

Automatic imaging with serialEM and synapseFinder

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1 Introduction

2 The SerialEM Interface

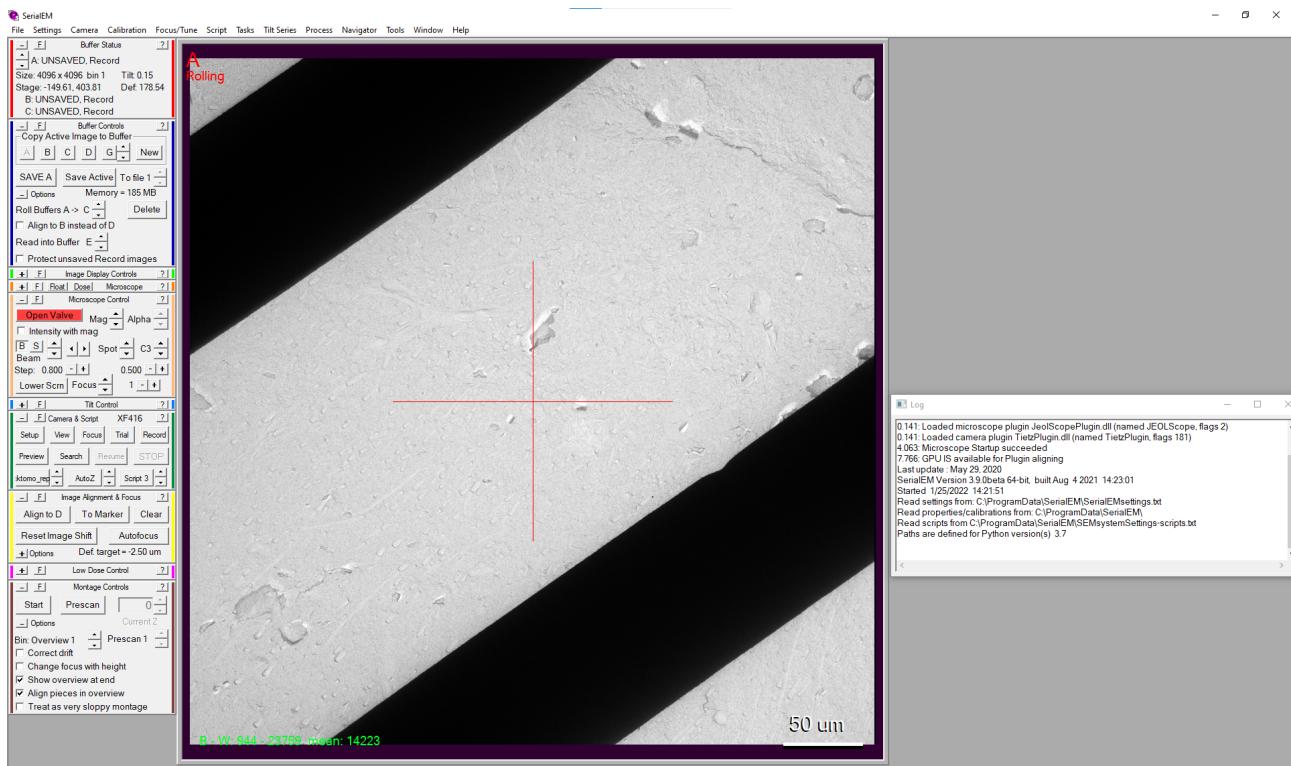


Figure 1: Text here

3 Take an overview image of the whole replica

