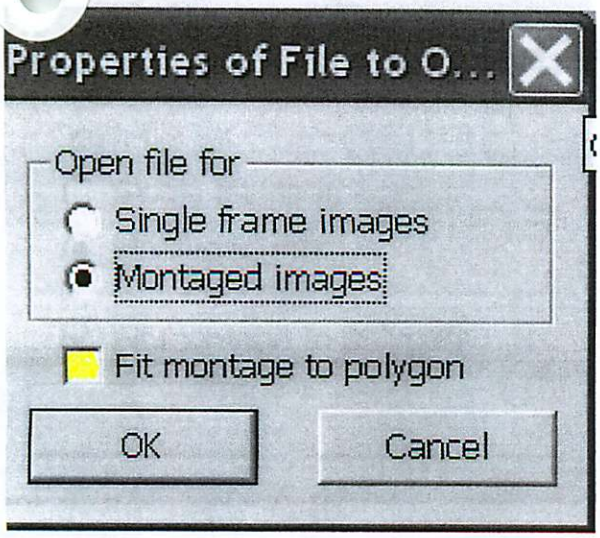
Note the appearance of the letter A in the dialogue line for item 1 in the dialogue box.


SerialEM
File Settings Camera Calibration Focus Macro Tasks Tilt Series Process Navigator Window Help
Buffer Status
A: UNSAVED, Record
Size: 4096 x 4096 bin 1 Tilt: 0.05
Stage: 48.35, 286.38 Def:
B: UNSAVED, Record
C: UNSAVED, Record
D: UNSAVED, Record
Buffer Controls
Copy Active Image to Buffer
A B C D E New
SAVE A Save Active To 1
Options Memory = 251 MB
Image Display Controls
Zoom 0.25 15740
Bri Black
Con 27260
Options White
Float Dose Microscope
0.0000 na 60 LM
Def -126.7 um IS 0.00 um
Reset
Obj 66.66% Spot 2
Tilt Control
Camera & Macro Controls
Setup View Focus Trial Record
Preview End Resume STOP
Navigator
Label: 1 Registration point 1 Corner point (C)
Color Green Draw Rotate when load For anchor map
#1 Note:
Acquire (A) Tilt series New file at item New file at group
Set: File Properties Imaging State TS Parameters Filename
Registration 1 Draw all reg. Draw none Collapse groups
Add Stage Pos Label Color X Y Z Type Reg. Acq. Note
Add Points
Add Polygon
Move Item
Update Z
Go To XY
Go To XYZ
Go To Marker
Load Map
1 Grn -152.4 275.3 127.5 Poly 1 A
A
1
50 um
1) = 23104

The next step is to select New file at item. This will bring up Properties of File to O... window.



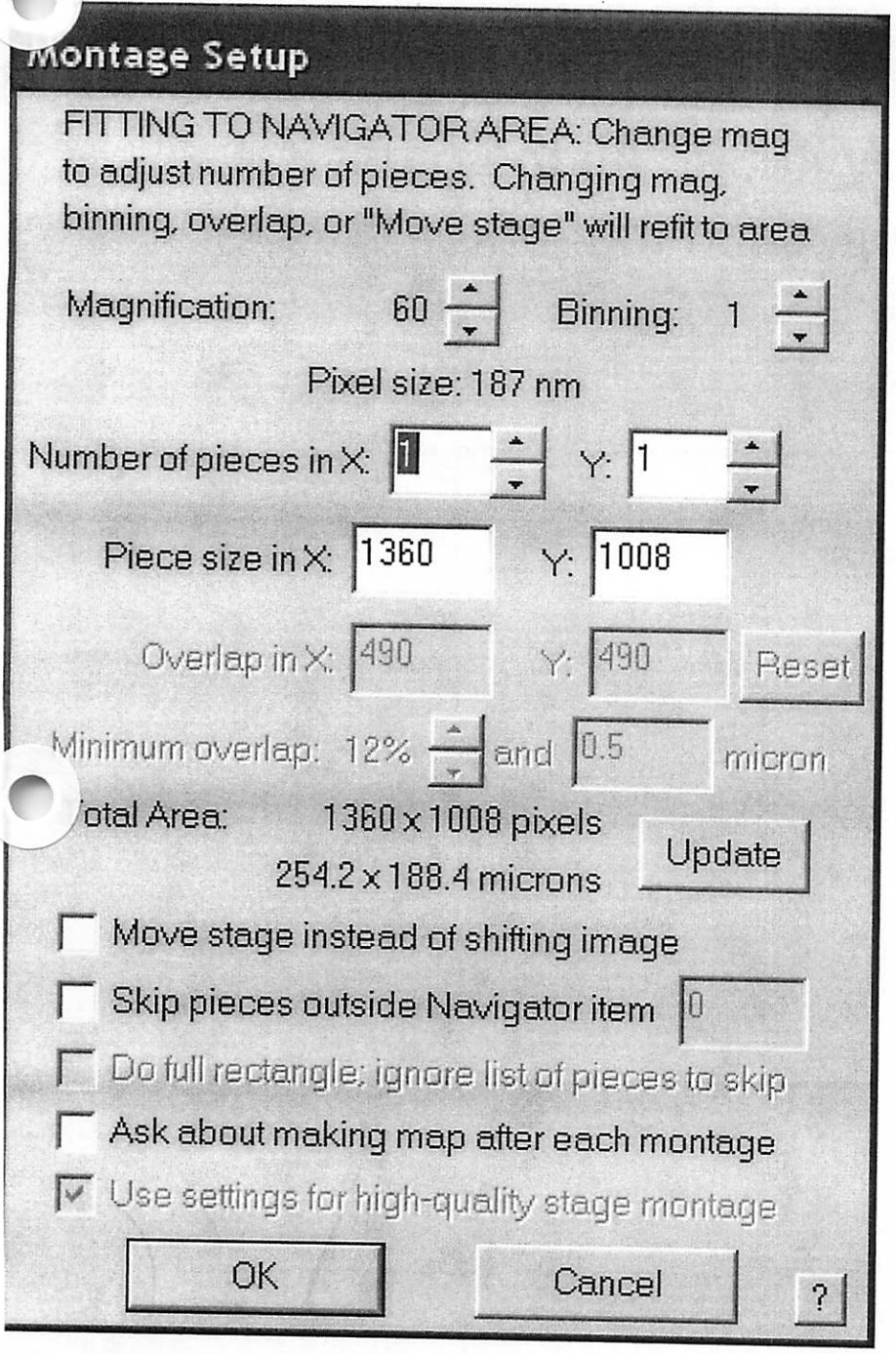
Select Montage Images.



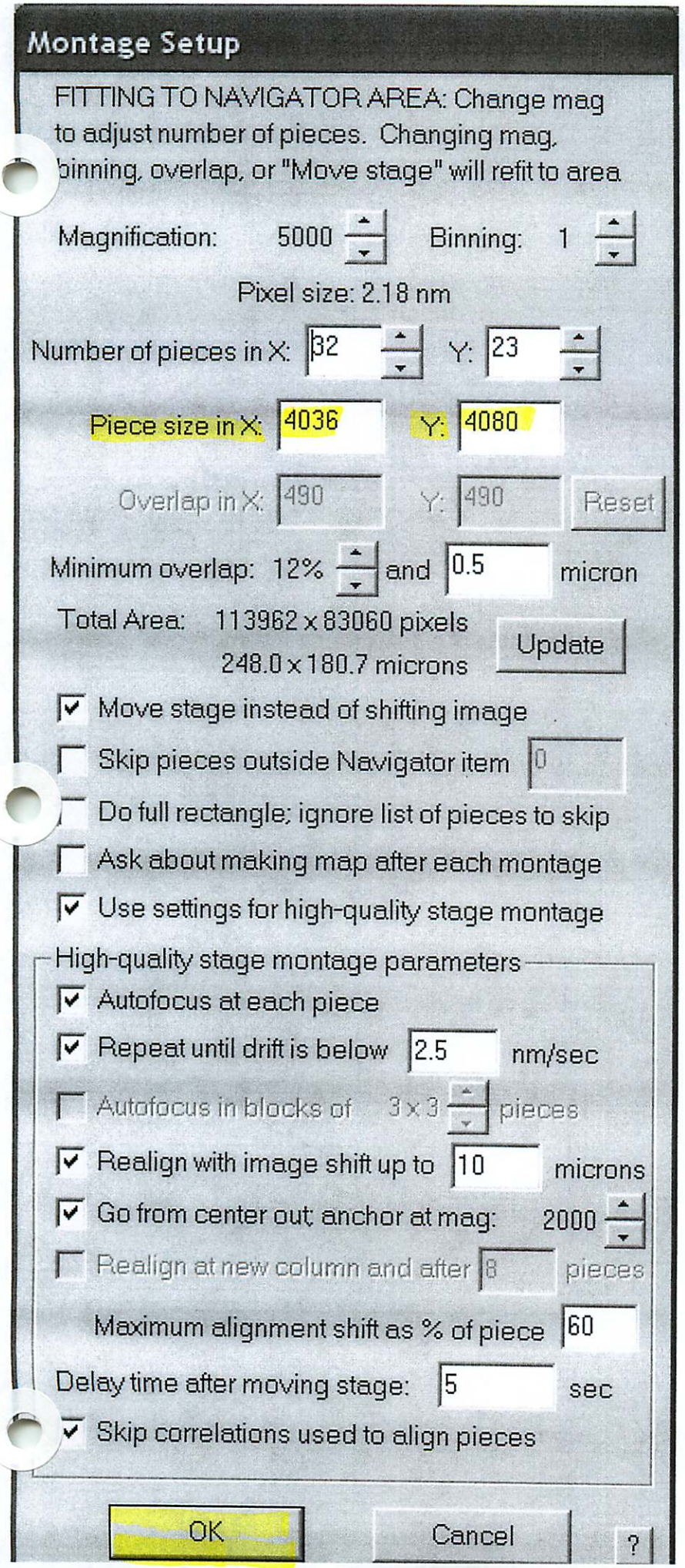
Select Fit montage to polygon.

Click OK.

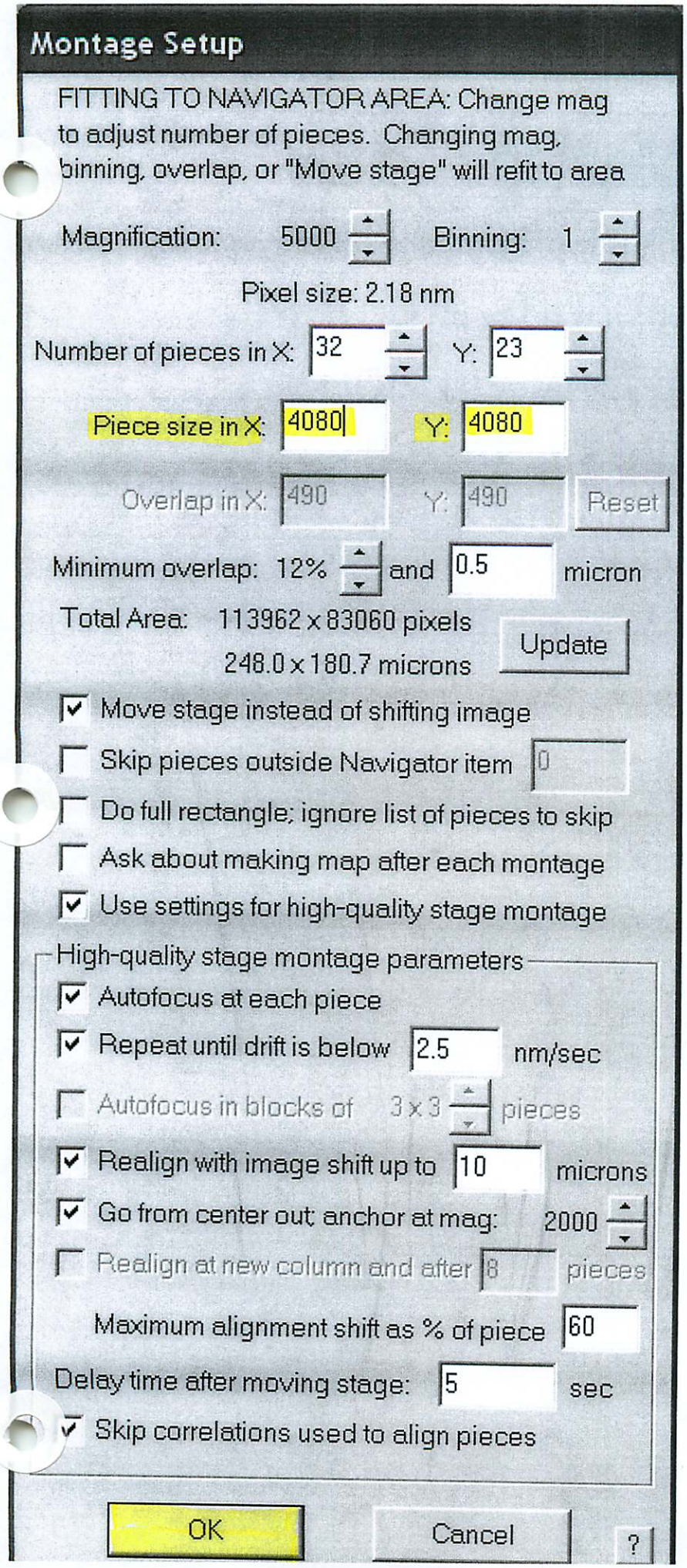
Upon clicking OK in Properties of File to O... window, the Montage Setup window is opened. In this version of the window increase the mag to 5000 (Magnification: 60) using the adjacent up button. As you do so the bottom of the window will expand downward (see next page).


Montage Setup
FITTING TO NAVIGATOR AREA: Change mag to adjust number of pieces. Changing mag, binning, overlap, or "Move stage" will refit to area
Magnification: 60
Binning: 1
Pixel size: 187 nm
Number of pieces in X: 1
Y: 1
Piece size in X: 1360
Y: 1008
Overlap in X: 490
Y: 490
Reset
Minimum overlap: 12%
and 0.5
micron
Total Area: 1360 x 1008 pixels
254.2 x 188.4 microns
Update
Move stage instead of shifting image
Skip pieces outside Navigator item 0
Do full rectangle; ignore list of pieces to skip
Ask about making map after each montage
Use settings for high-quality stage montage
OK
Cancel
?

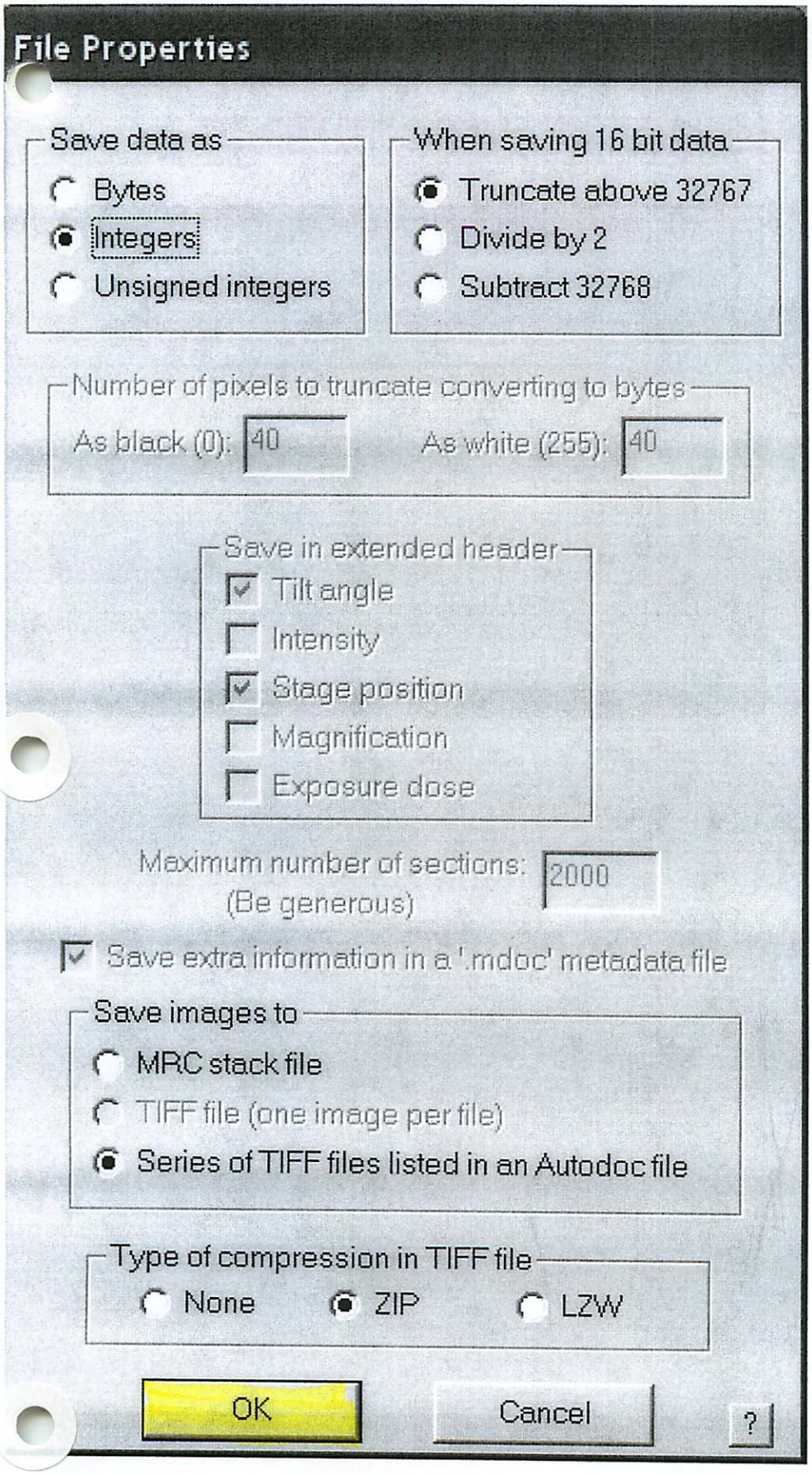
In the expanded Montage Setup window the only item that needs to be corrected is Pixel size in X: Y: ..... the number of pixels should be 4080 by 4080. All the other settings are default such that once selected they appear so in subsequent captures. Click OK to proceed.