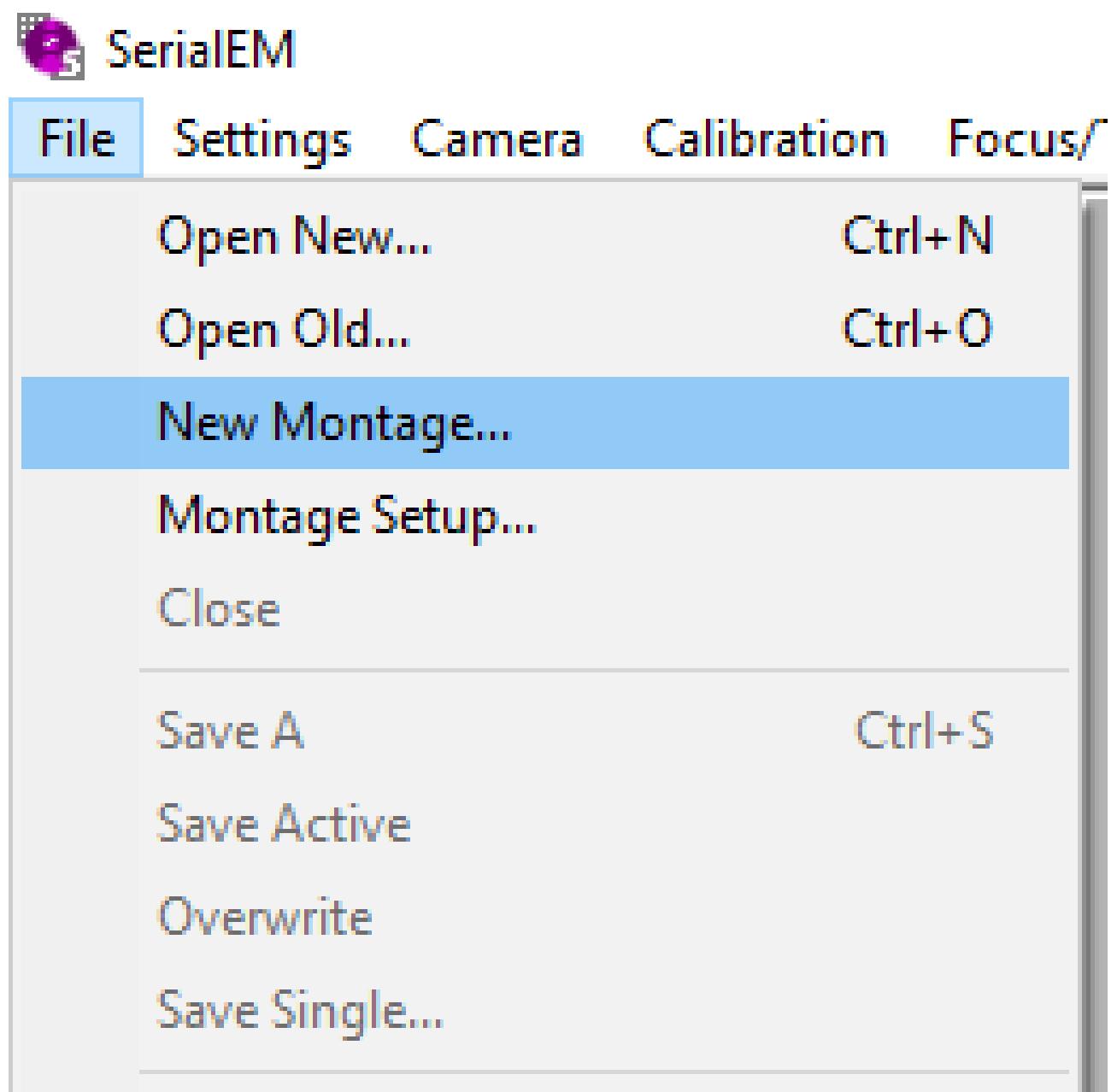


Figure 2: NewMontage

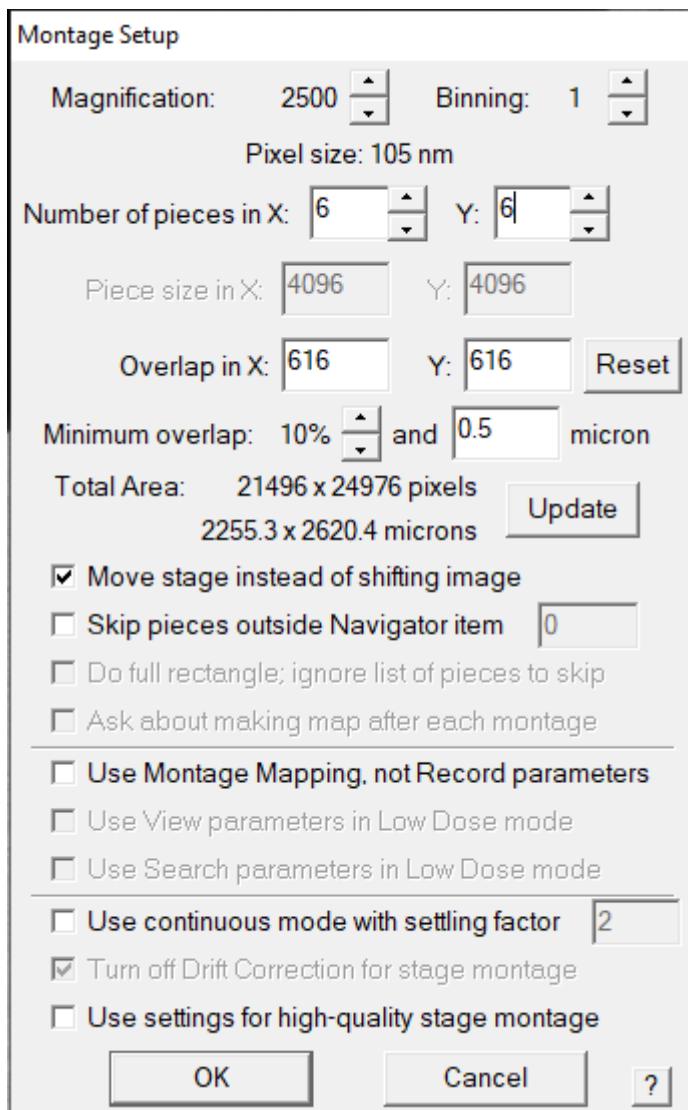


Figure 3: Text

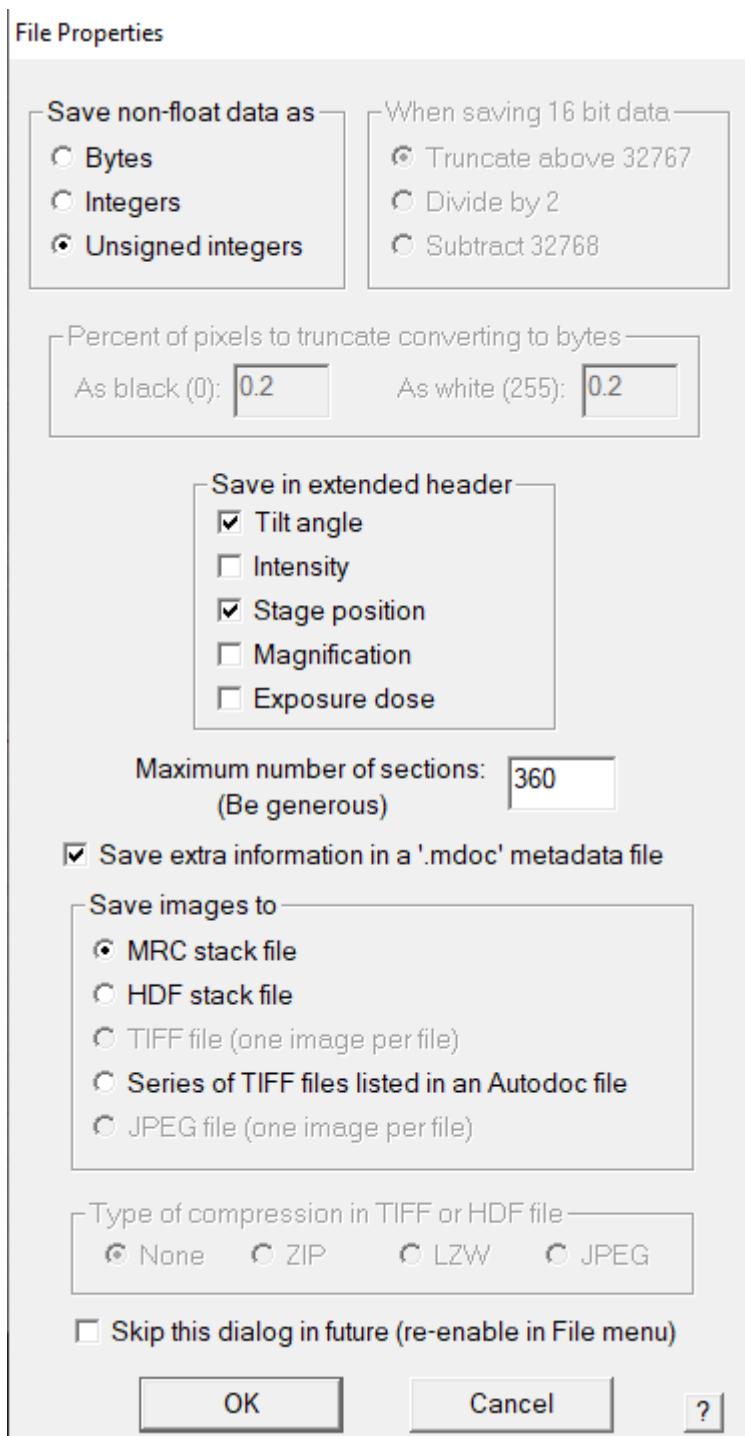


Figure 4: Text

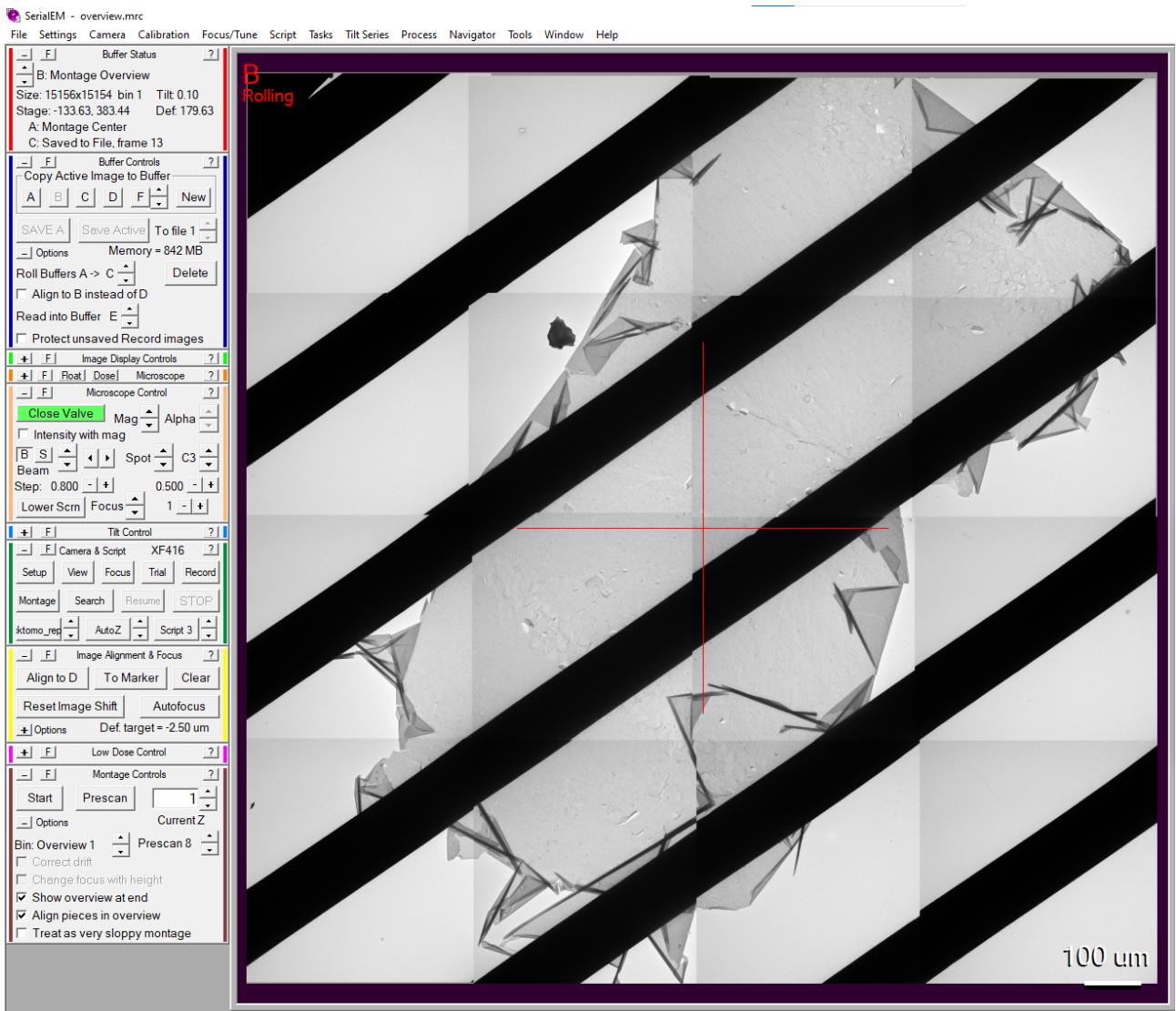


Figure 5: Text

4 Make overview a map and align with higher mag