Montage Setup
FITTING TO NAVIGATOR AREA: Change mag to adjust number of pieces. Changing mag, binning, overlap, or "Move stage" will refit to area
Magnification: 5000
Binning: 1
Pixel size: 2.18 nm
Number of pieces in X: 32
Y: 23
Piece size in X: 4036
Y: 4080
Overlap in X: 490
Y: 490
Reset
Minimum overlap: 12%
and 0.5
micron
Total Area: 113962 x 83060 pixels
248.0 x 180.7 microns
Update
Move stage instead of shifting image
Skip pieces outside Navigator item 0
Do full rectangle; ignore list of pieces to skip
Ask about making map after each montage
Use settings for high-quality stage montage
High-quality stage montage parameters
Autofocus at each piece
Repeat until drift is below 2.5 nm/sec
Autofocus in blocks of 3 x 3 pieces
Realign with image shift up to 10 microns
Go from center out; anchor at mag: 2000
Realign at new column and after 8 pieces
Maximum alignment shift as % of piece 60
Delay time after moving stage: 5 sec
Skip correlations used to align pieces
OK
Cancel
?

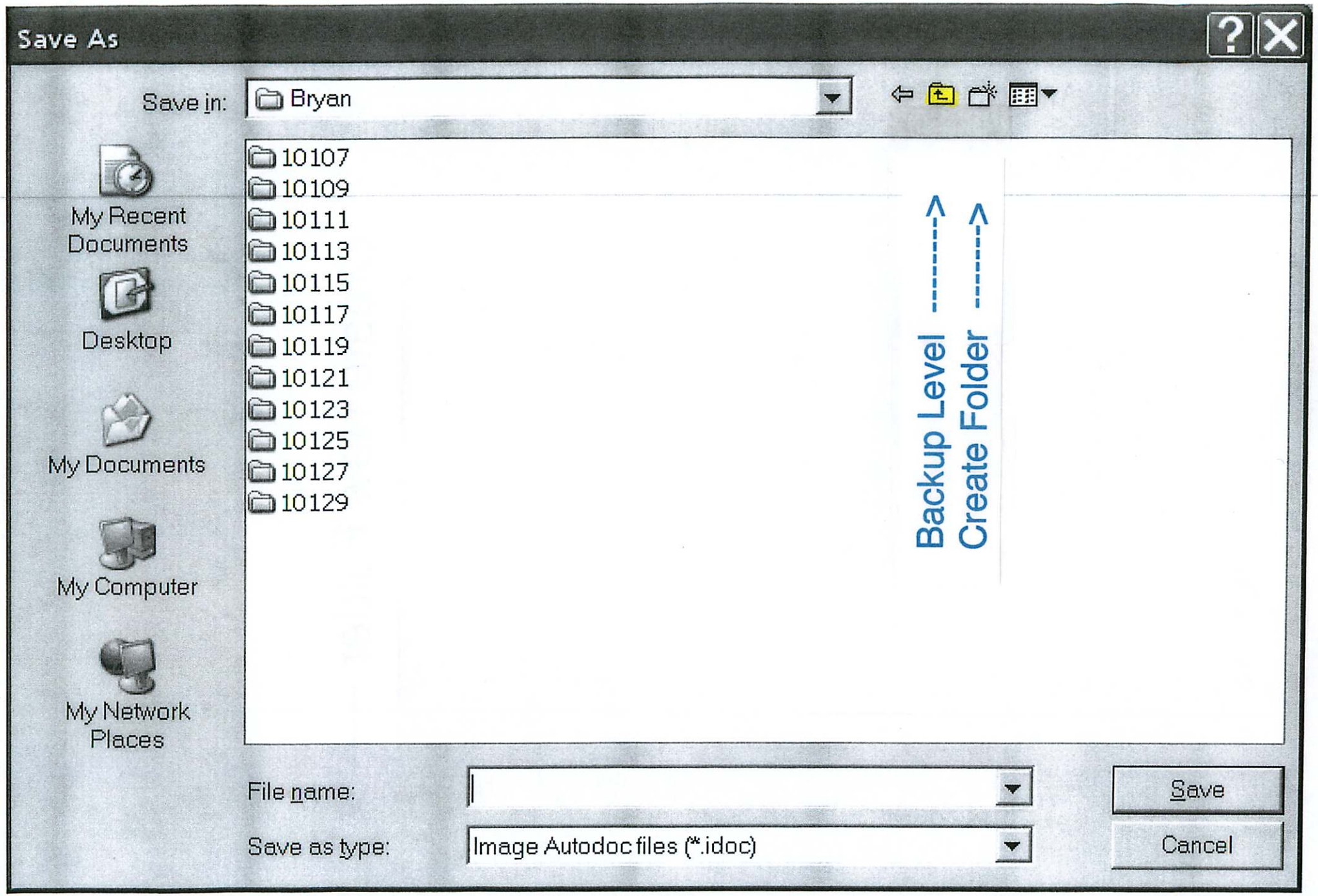
4080 by 4080 ---------->


Montage Setup
FITTING TO NAVIGATOR AREA: Change mag to adjust number of pieces. Changing mag, binning, overlap, or "Move stage" will refit to area
Magnification: 5000
Binning: 1
Pixel size: 2.18 nm
Number of pieces in X: 32
Y: 23
Piece size in X: 4080
Y: 4080
Overlap in X: 490
Y: 490
Reset
Minimum overlap: 12%
and 0.5
micron
Total Area: 113962 x 83060 pixels
248.0 x 180.7 microns
Update
Move stage instead of shifting image
Skip pieces outside Navigator item 0
Do full rectangle; ignore list of pieces to skip
Ask about making map after each montage
Use settings for high-quality stage montage
High-quality stage montage parameters
Autofocus at each piece
Repeat until drift is below 2.5 nm/sec
Autofocus in blocks of 3 x 3 pieces
Realign with image shift up to 10 microns
Go from center out; anchor at mag: 2000
Realign at new column and after 8 pieces
Maximum alignment shift as % of piece 60
Delay time after moving stage: 5 sec
Skip correlations used to align pieces
OK
Cancel
?

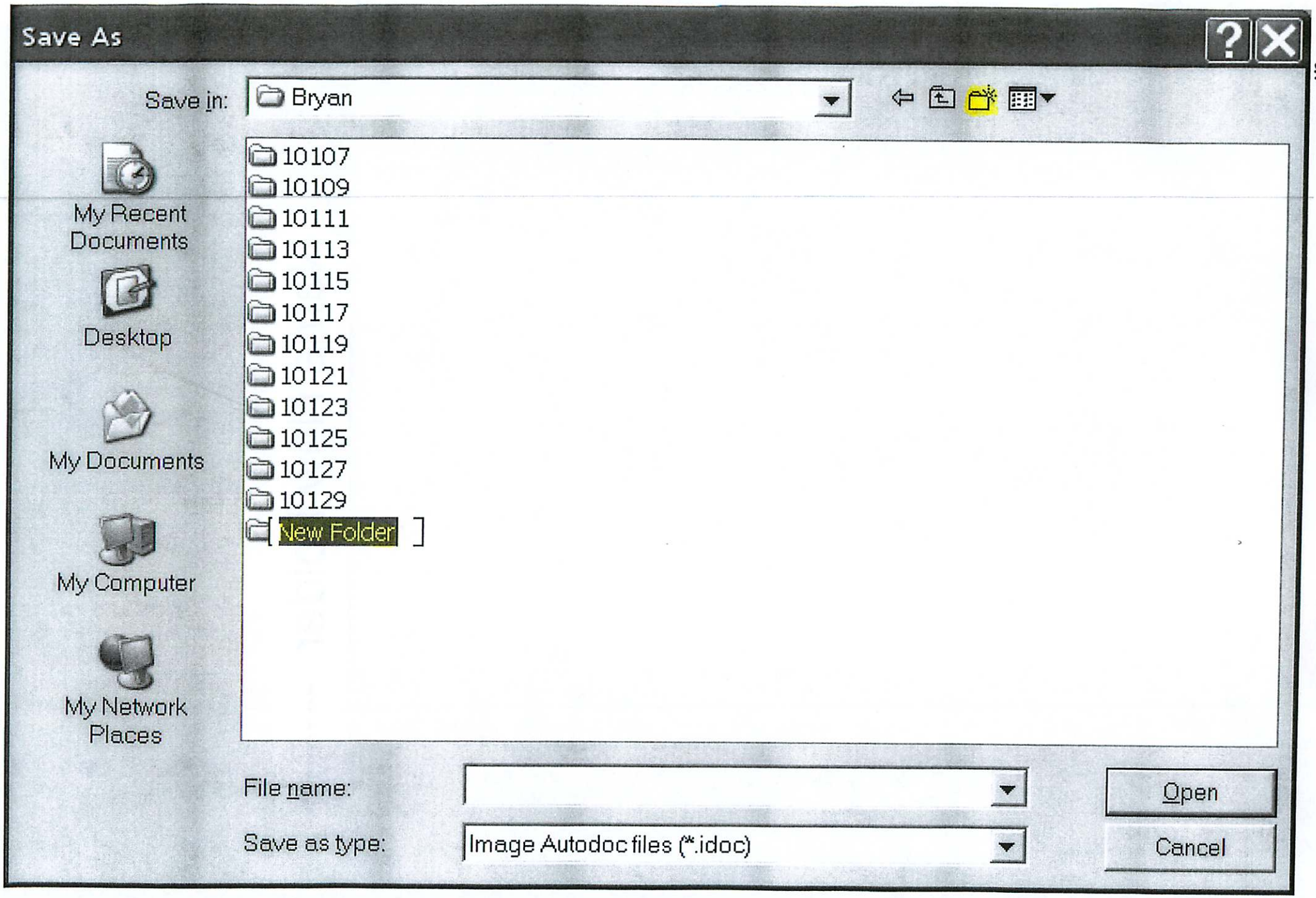
Clicking OK in Montage Setup window opens File Properties window. All selections are default. Important one is Save images to: Series of TIFF files listed in an Autodoc file. Click OK to open Save As window.


File Properties
Save data as
Bytes
Integers
Unsigned integers
When saving 16 bit data
Truncate above 32767
Divide by 2
Subtract 32768
Number of pixels to truncate converting to bytes
As black (0): 40
As white (255): 40
Save in extended header
Tilt angle
Intensity
Stage position
Magnification
Exposure dose
Maximum number of sections:
(Be generous)
2000
Save extra information in a '.mdoc' metadata file
Save images to
MRC stack file
TIFF file (one image per file)
Series of TIFF files listed in an Autodoc file
Type of compression in TIFF file
None
ZIP
LZW
OK
Cancel
?

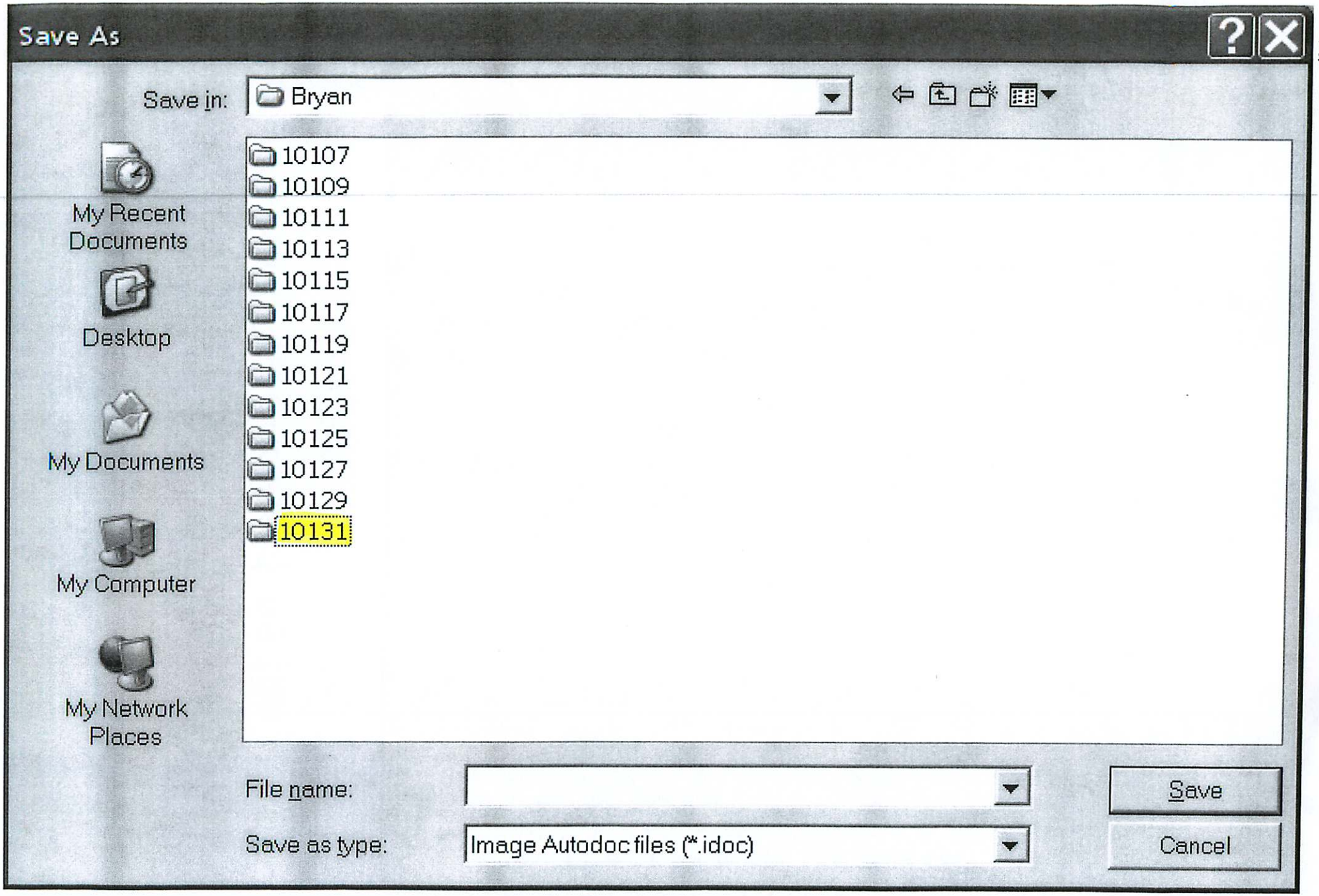
Create new folder in which the images from the present capture will collect.