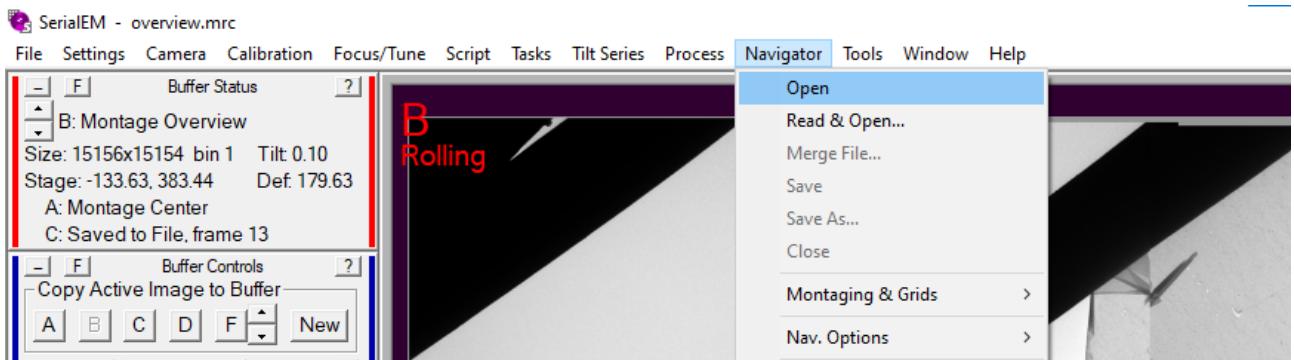


Figure 6: Text

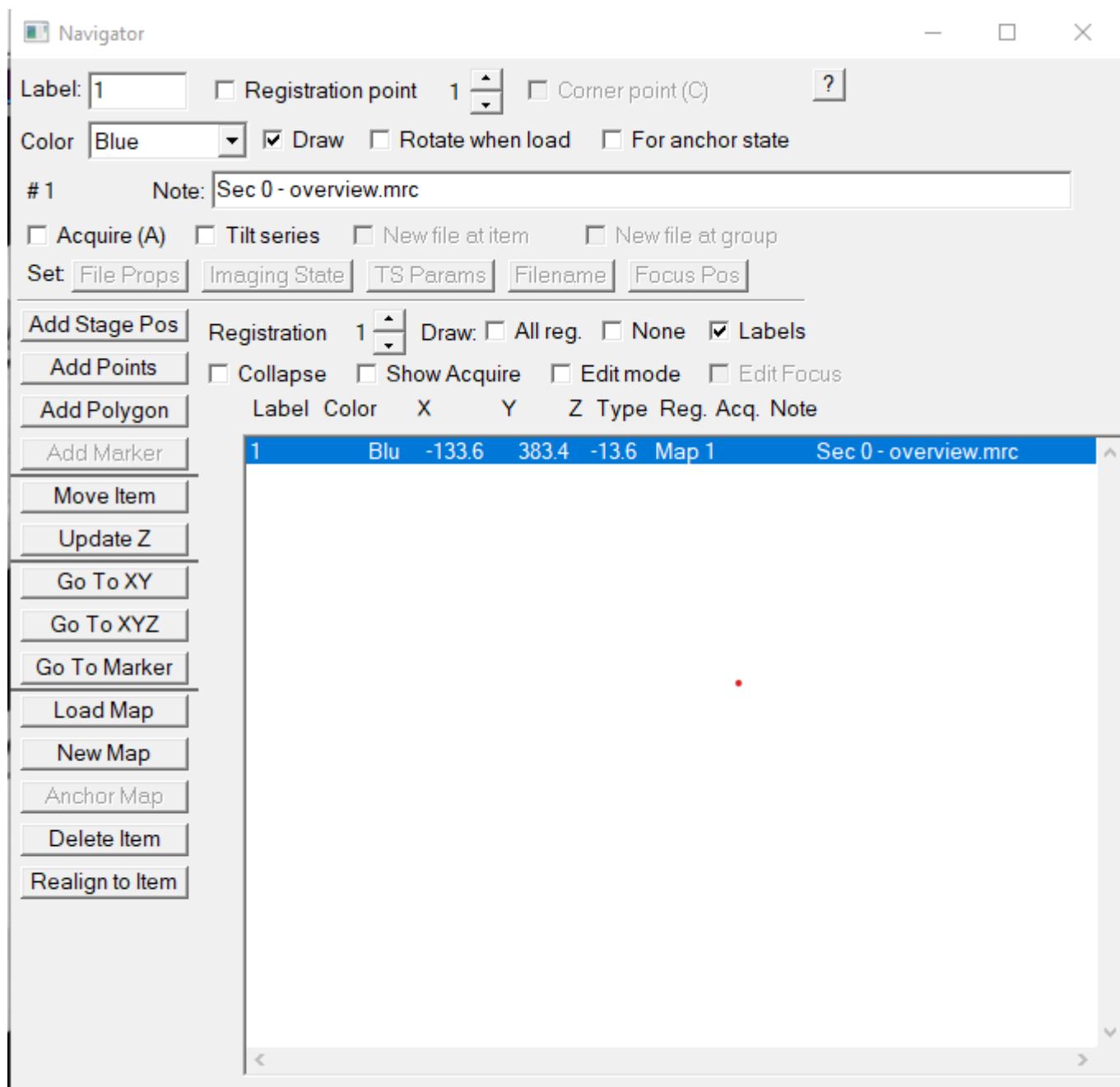


Figure 7: Text

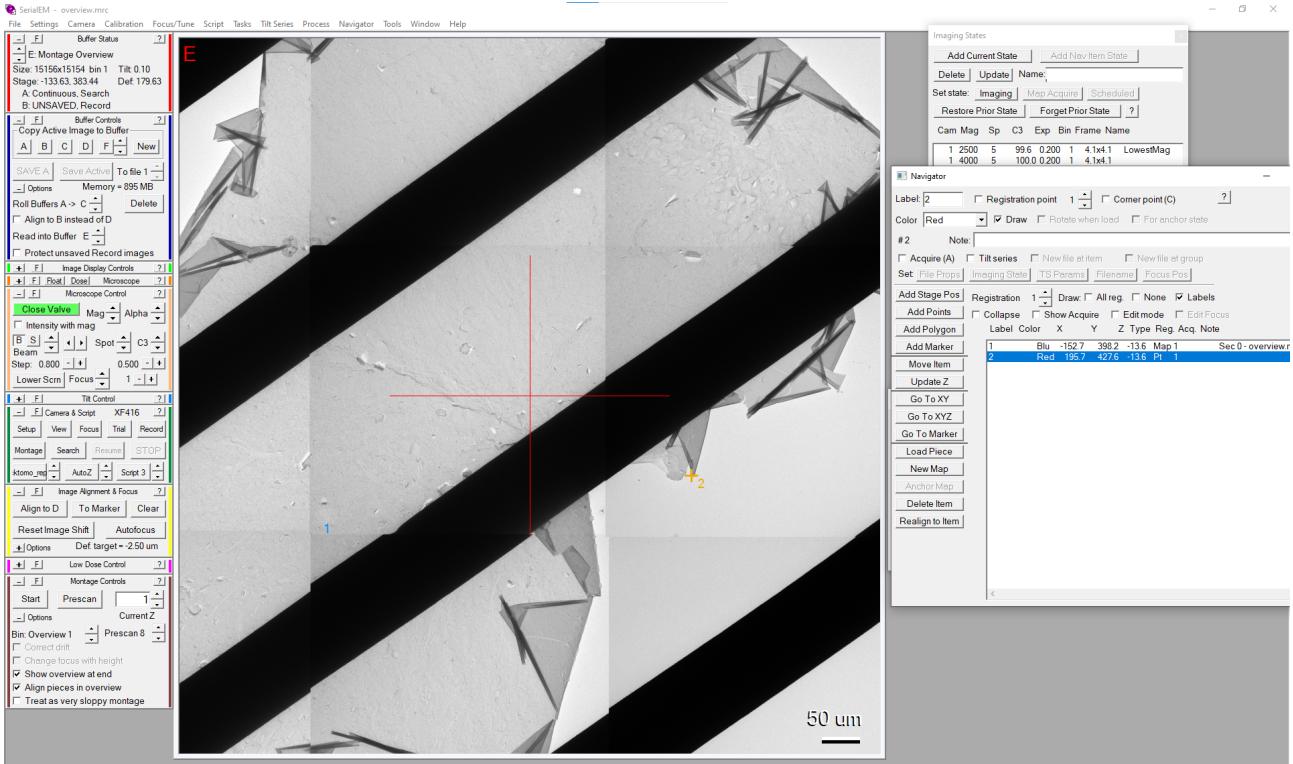


Figure 8: Text

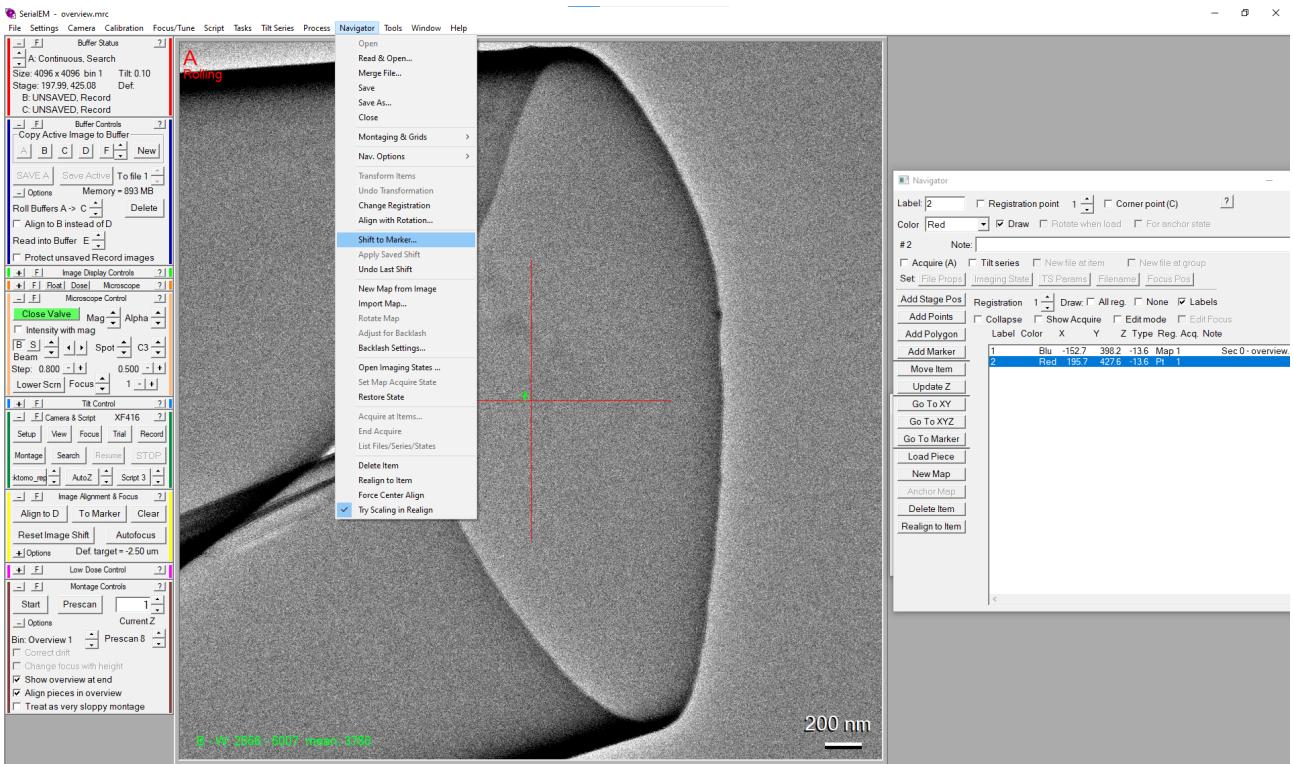


Figure 9: Text

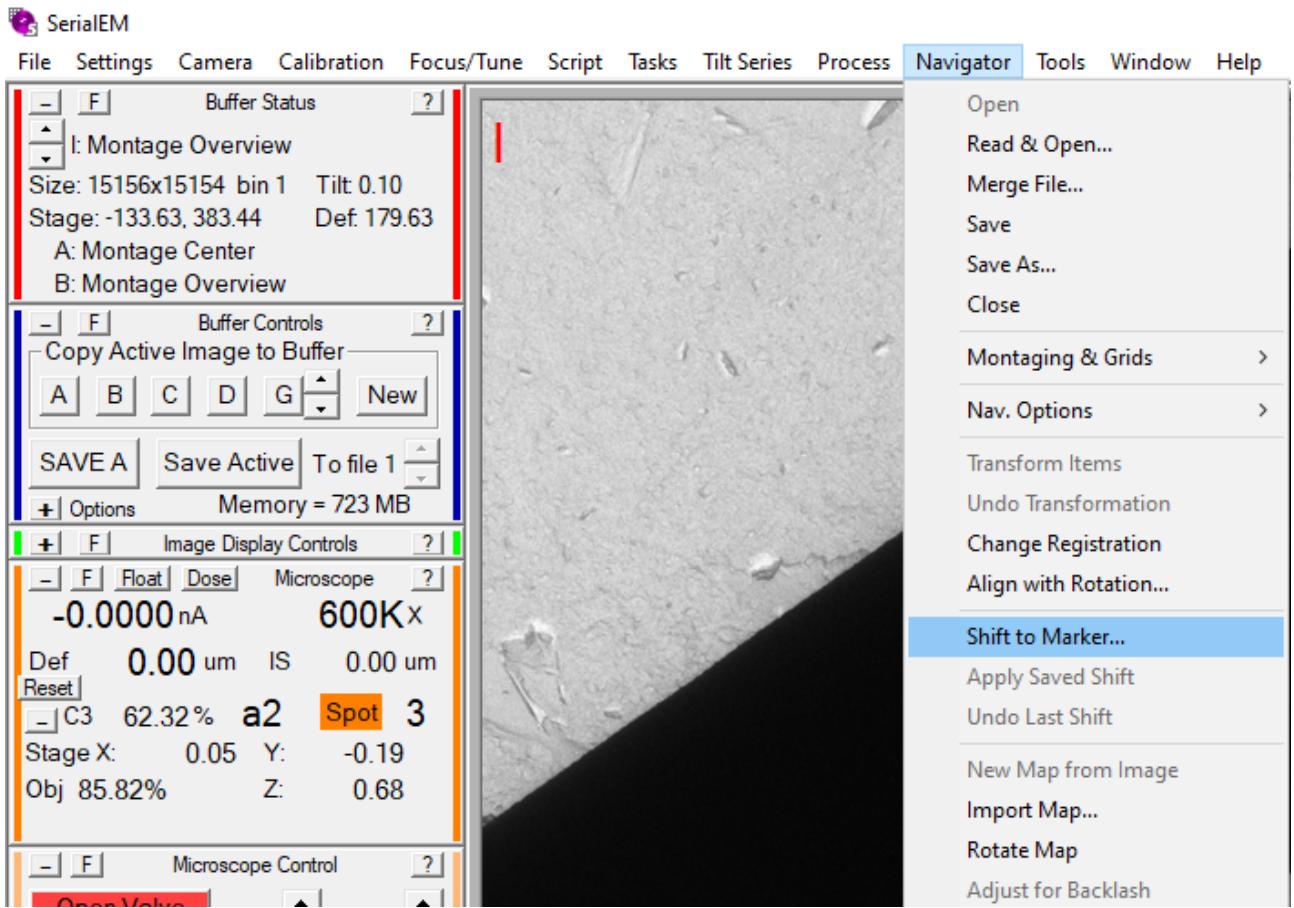


Figure 10: Text

Shift To Marker

X

The shift between the current item and the marker point is 0.11, 0.04 microns

All selected items at registration 1 will be shifted by that amount

Which items to shift

- All items at the registration of the image with m
- All maps at 200000x and items marked on them
- Only maps at 200000x and marked items that have not been shifted before

Saving shift for reuse

- Do not save shift for reuse
- Save shift to apply again from 200000x to 200000x or other second mag
- Add to saved shift from 200000x to 200000x

OK

Cancel

?