Save As
Save in:
Bryan
My Recent Documents
Desktop
My Documents
My Computer
My Network Places
10107
10109
10111
10113
10115
10117
10119
10121
10123
10125
10127
10129
Backup Level ------->
Create Folder ------->
File name:
Save as type:
Image Autodoc files (*.idoc)
Save
Cancel

Create New Folder ------>

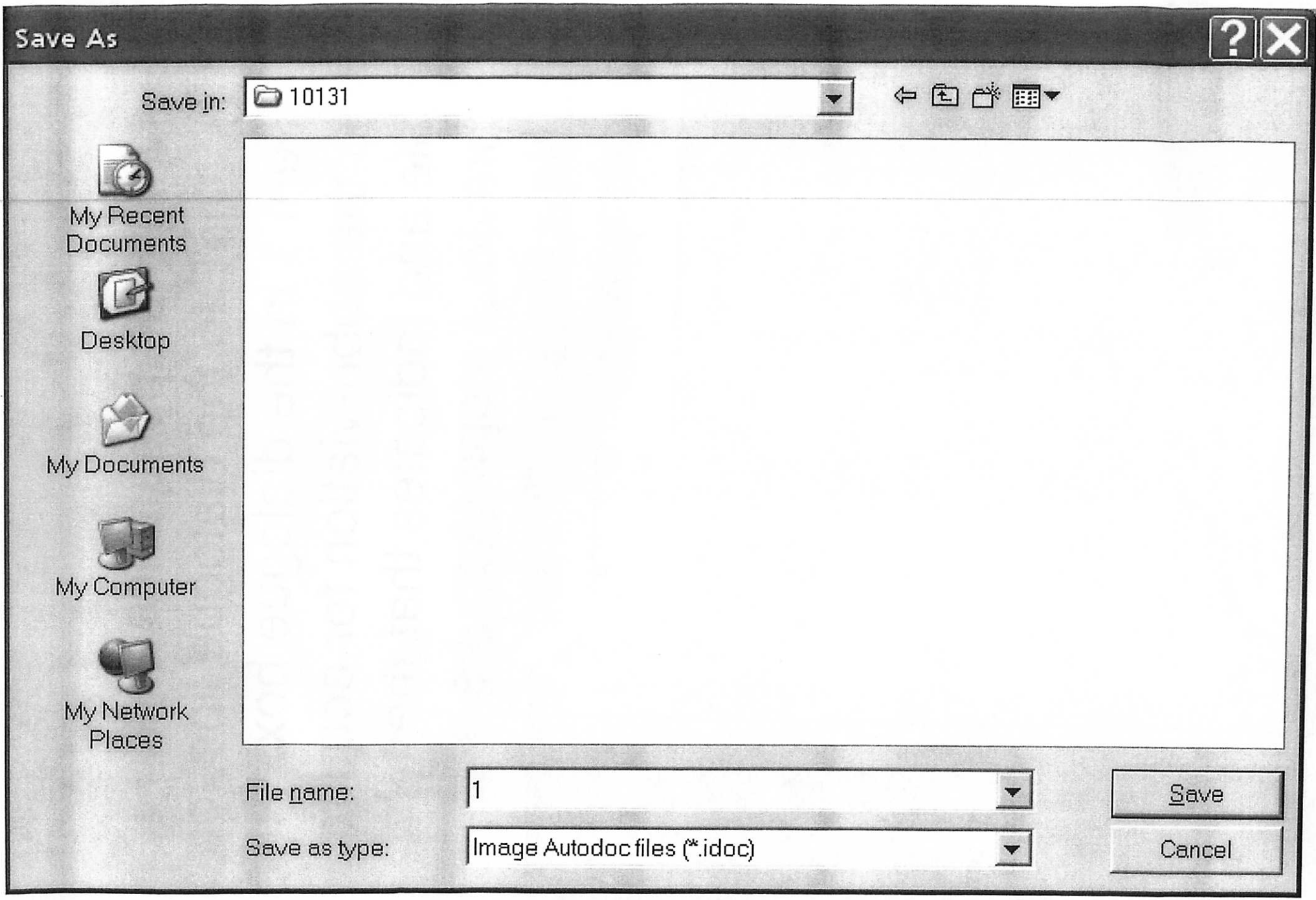

Save As
Save in:
Bryan
My Recent Documents
Desktop
My Documents
My Computer
My Network Places
10107
10109
10111
10113
10115
10117
10119
10121
10123
10125
10127
10129
New Folder
File name:
Save as type:
Image Autodoc files (*.idoc)
Open
Cancel

Name New Folder ------>

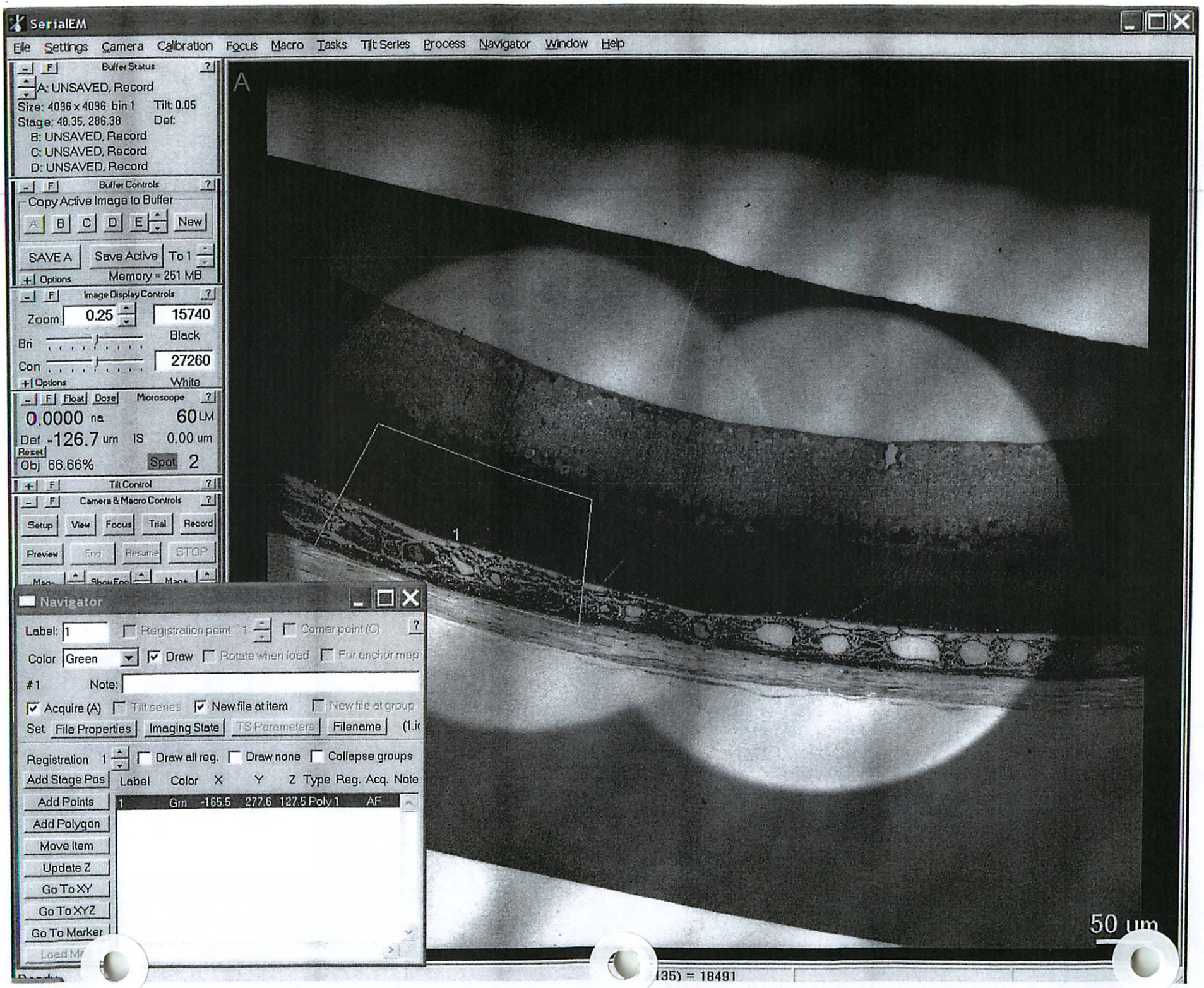

Save As
Save in:
Bryan
10107
10109
10111
10113
10115
10117
10119
10121
10123
10125
10127
10129
10131
My Recent Documents
Desktop
My Documents
My Computer
My Network Places
File name:
Save as type:
Image Autodoc files (*.idoc)
Save
Cancel

Open New Folder ------>

Select file name. Have been routinely choosing 1 as file name for all captures.


Save As
Save in:
10131
My Recent Documents
Desktop
My Documents
My Computer
My Network Places
File name:
1
Save as type:
Image Autodoc files (*.idoc)
Save
Cancel

Note the appearance of the letter F in the dialogue line for item 1 in the dialogue box. AF is the abbreviation for acquire file and indicates that the two previous steps (Acquire and New file at item) are completed.


SerialEM
File Settings Camera Calibration Focus Macro Tasks Tilt Series Process Navigator Window Help
Buffer Status
A: UNSAVED, Record
Size: 4096 x 4096 bin 1 Tilt: 0.05
Stage: 48.35, 286.38 Def:
B: UNSAVED, Record
C: UNSAVED, Record
D: UNSAVED, Record
Buffer Controls
Copy Active Image to Buffer
A B C D E New
SAVE A Save Active To 1
Options Memory = 251 MB
Image Display Controls
Zoom 0.25 15740
Bri Black
Con 27260
Options White
Float Dose Microscope
0.0000 na 60 LM
Def -126.7 um IS 0.00 um
Reset
Obj 66.66% Spot 2
Tilt Control
Camera & Macro Controls
Setup View Focus Trial Record
Preview End Resume STOP
Navigator
Label: 1 Registration point 1 Corner point (C)
Color Green Draw Rotate when loaded For anchor map
#1 Note:
Acquire (A) Tilt series New file at item New file at group
Set File Properties Imaging State TS Parameters Filename
Registration 1 Draw all reg. Draw none Collapse groups
Add Stage Pos Label Color X Y Z Type Reg. Acq. Note
Add Points
Add Polygon
Move Item
Update Z
Go To XY
Go To XYZ
Go To Marker
1 Grn -165.5 277.6 127.5 Poly 1 AF
A
1
50 um
135) = 18491

Change the magnification to 5,000X before proceeding to the next step, image shift calibration. *Make sure the objective aperture is inserted before increasing the magnification*.

*

If the magnification is not changed from 60X to 5000X before performing the image shift calibration, SerialEM is corrupted and will not function until the following correction is made:

Carl,

I think you are suffering from that bad initial image shift calibration at 60x - I'm glad you mentioned it. This gets remembered in the file SEMshortTermCal.txt in the C:\Program Files\SerialEM folder

Exit SerialEM and rename that file to something else so that I can see what
is in there at some point. Restart SerialEM and it should behave OK.

We can add a property setting to have the program always ignore the short term
cals, which would probably make sense in your case.

David

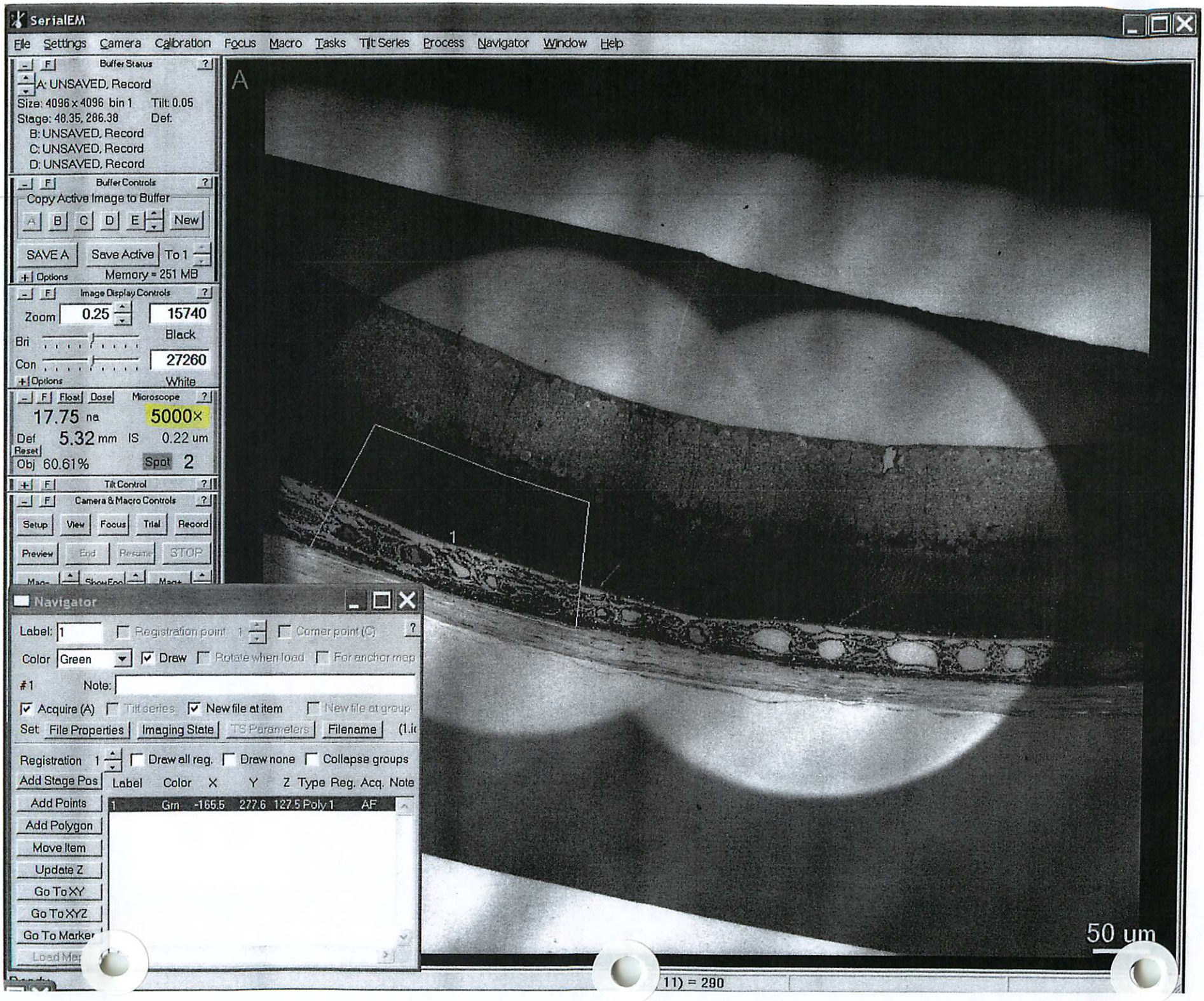
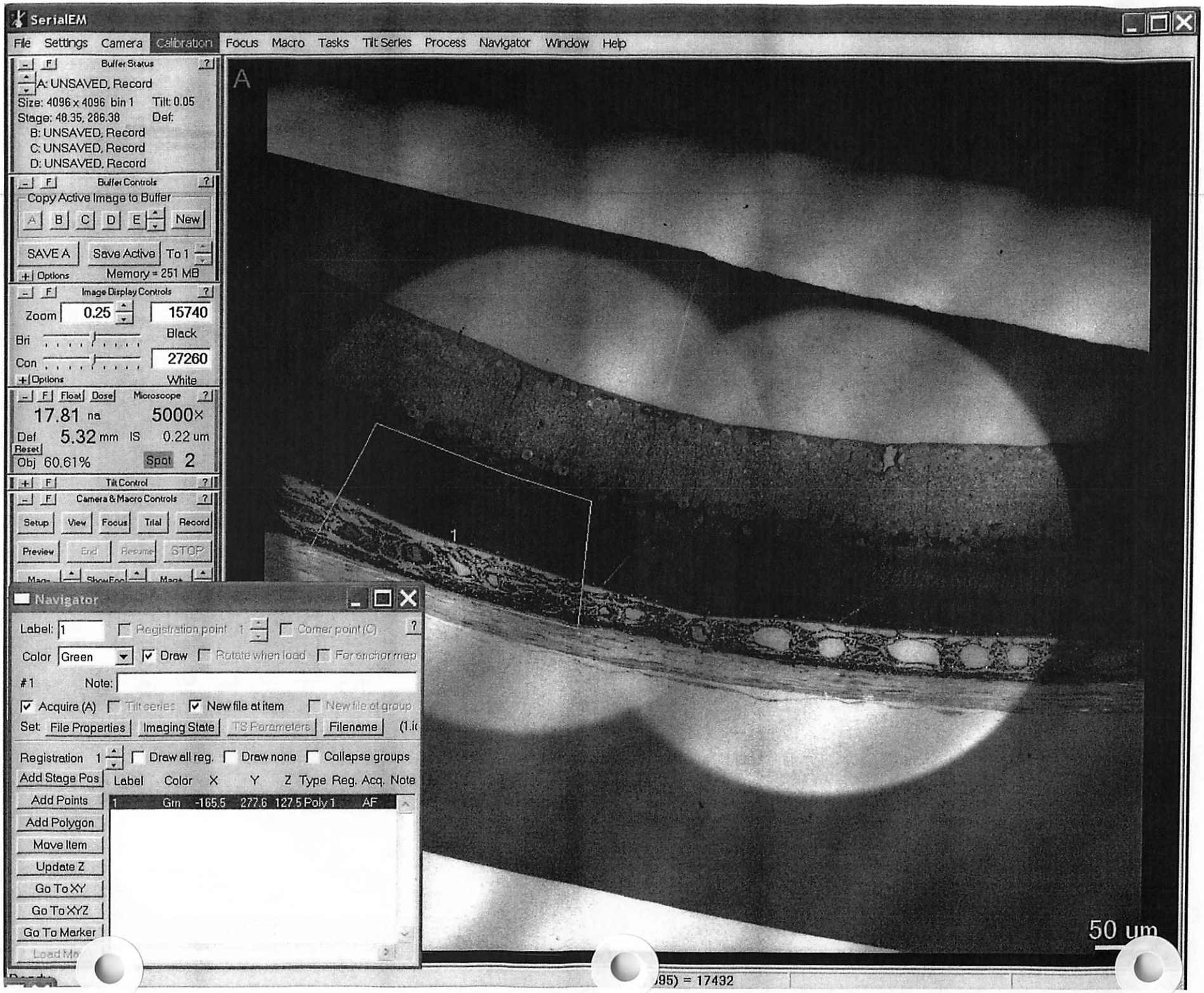
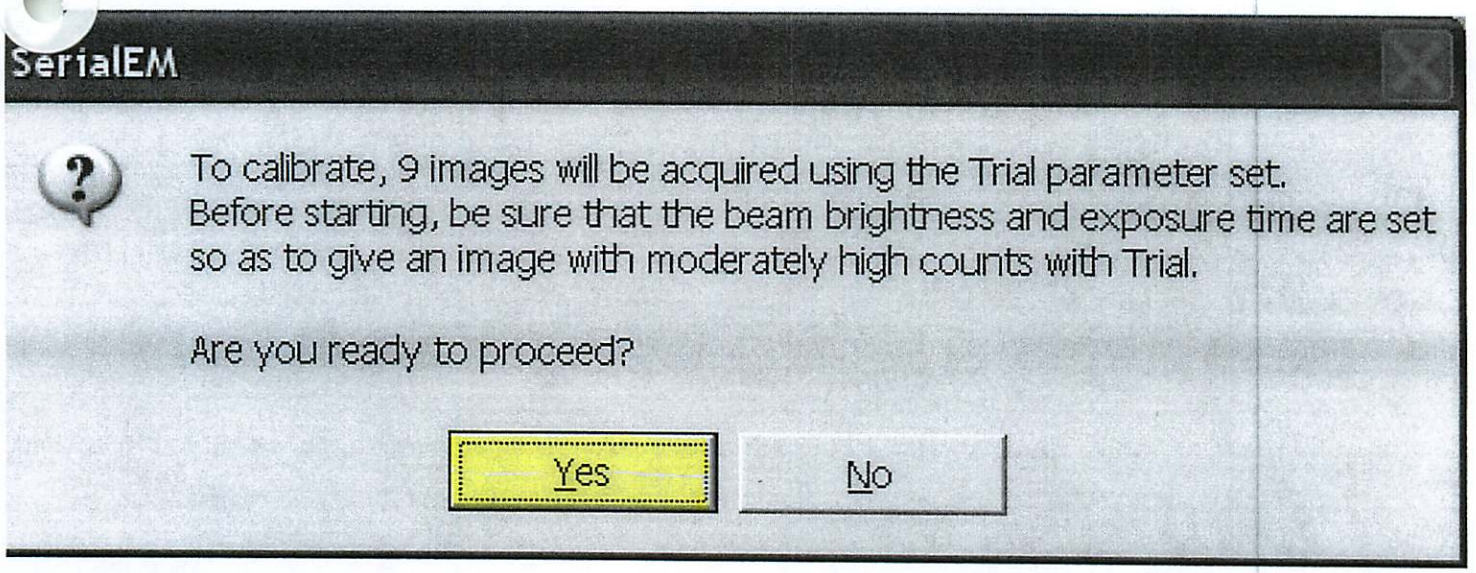

SerialEM
File Settings Camera Calibration Focus Macro Tasks Tilt Series Process Navigator Window Help
Buffer Status
A: UNSAVED, Record
Size: 4096 x 4096 bin 1 Tilt: 0.05
Stage: 48.35, 286.38 Def:
B: UNSAVED, Record
C: UNSAVED, Record
D: UNSAVED, Record
Buffer Controls
Copy Active Image to Buffer
A B C D E New
SAVE A Save Active To 1
Options Memory = 251 MB
Image Display Controls
Zoom 0.25 15740
Bri Black
Con 27260
Options White
Float Dose Microscope
17.75 na 5000×
Def 5.32 mm IS 0.22 um
Reset
Obj 60.61% Spot 2
Tilt Control
Camera & Macro Controls
Setup View Focus Trial Record
Preview End Resume STOP
A
1
Navigator
Label: 1 Registration point 1 Corner point (C)
Color Green Draw Rotate when load For anchor map
#1 Note:
Acquire (A) Tilt series New file at item New file at group
Set: File Properties Imaging State TS Parameters Filename
Registration 1 Draw all reg. Draw none Collapse groups
Add Stage Pos
Label Color X Y Z Type Reg. Acq. Note
1 Grn -165.5 272.6 127.5 Poly 1 AF
Add Points
Add Polygon
Move Item
Update Z
Go To XY
Go To XYZ
Go To Marker
50 um
11) = 290

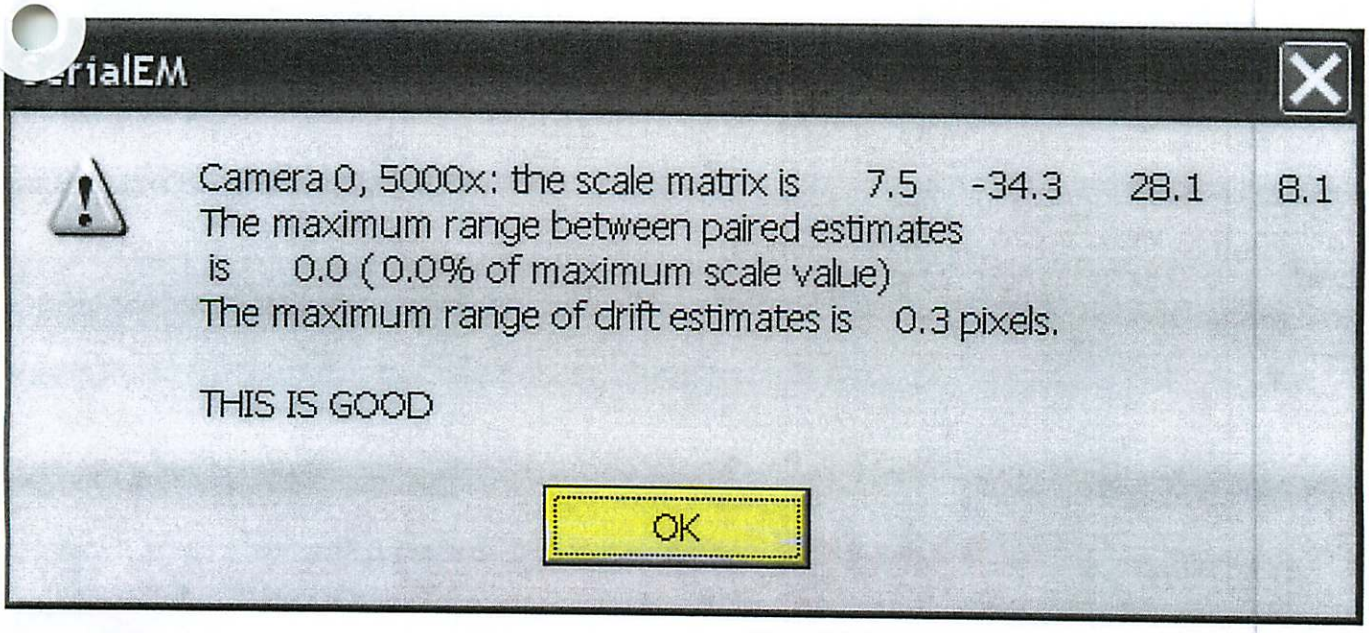
Image Shift calibration is found in the drop down menu under Calibration in the SerialEM menu.


SerialEM
File Settings Camera Calibration Focus Macro Tasks Tilt Series Process Navigator Window Help
Buffer Status
A: UNSAVED, Record
Size: 4096 x 4096 bin 1 Tilt: 0.05
Stage: 48.35, 286.38 Def:
B: UNSAVED, Record
C: UNSAVED, Record
D: UNSAVED, Record
Buffer Controls
Copy Active Image to Buffer
A B C D E New
SAVE A Save Active To 1
Options Memory = 251 MB
Image Display Controls
Zoom 0.25 15740
Bri Black
Con 27260
Options White
Float Dose Microscope
17.81 na 5000×
Def 5.32 mm IS 0.22 um
Reset
Obj 60.61% Spot 2
Tilt Control
Camera & Macro Controls
Setup View Focus Trial Record
Preview End Resume STOP
Navigator
Label: 1 Registration point 1 Corner point (C)
Color Green Draw Rotate when load For anchor map
#1 Note:
Acquire (A) Tilt series New file at item New file at group
Set: File Properties Imaging State TS Parameters Filename
Registration 1 Draw all reg. Draw none Collapse groups
Add Stage Pos Label Color X Y Z Type Reg. Acq. Note
Add Points
Add Polygon
Move Item
Update Z
Go To XY
Go To XYZ
Go To Marker
1 Grn -165.5 277.6 127.5 Poly 1 AF
A
1
50 um
095) = 17432

Before clicking Yes and proceeding with the image shift calibration, again make sure the mag is set at 5,000X. Also, make sure the objective aperture is centered and the beam is both centered and condensed until the current density is in the range of 100 pA/cm2 with the small fluorescent focusing screen in place. The current density reading is found in the drop-down Screen panel located in the left-hand TEM Systems Task Bar of the TEM Center for JEOL-1400 window.

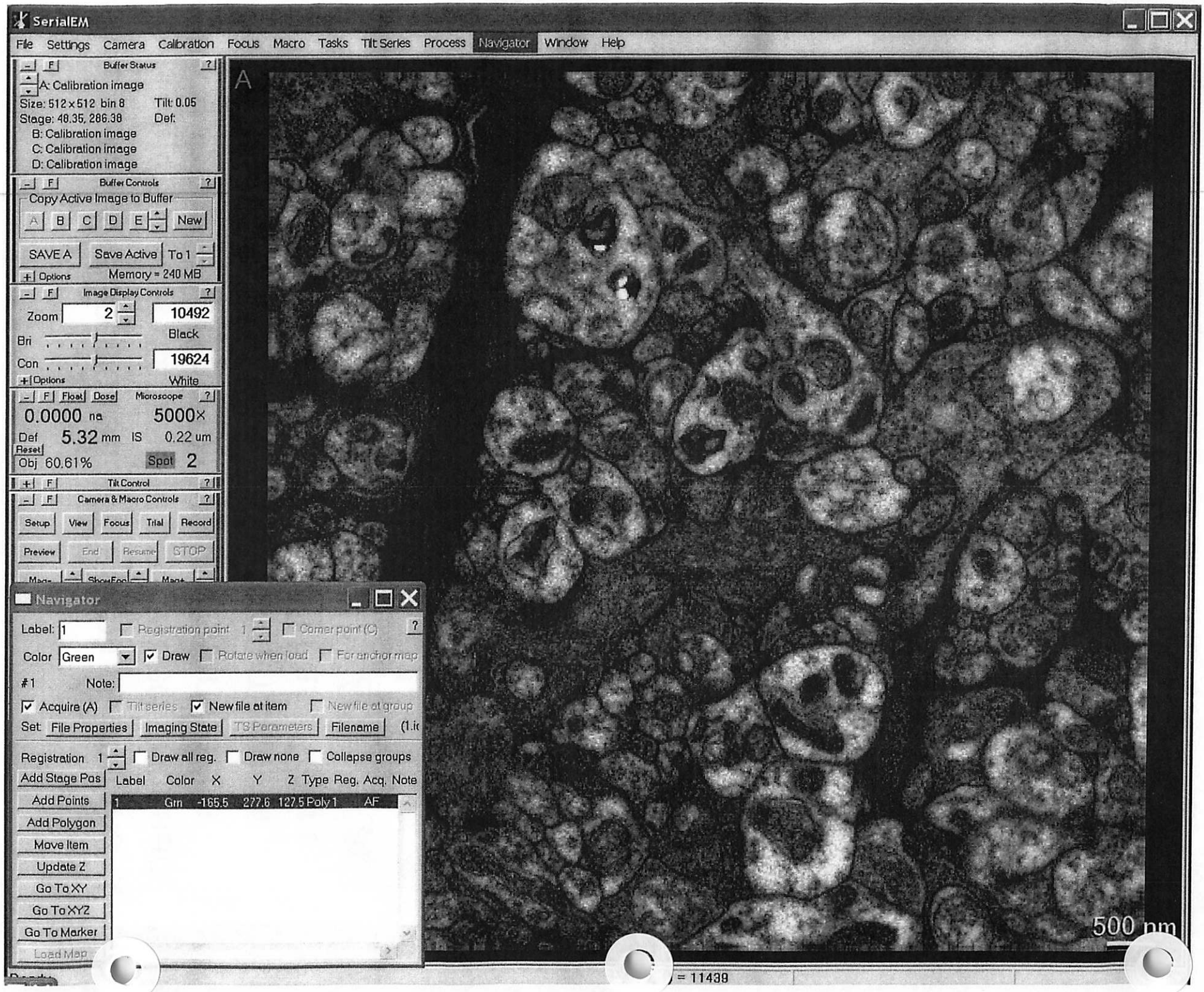
* 100 pA/cm2 is the current density routinely chosen for capture.


SerialEM
To calibrate, 9 images will be acquired using the Trial parameter set.
Before starting, be sure that the beam brightness and exposure time are set so as to give an image with moderately high counts with Trial.
Are you ready to proceed?
Yes
No

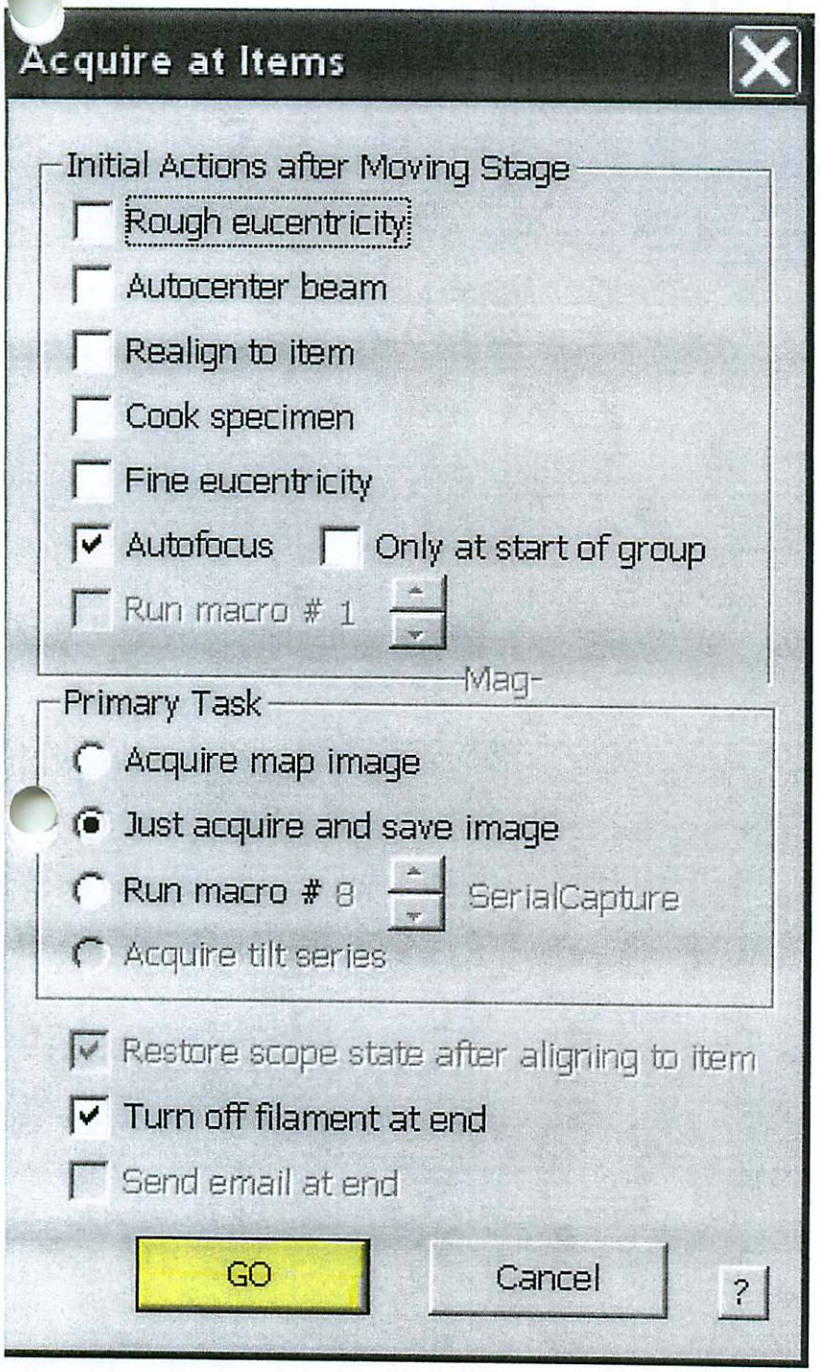

SerialEM
Camera 0, 5000x: the scale matrix is 7.5 -34.3 28.1 8.1
The maximum range between paired estimates
is 0.0 (0.0% of maximum scale value)
The maximum range of drift estimates is 0.3 pixels.
THIS IS GOOD
OK