Figure 11: Text

5 Take intermediate magnification image of your region of interest

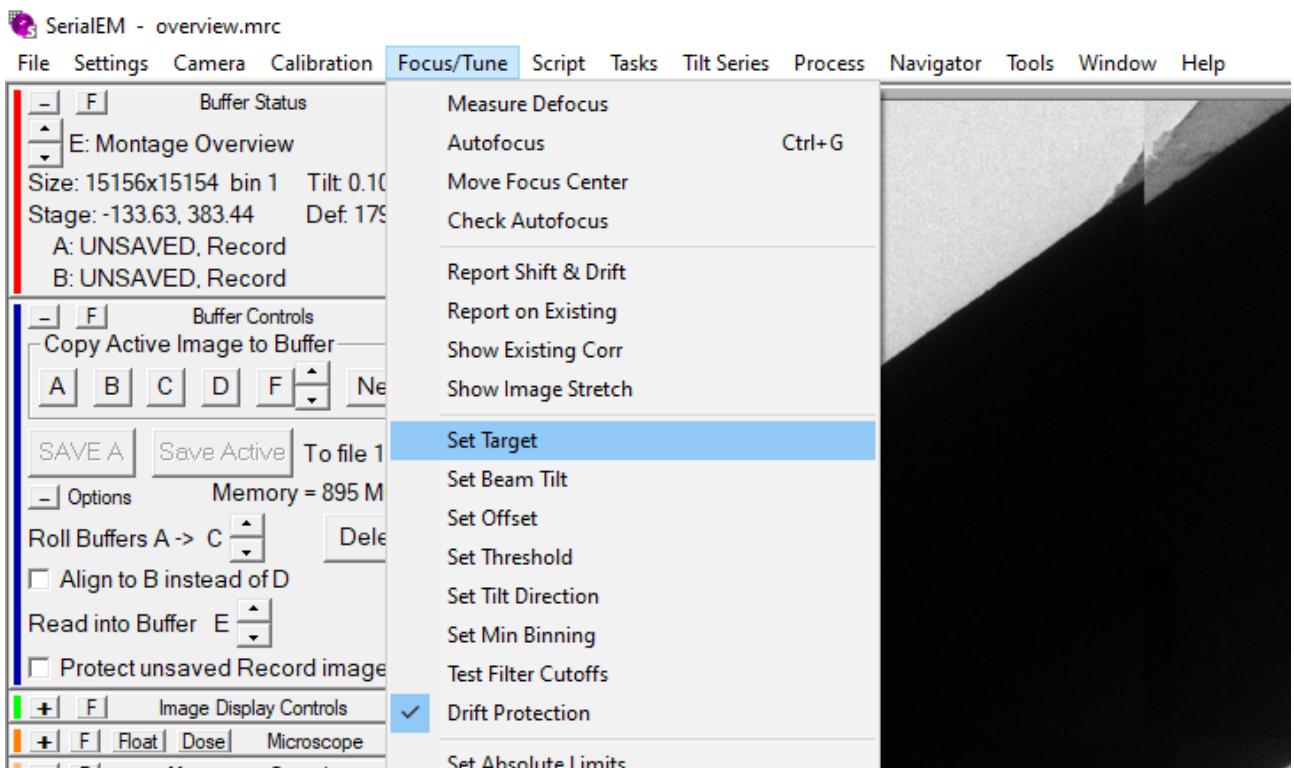


Figure 12: Text

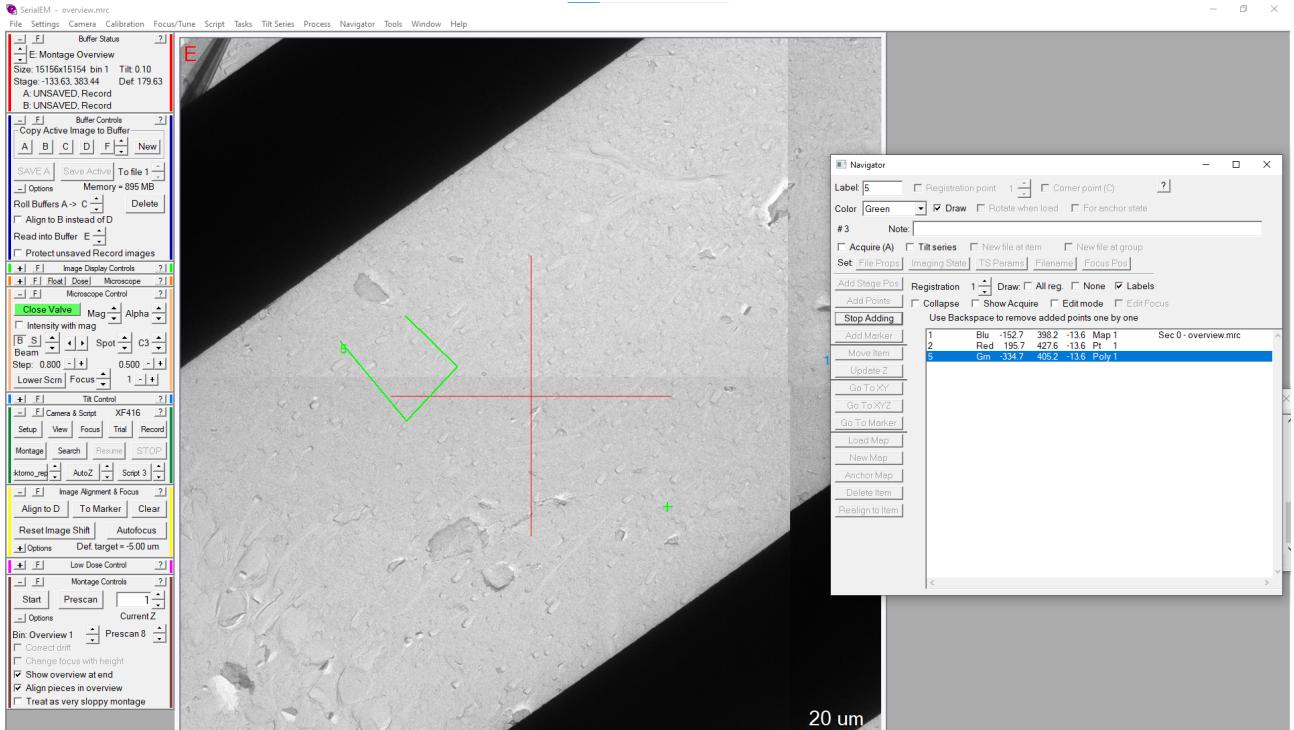


Figure 13: Text

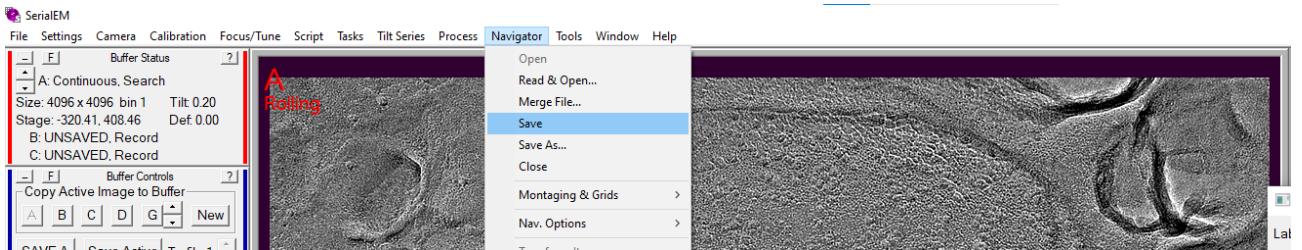