Navigator Menu ------->

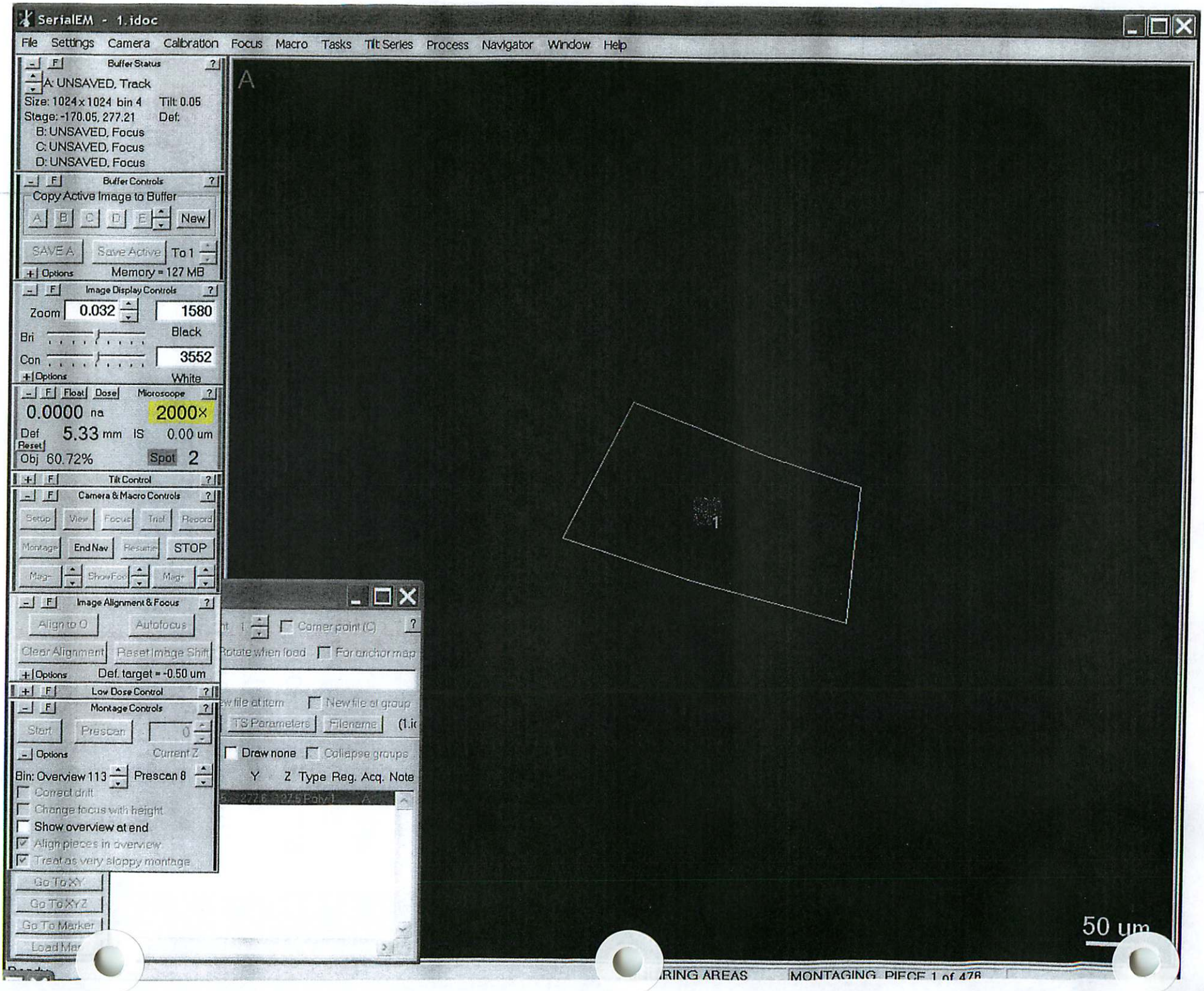
(see next page)


SerialEM
File Settings Camera Calibration Focus Macro Tasks Tilt Series Process Navigator Window Help
Buffer Status
A: Calibration image
Size: 512 x 512 bin 8
Tilt: 0.05
Stage: 48.35, 286.38
Def:
B: Calibration image
C: Calibration image
D: Calibration image
Buffer Controls
Copy Active Image to Buffer
A B C D E New
SAVE A
Save Active
To 1
Options
Memory = 240 MB
Image Display Controls
Zoom 2
10492
Bri
Black
Con
19624
Options
White
Float Dose Microscope
0.0000 na
5000×
Def 5.32 mm IS 0.22 um
Reset
Obj 60.61%
Spot 2
Tilt Control
Camera & Macro Controls
Setup View Focus Trial Record
Preview End Resume STOP
Navigator
Label: 1
Registration point 1
Corner point (C)
Color Green
Draw
Rotate when load
For anchor map
#1 Note:
Acquire (A)
Tilt series
New file at item
New file at group
Set: File Properties Imaging State TS Parameters Filename
Registration 1
Draw all reg.
Draw none
Collapse groups
Add Stage Pos
Label Color X Y Z Type Reg. Acq. Note
Add Points
Add Polygon
Move Item
Update Z
Go To XY
Go To XYZ
Go To Marker
Load Map
1 Grn -165.5 277.6 127.5 Poly 1 AF
A
500 nm
= 11439

With the scope ready for capture and the image shift calibration performed, proceed with the next and final step, starting the capture. Go to the drop-down menu for Navigator in the upper Menu bar. Select Acquire at Points to pull up the Acquire at Items window. The settings are default. *Make sure to select Turn of filament at end.*


Acquire at Items
Initial Actions after Moving Stage
Rough eucentricity
Autocenter beam
Realign to item
Cook specimen
Fine eucentricity
Autofocus
Only at start of group
Run macro # 1
Mag-
Primary Task
Acquire map image
Just acquire and save image
Run macro # 8
SerialCapture
Acquire tilt series
Restore scope state after aligning to item
Turn off filament at end
Send email at end
GO
Cancel
?

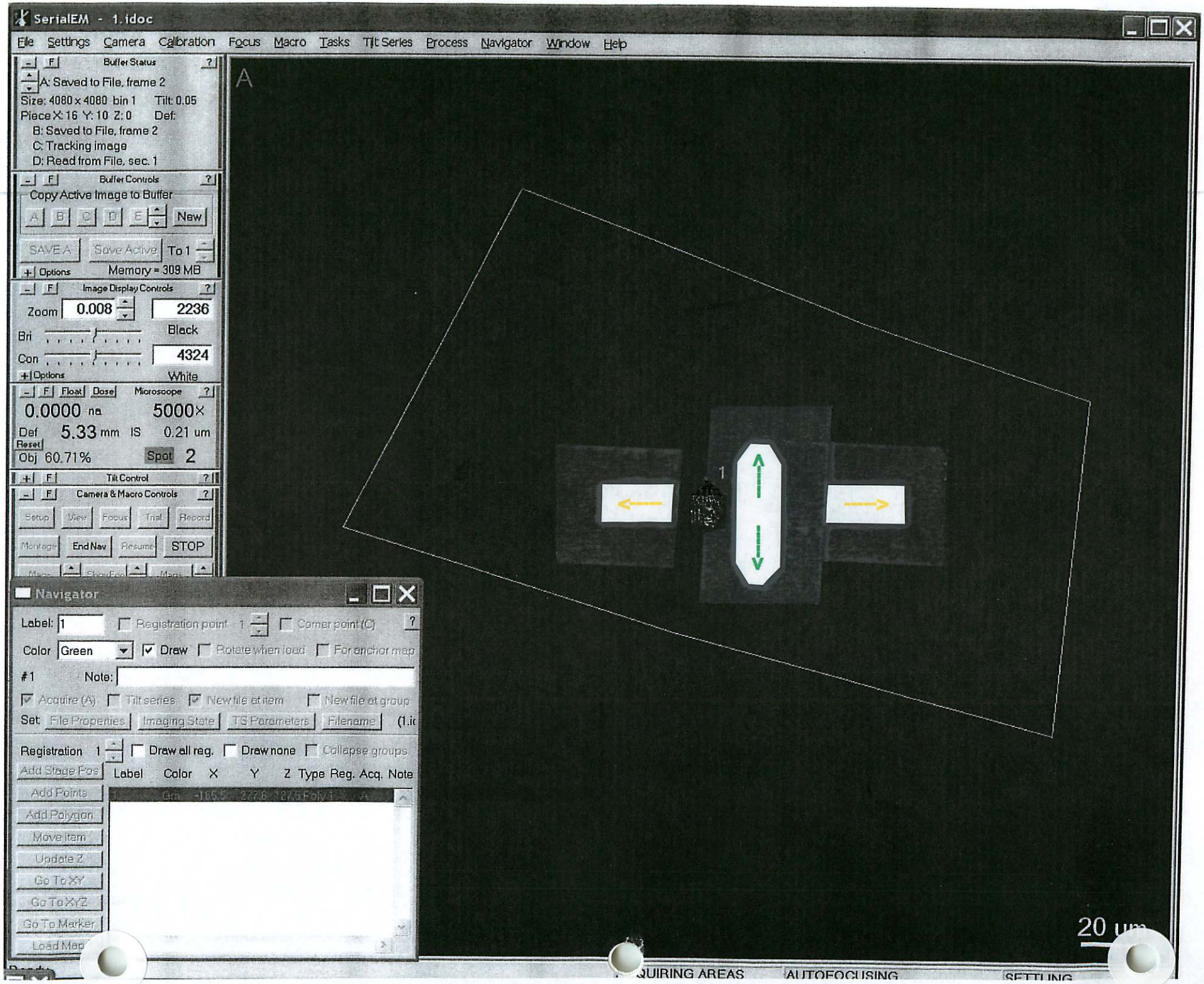
Initial image shift calibration capture at 2,000X.


SerialEM - 1.idoc
File Settings Camera Calibration Focus Macro Tasks Tilt Series Process Navigator Window Help
Buffer Status
A: UNSAVED, Track
Size: 1024 x 1024 bin 4 Tilt: 0.05
Stage: -170.05, 277.21 Def:
B: UNSAVED, Focus
C: UNSAVED, Focus
D: UNSAVED, Focus
Buffer Controls
Copy Active Image to Buffer
A B C D E New
SAVE A Save Active To 1
Options Memory = 127 MB
Image Display Controls
Zoom 0.032 1580
Bri Black
Con 3552
Options White
Float Dose Microscope
0.0000 na 2000×
Def 5.33 mm IS 0.00 um
Reset
Obj 60.72% Spot 2
Tilt Control
Camera & Macro Controls
Setup View Focus Trial Record
Montage End Nav Resume STOP
Image Alignment & Focus
Align to 0 Autofocus
Clear Alignment Reset Image Shift
Options Def. target = -0.50 um
Low Dose Control
Montage Controls
Start Prescan 0
Options Current Z
Bin: Overview 113 Prescan 8
Correct drift
Change focus with height
Show overview at end
Align pieces in overview
Treat as very sloppy montage
Go To XY
Go To XYZ
Go To Marker
Corner point (C)
Rotate when load
For anchor map
New file at item
New file at group
TS Parameters
Filename
Draw none
Collapse groups
Y Z Type Reg. Acq. Note
A
1
50 um
MONTAGING PIECE 1 of 478

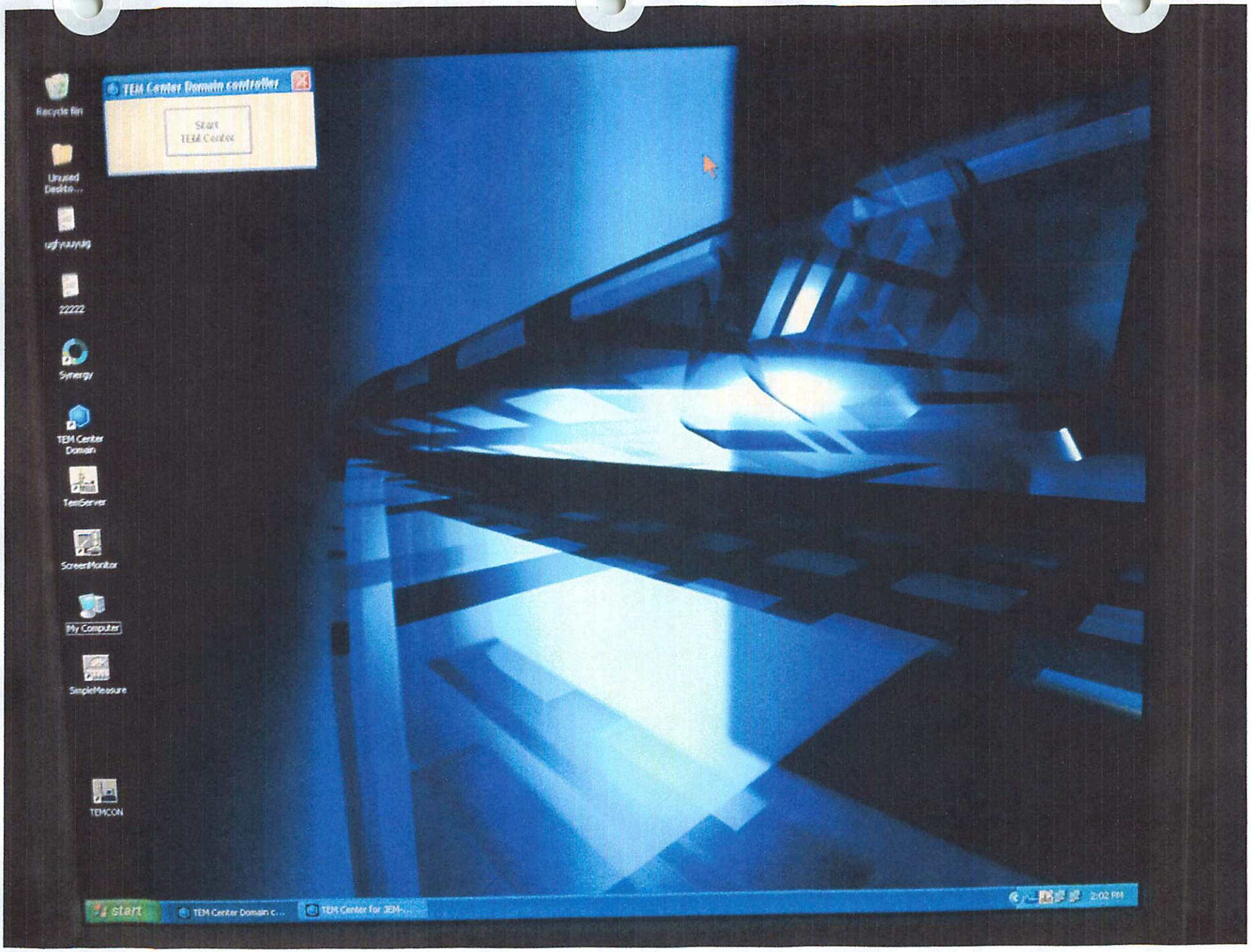
The capture starts at a midpoint in the polygon drawn around the area to be captured. The initial segment of the first row is captured in a downward direction from this point. The remaining segment of the first row is completed by capturing in an upward direction from the midpoint. The capture moves one row to the left and proceeds in the same pattern as the first. After the left-hand side of the polygon has been captured, the right-hand side is captured in the same manner.

* **Position the capture so that the long axis is in the horizontal plane.**

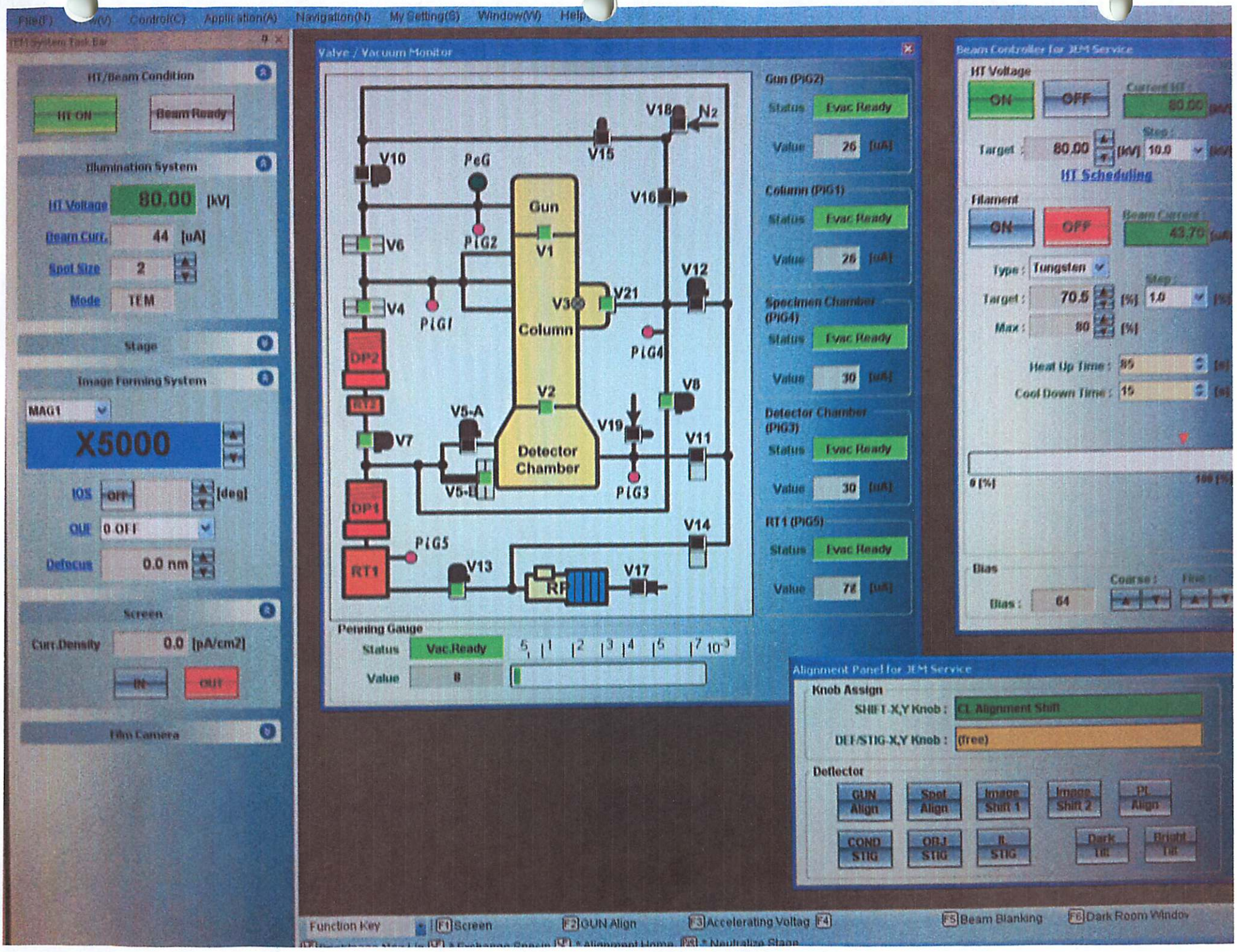
Watch the capture of the first row in both the downward (south) and upward (north) direction to insure that the area of interest is within the capture polygon. This is necessary because of the shift from low mag (60X) in which the polygon is drawn to high mag (5,000X) in which the capture is made. This shift is primarily from south to north and varies from 10 to 20 um.

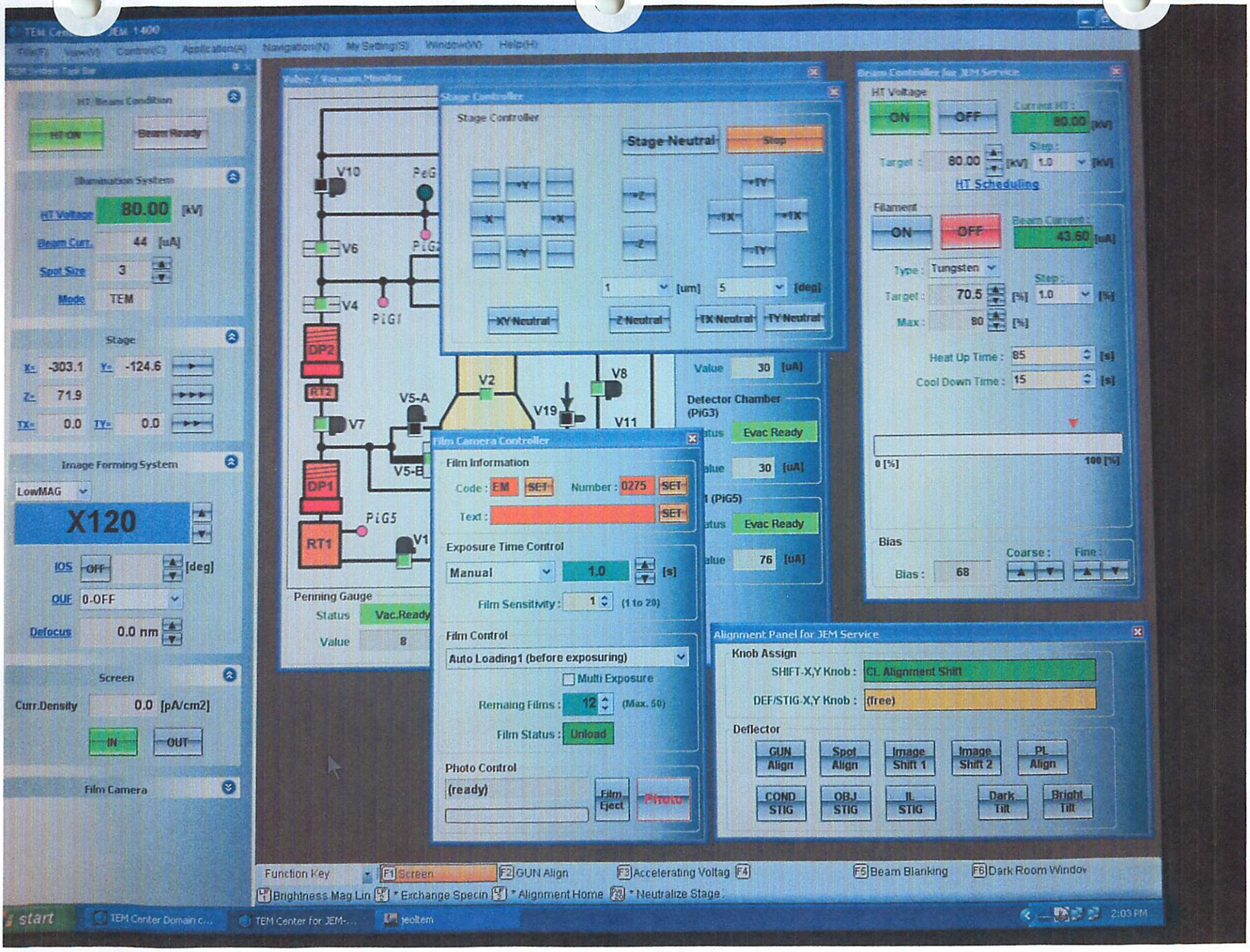

SerialEM - 1.idoc
File Settings Camera Calibration Focus Macro Tasks Tilt Series Process Navigator Window Help
Buffer Status
A: Saved to File, frame 2
Size: 4080 x 4080 bin 1 Tilt: 0.05
Piece X: 16 Y: 10 Z: 0 Def:
B: Saved to File, frame 2
C: Tracking image
D: Read from File, sec. 1
Buffer Controls
Copy Active Image to Buffer
A B C D E New
SAVE A Save Active To 1
Options Memory = 309 MB
Image Display Controls
Zoom 0.008 2236
Bri Black
Con 4324
Options White
Float Dose Microscope
0.0000 na 5000×
Def 5.33 mm IS 0.21 um
Reset
Obj 60.71% Spot 2
Tilt Control
Camera & Macro Controls
Setup View Focus Trial Record
Montage End Nav Resume STOP
Navigator
Label: 1 Registration point 1 Corner point (C)
Color Green Draw Rotate when load For anchor map
#1 Note:
Acquire (A) Tilt series New file at item New file at group
Set: File Properties Imaging State TS Parameters Filename
Registration 1 Draw all reg. Draw none Collapse groups
Add Stage Pos Label Color X Y Z Type Reg. Acq. Note
Add Points
Add Polygon
Move Item
Update Z
Go To XY
Go To XYZ
Go To Marker
Load Map
A
1
20 um
QUIRING AREAS
AUTOFOCUSING
SETTLING

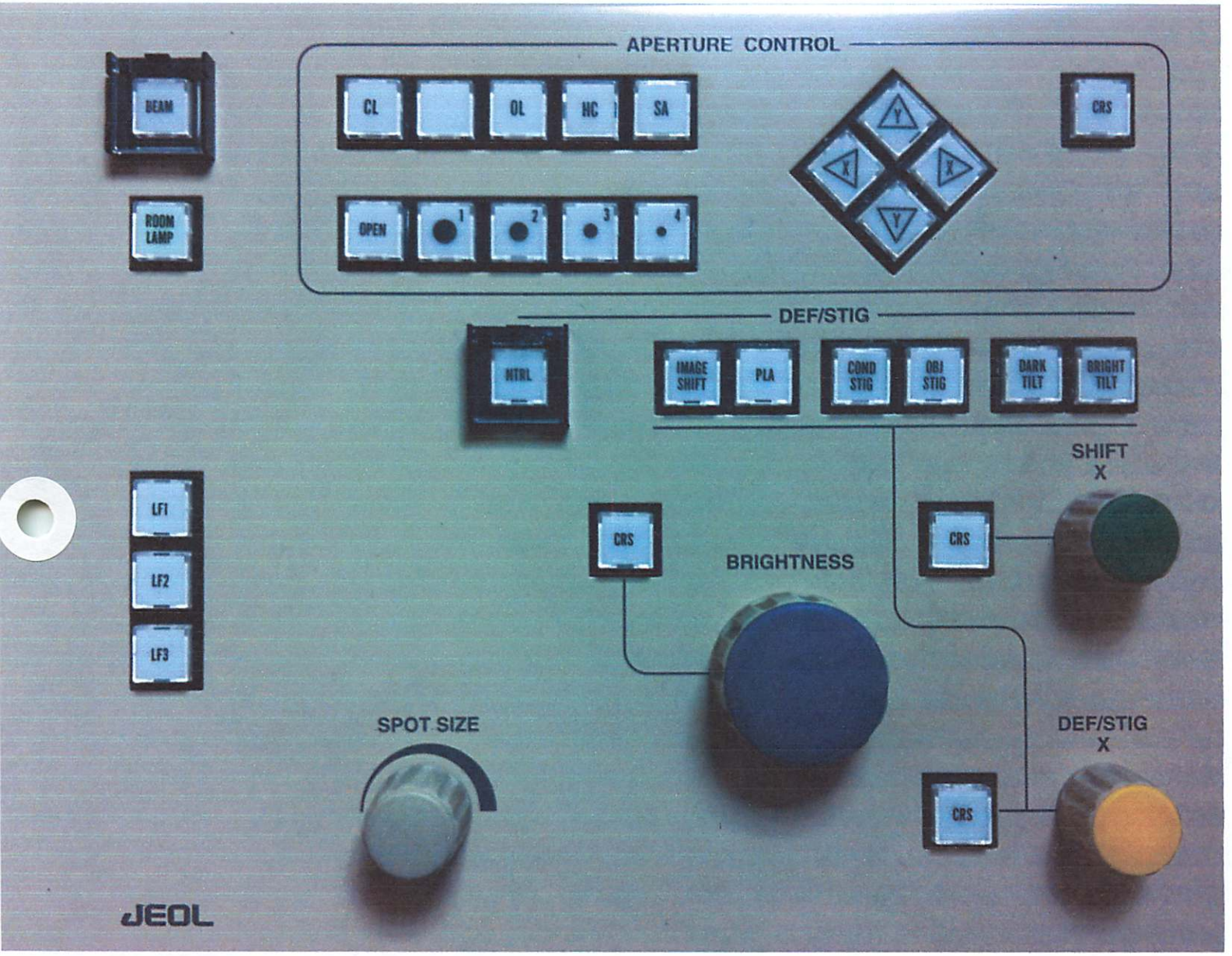
PANELS and SCREENS


TEM Center Domain controller
Start
TEM Center
Recycle Bin
Unused
Desktop...
ugfyaaryug
22222
Synergy
TEM Center
Domain
TemServer
ScreenMonitor
My Computer
SimpleMeasure
TEMCON
start
TEM Center Domain c...
TEM Center for JEM-...
2:02 PM

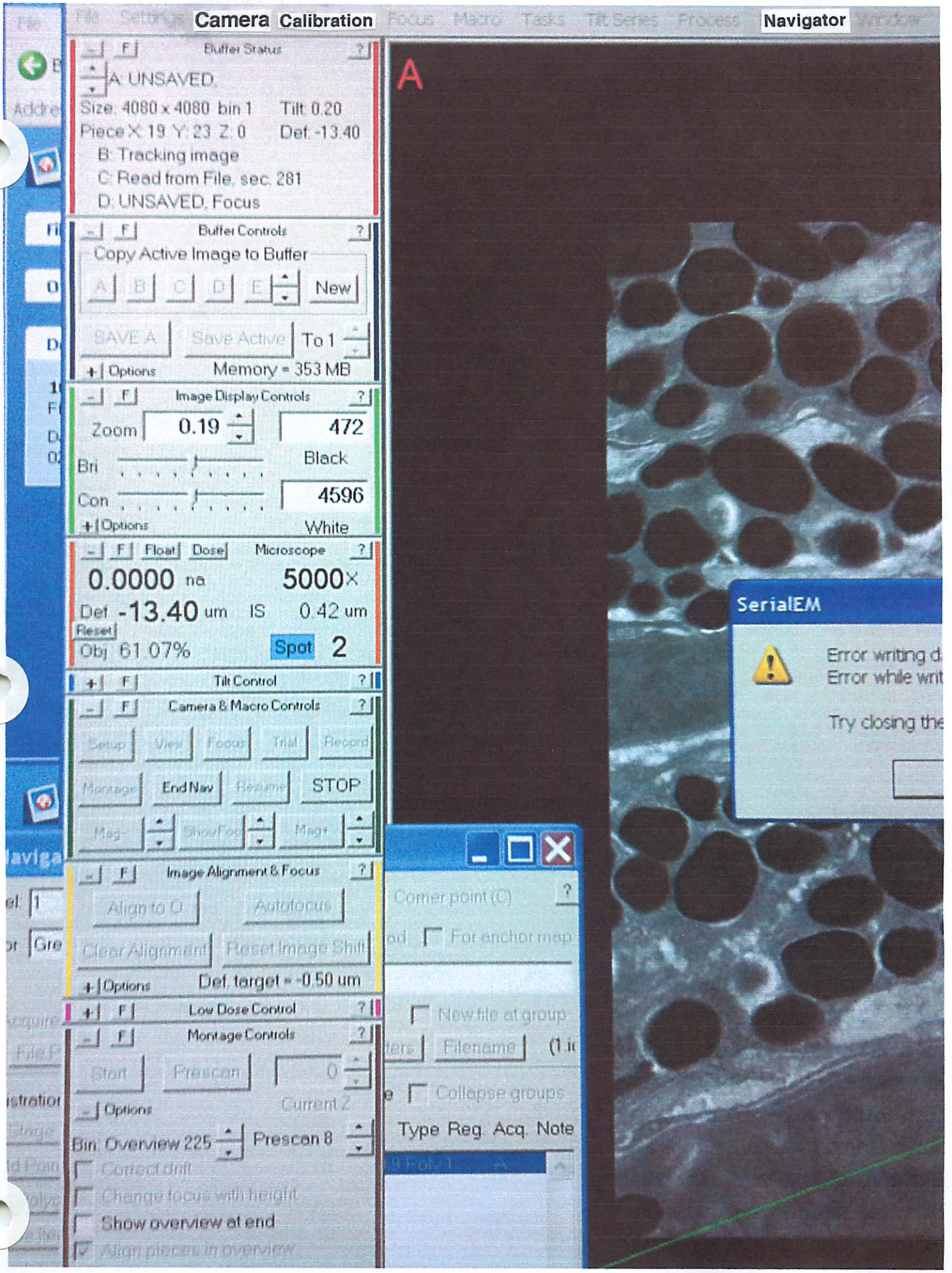

TEM Center Domain controller
Start TEM Center
Synergy
TEM Center Domain
TemServer
ScreenMonitor
TEMCON
Controller for JEM-1400
File Dialogue Monitor Property Option Maintenance Display JEOLS Help
HT SPC Photo Status VAC
F1: F2: F3: F4: F5: F6:
EM-21010/2131HTR : High Tilt Holder
HT
Beam Ready
Acc.80.00 kV
Beam Current 44 µA
TEM Spot 3
LOWMAG : X120
Defocus : 0.0 nm
Cur. Dens. 0.0 pA/cm2
Exp. Time 1 sec M
X= -303.1 µm
Y= -124.6 µm
Z= 71.9 µm
TX= 0.0 deg
TY= 0.0 deg
SF Refresh
Stage Neutral
Connected
start
TEM Center Domain c...
TEM Center for JEM-...
jeoltem
2:02 PM

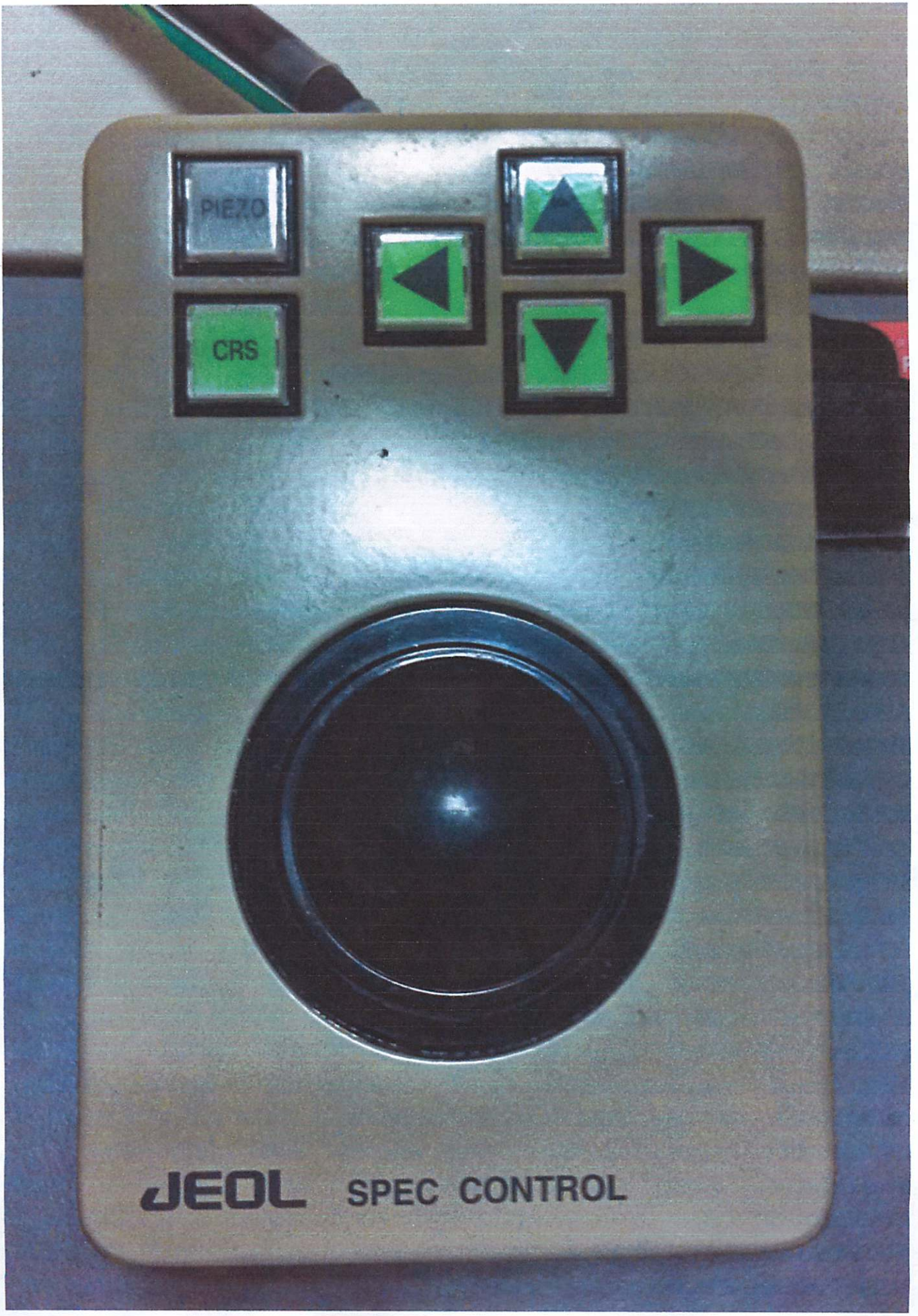

File(F) Control(C) Application(A) Navigation(N) My Setting(S) Window(W) Help
HT/Beam Condition
HT ON
Beam Ready
Illumination System
HT Voltage 80.00 [kV]
Beam Curr. 44 [uA]
Spot Size 2
Mode TEM
Stage
Image Forming System
MAG1
X5000
IOS OFF [deg]
OUE 0 OFF
Defocus 0.0 nm
Screen
Curr.Density 0.0 [pA/cm2]
IN
OUT
Film Camera
Valve / Vacuum Monitor
V18
N2
V10
PeG
V15
V16
Gun
V1
V6
PIG2
V12
V4
PIG1
V3
V21
Column
PIG4
DP2
V2
V8
V5-A
V19
V11
V7
Detector Chamber
V5-B
PIG3
DP1
V14
PIG5
RT1
V13
RP
V17
Penning Gauge
Status Vac.Ready
Value 8
Gun (PiG2)
Status Evac Ready
Value 26 [uA]
Column (PIG1)
Status Evac Ready
Value 26 [uA]
Specimen Chamber (PiG4)
Status Evac Ready
Value 30 [uA]
Detector Chamber (PIG3)
Status Evac Ready
Value 30 [uA]
RT1 (PiG5)
Status Evac Ready
Value 78 [uA]
Beam Controller for JEM Service
HT Voltage
ON
OFF
Target 80.00 [kV]
HT Scheduling
Filament
ON
OFF
Type : Tungsten
Target : 70.5 [%]
Max : 80 [%]
Heat Up Time : 85 [s]
Cool Down Time : 15 [s]
Bias
Bias : 64
Alignment Panel for JEM Service
Knob Assign
SHIFT-X,Y Knob : CL Alignment Shift
DEF/STIG-X,Y Knob : (free)
Deflector
GUN Align
Spot Align
Image Shift 1
Image Shift 2
PL Align
COND STIG
OBJ STIG
IL STIG
Dark Tilt
Bright Tilt
Function Key
F1 Screen
F2 GUN Align
F3 Accelerating Voltag
F4
F5 Beam Blanking
F6 Dark Room Windo


JEM 1400
HT Beam Condition
HT ON
Beam Ready
Illumination System
HT Voltage 80.00 [kV]
Beam Curr. 44 [uA]
Spot Size 3
Mode TEM
Stage
X= -303.1 Y= -124.6
Z= 71.9
TX= 0.0 TY= 0.0
Image Forming System
LowMAG
X120
IOS OFF [deg]
OUF 0.OFF
Defocus 0.0 nm
Screen
Curr.Density 0.0 [pA/cm2]
IN OUT
Film Camera
Stage Controller
Stage Neutral
Stop
XY Neutral
Z Neutral
TX Neutral
TY Neutral
Penning Gauge
Status Vac.Ready
Value 8
Value 30 [uA]
Detector Chamber (PiG3)
Evac Ready
Value 30 [uA]
Evac Ready
Value 76 [uA]
Film Camera Controller
Film Information
Code : EM SET Number : 0275 SET
Text : SET
Exposure Time Control
Manual 1.0 [s]
Film Sensitivity : 1 (1 to 20)
Film Control
Auto Loading1 (before exposuring)
Multi Exposure
Remaing Films : 12 (Max. 50)
Film Status : Unload
Photo Control
(ready)
Film Eject
Photo
HT Voltage
ON OFF
Current HT : 80.00 [kV]
Target : 80.00 [kV] Step : 1.0 [kV]
HT Scheduling
Filament
ON OFF
Beam Current : 43.60 [uA]
Type : Tungsten
Target : 70.5 [%] Step : 1.0 [%]
Max : 80 [%]
Heat Up Time : 85 [s]
Cool Down Time : 15 [s]
0 [%] 100 [%]
Bias
Bias : 68
Coarse : Fine :
Alignment Panel for JEM Service
Knob Assign
SHIFT-X,Y Knob : CL Alignment Shift
DEF/STIG-X,Y Knob : (free)
Deflector
GUN Align
Spot Align
Image Shift 1
Image Shift 2
PL Align
COND STIG
OBJ STIG
IL STIG
Dark Tilt
Bright Tilt
Function Key
F1 Screen
F2 GUN Align
F3 Accelerating Voltag
F4
F5 Beam Blanking
F6 Dark Room Windov
Brightness Mag Lin
* Exchange Specin
* Alignment Home
* Neutralize Stage
start
TEM Center Domain c...
TEM Center for JEM-...
jeoltem
2:03 PM

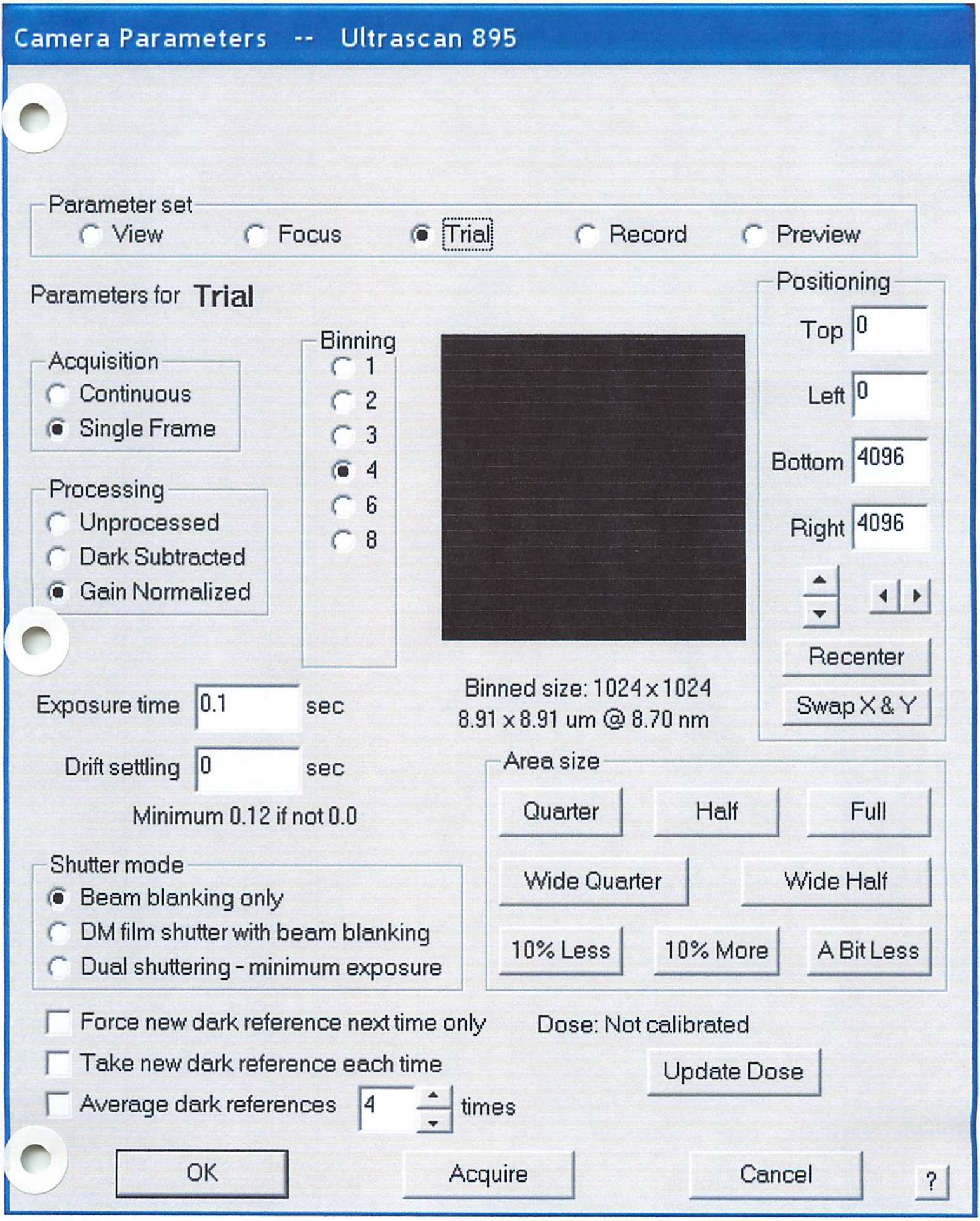

APERTURE CONTROL
BEAM
CL
OL
HC
SA
CRS
ROOM LAMP
OPEN
1
2
3
4
Y
X
X
Y
DEF/STIG
NTRL
IMAGE SHIFT
PLA
COND STIG
OBJ STIG
DARK TILT
BRIGHT TILT
SHIFT X
LF1
LF2
LF3
CRS
CRS
BRIGHTNESS
SPOT SIZE
DEF/STIG X
CRS
JEOL

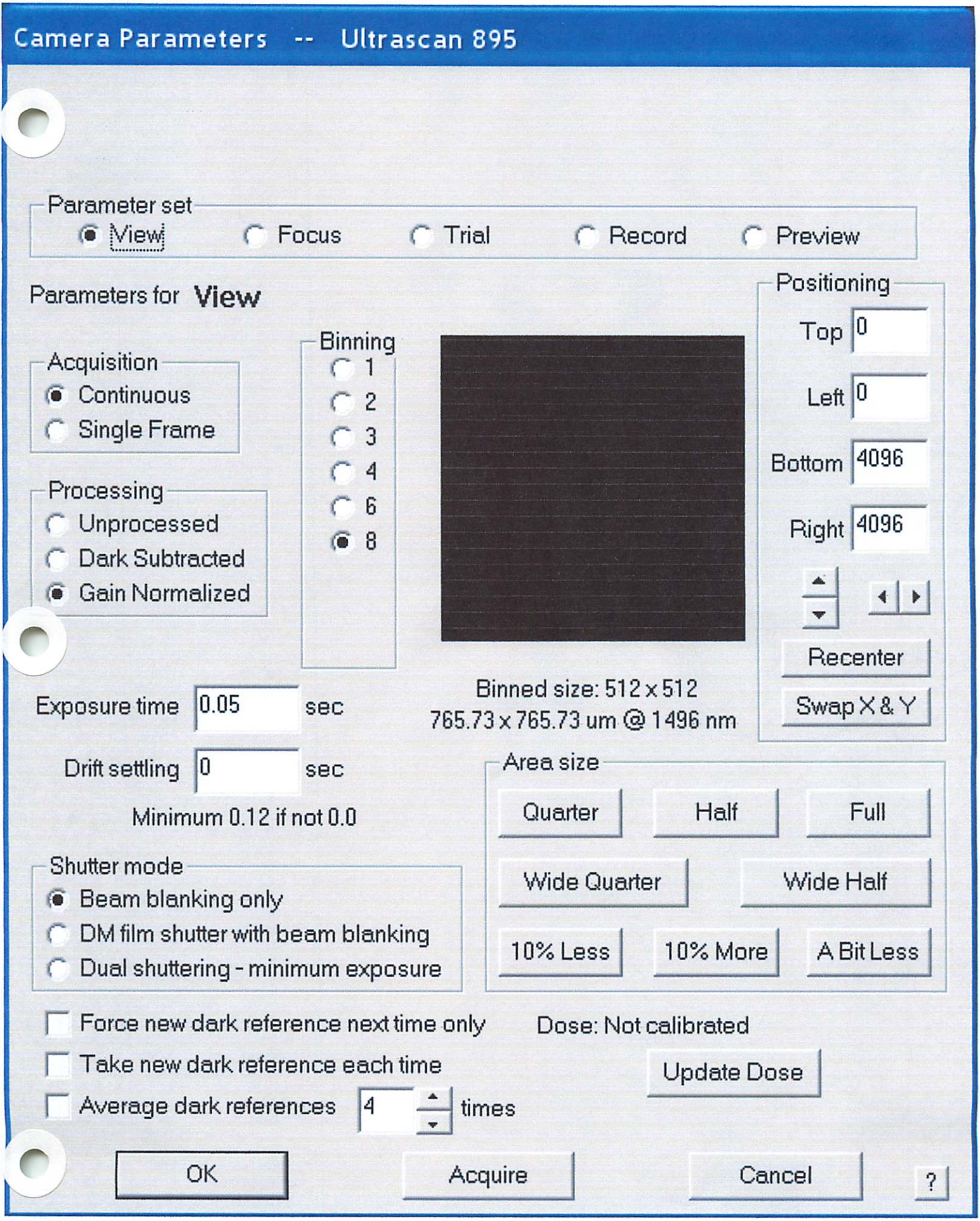

GL WOBB
HT WOBB
F1
F2
F3
F4
F5
F6
800>
50>1K
MAG 1
MAG 2
LOW MAG
DIFF
IOS/DIFF FOCUS
STO FOCUS
MAG/CAML
SHIFT Y
IOS
EXP TIME
IMAGE WOBB X
IMAGE WOBB Y
AUTO
FINE
OBJ FOCUS
COARSE
DEF/STIG Y
CRS
PHOTO
JEOL

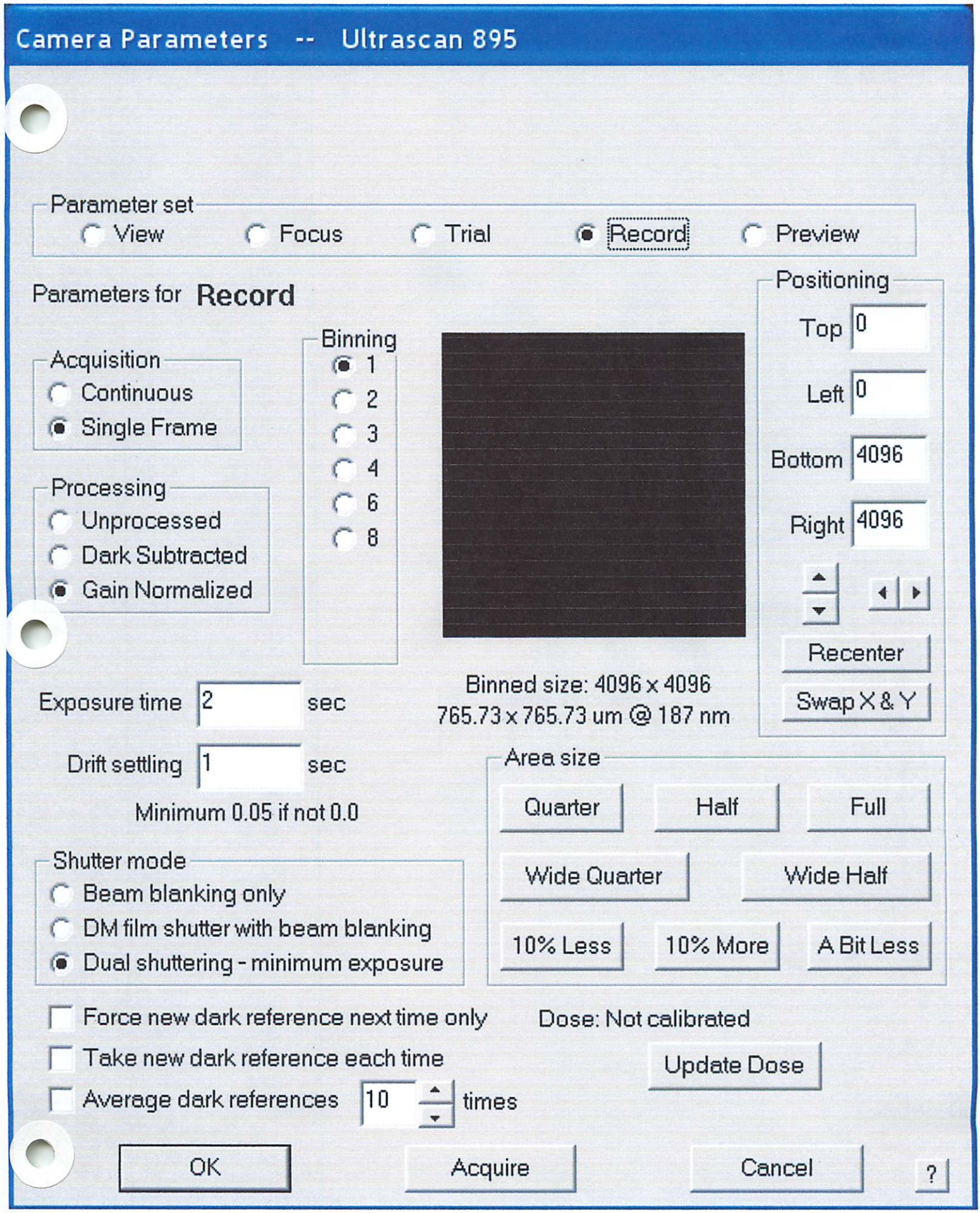

Camera
Calibration
Navigator
Buffer Status
A: UNSAVED.
Size: 4080 x 4080 bin 1
Tilt: 0.20
Piece X: 19 Y: 23 Z: 0
Def: -13.40
B: Tracking image
C: Read from File, sec. 281
D: UNSAVED, Focus
Buffer Controls
Copy Active Image to Buffer
A B C D E
New
SAVE A
Save Active
To 1
Options
Memory = 353 MB
Image Display Controls
Zoom
0.19
472
Bri
Black
Con
4596
Options
White
Float
Dose
Microscope
0.0000 na
5000×
Def -13.40 um
IS 0.42 um
Reset
Obj 61.07%
Spot 2
Tilt Control
Camera & Macro Controls
Setup
View
Focus
Trial
Record
Montage
End Nav
Resume
STOP
Image Alignment & Focus
Align to O
Autofocus
Clear Alignment
Reset Image Shift
Options
Def. target = -0.50 um
Low Dose Control
Montage Controls
Start
Prescan
Current Z
Options
Bin: Overview 225
Prescan 8
Correct drift
Change focus with height
Show overview at end
Align pieces in overview
A
SerialEM
Error writing d
Error while writ
Try closing the
Corner point (C)
For anchor map
New file at group
Filename
Collapse groups
Type Reg. Acq. Note

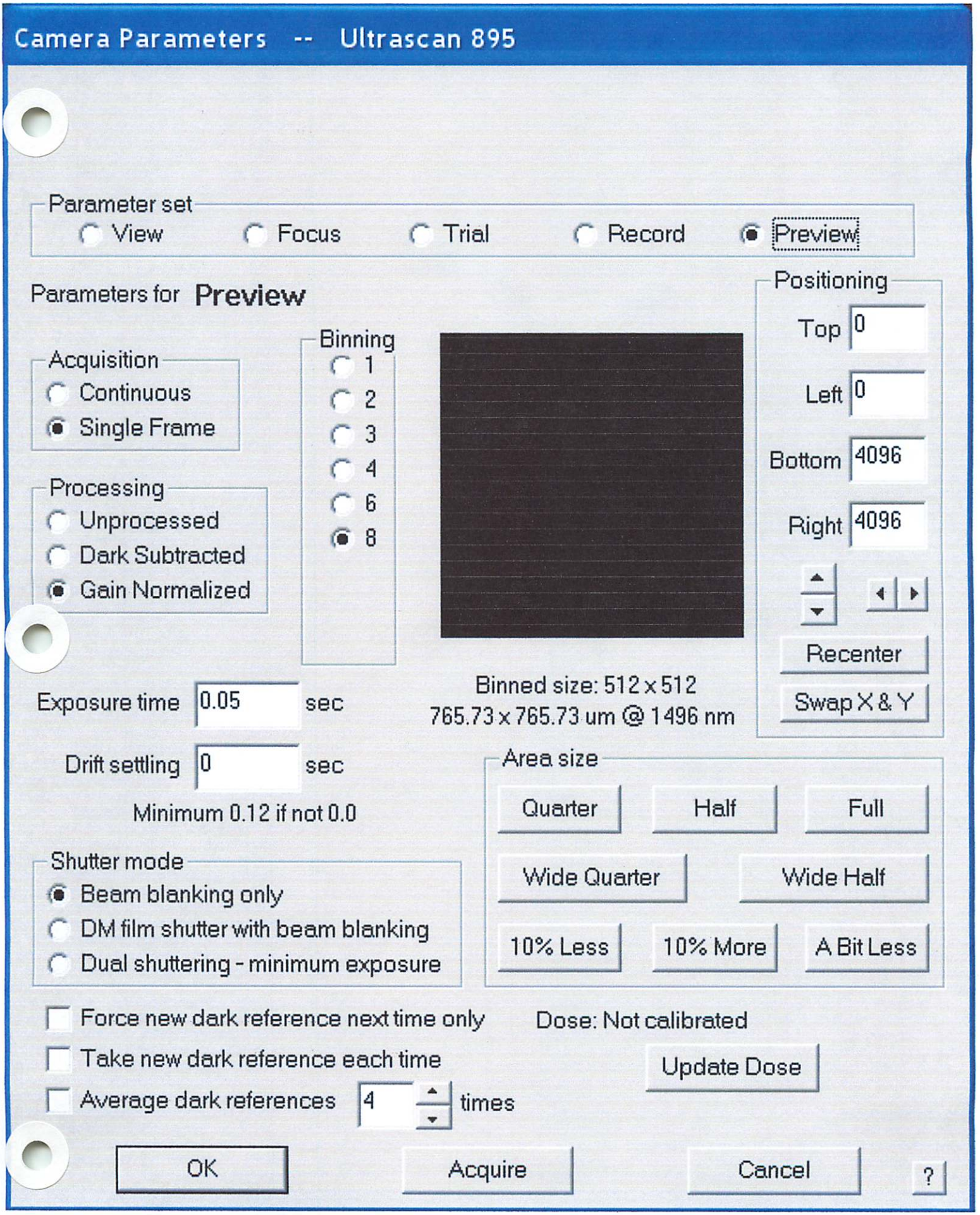

PIEZO
CRS
JEOL
SPEC CONTROL

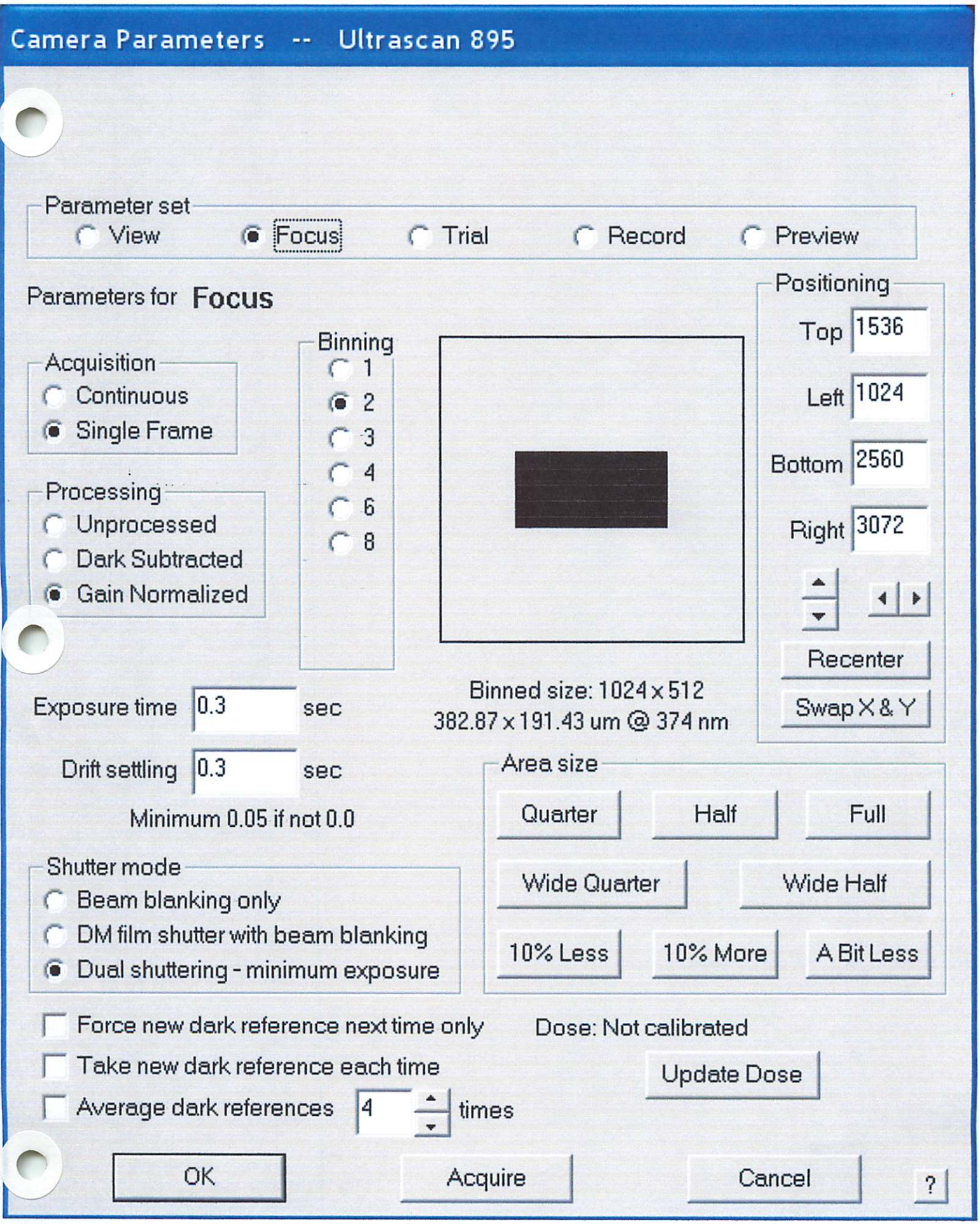
CAMERA SETUP WINDOWS


Camera Parameters -- Ultrascan 895
Parameter set
View
Focus
Trial
Record
Preview
Parameters for Trial
Positioning
Top 0
Left 0
Bottom 4096
Right 4096
Recenter
Swap X & Y
Acquisition
Continuous
Single Frame
Binning
1
2
3
4
6
8
Processing
Unprocessed
Dark Subtracted
Gain Normalized
Binned size: 1024 x 1024
8.91 x 8.91 um @ 8.70 nm
Exposure time 0.1 sec
Drift settling 0 sec
Minimum 0.12 if not 0.0
Area size
Quarter
Half
Full
Wide Quarter
Wide Half
10% Less
10% More
A Bit Less
Shutter mode
Beam blanking only
DM film shutter with beam blanking
Dual shuttering - minimum exposure
Force new dark reference next time only
Take new dark reference each time
Average dark references 4 times
Dose: Not calibrated
Update Dose
OK
Acquire
Cancel
?


Camera Parameters -- Ultrascan 895
Parameter set
View
Focus
Trial
Record
Preview
Parameters for View
Positioning
Top 0
Left 0
Bottom 4096
Right 4096
Recenter
Swap X & Y
Acquisition
Continuous
Single Frame
Binning
1
2
3
4
6
8
Processing
Unprocessed
Dark Subtracted
Gain Normalized
Binned size: 512 x 512
765.73 x 765.73 um @ 1496 nm
Exposure time 0.05 sec
Drift settling 0 sec
Minimum 0.12 if not 0.0
Area size
Quarter
Half
Full
Wide Quarter
Wide Half
10% Less
10% More
A Bit Less
Shutter mode
Beam blanking only
DM film shutter with beam blanking
Dual shuttering - minimum exposure
Force new dark reference next time only
Take new dark reference each time
Average dark references 4 times
Dose: Not calibrated
Update Dose
OK
Acquire
Cancel
?


Camera Parameters -- Ultrascan 895
Parameter set
View
Focus
Trial
Record
Preview
Parameters for Record
Positioning
Top 0
Left 0
Bottom 4096
Right 4096
Recenter
Swap X & Y
Acquisition
Continuous
Single Frame
Binning
1
2
3
4
6
8
Processing
Unprocessed
Dark Subtracted
Gain Normalized
Binned size: 4096 x 4096
765.73 x 765.73 um @ 187 nm
Exposure time 2 sec
Drift settling 1 sec
Minimum 0.05 if not 0.0
Area size
Quarter
Half
Full
Wide Quarter
Wide Half
10% Less
10% More
A Bit Less
Shutter mode
Beam blanking only
DM film shutter with beam blanking
Dual shuttering - minimum exposure
Force new dark reference next time only
Take new dark reference each time
Average dark references 10 times
Dose: Not calibrated
Update Dose
OK
Acquire
Cancel
?


Camera Parameters -- Ultrascan 895
Parameter set
View
Focus
Trial
Record
Preview
Parameters for Preview
Positioning
Top 0
Left 0
Bottom 4096
Right 4096
Recenter
Swap X & Y
Acquisition
Continuous
Single Frame
Binning
1
2
3
4
6
8
Processing
Unprocessed
Dark Subtracted
Gain Normalized
Binned size: 512 x 512
765.73 x 765.73 um @ 1496 nm
Exposure time 0.05 sec
Drift settling 0 sec
Minimum 0.12 if not 0.0
Area size
Quarter
Half
Full
Wide Quarter
Wide Half
10% Less
10% More
A Bit Less
Shutter mode
Beam blanking only
DM film shutter with beam blanking
Dual shuttering - minimum exposure
Force new dark reference next time only
Take new dark reference each time
Average dark references 4 times
Dose: Not calibrated
Update Dose
OK
Acquire
Cancel
?


Camera Parameters -- Ultrascan 895
Parameter set
View
Focus
Trial
Record
Preview
Parameters for Focus
Acquisition
Continuous
Single Frame
Processing
Unprocessed
Dark Subtracted
Gain Normalized
Binning
1
2
3
4
6
8
Positioning
Top 1536
Left 1024
Bottom 2560
Right 3072
Recenter
Swap X & Y
Exposure time 0.3 sec
Drift settling 0.3 sec
Minimum 0.05 if not 0.0
Binned size: 1024 x 512
382.87 x 191.43 um @ 374 nm
Area size
Quarter
Half
Full
Wide Quarter
Wide Half
10% Less
10% More
A Bit Less
Shutter mode
Beam blanking only
DM film shutter with beam blanking
Dual shuttering - minimum exposure
Force new dark reference next time only
Take new dark reference each time
Average dark references 4 times
Dose: Not calibrated
Update Dose
OK
Acquire
Cancel
?