Figure 14: This file should be saved in the same folder as your images

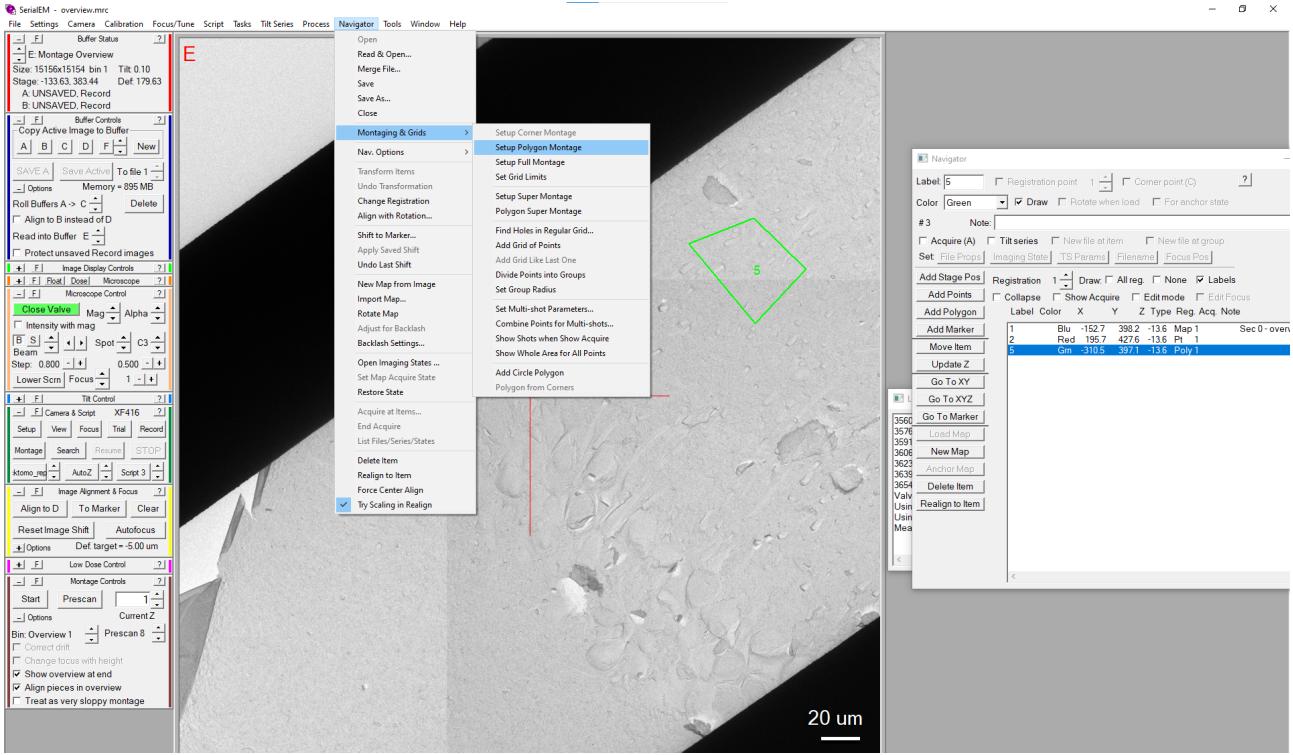


Figure 15: Text

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: 200000 Binning: 1

Pixel size: 1.03 nm

Number of pieces in X: Y:

Piece size in X: Y:

Overlap in X: Y:

Minimum overlap: 10% and micron

Total Area: 41232 x 44512 pixels
42.3 x 45.7 microns

Move stage instead of shifting image

Skip pieces outside Navigator item

Do full rectangle; ignore list of pieces to skip

Ask about making map after each montage

Use Montage Mapping, not Record parameters

Use View parameters in Low Dose mode

Use Search parameters in Low Dose mode

Use continuous mode with settling factor

Turn off Drift Correction for stage montage

Use settings for high-quality stage montage

High-quality stage montage parameters

Autofocus at each piece

Repeat until drift is below nm/sec

Autofocus in blocks of pieces

Realign with image shift up to microns

Go from center out; anchor at mag: 40000

Maximum alignment shift as % of piece

Delay time after moving stage: sec

Do not blank beam during Record readout

Skip correlations used to align pieces

Figure 16: Text

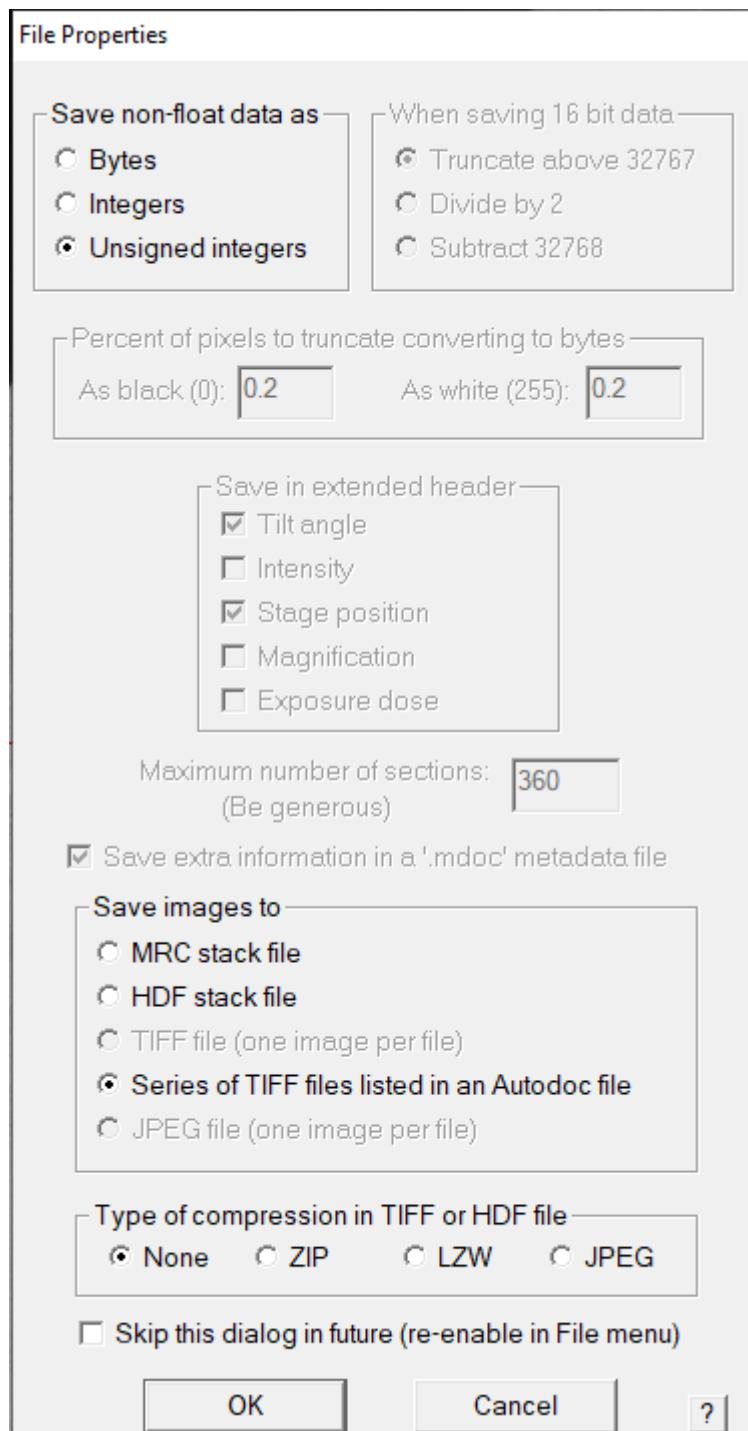


Figure 17: Text

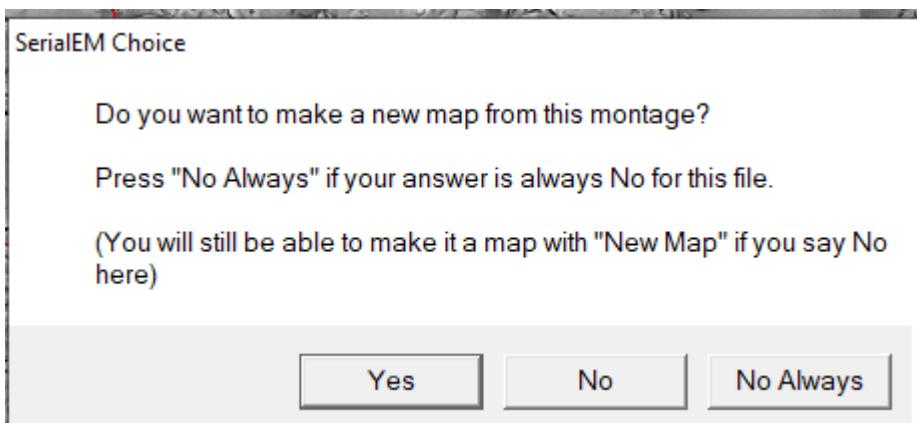


Figure 18: Text

6 Automatically find the features of interest - Using the GPU on the microscope PC

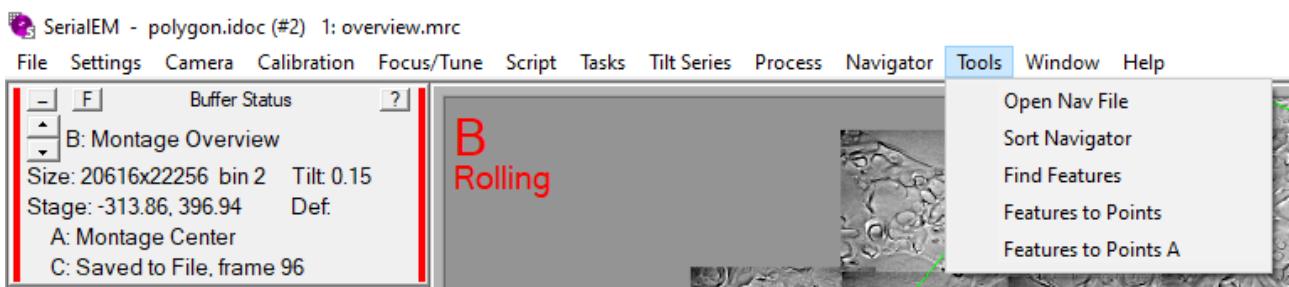


Figure 19: Text

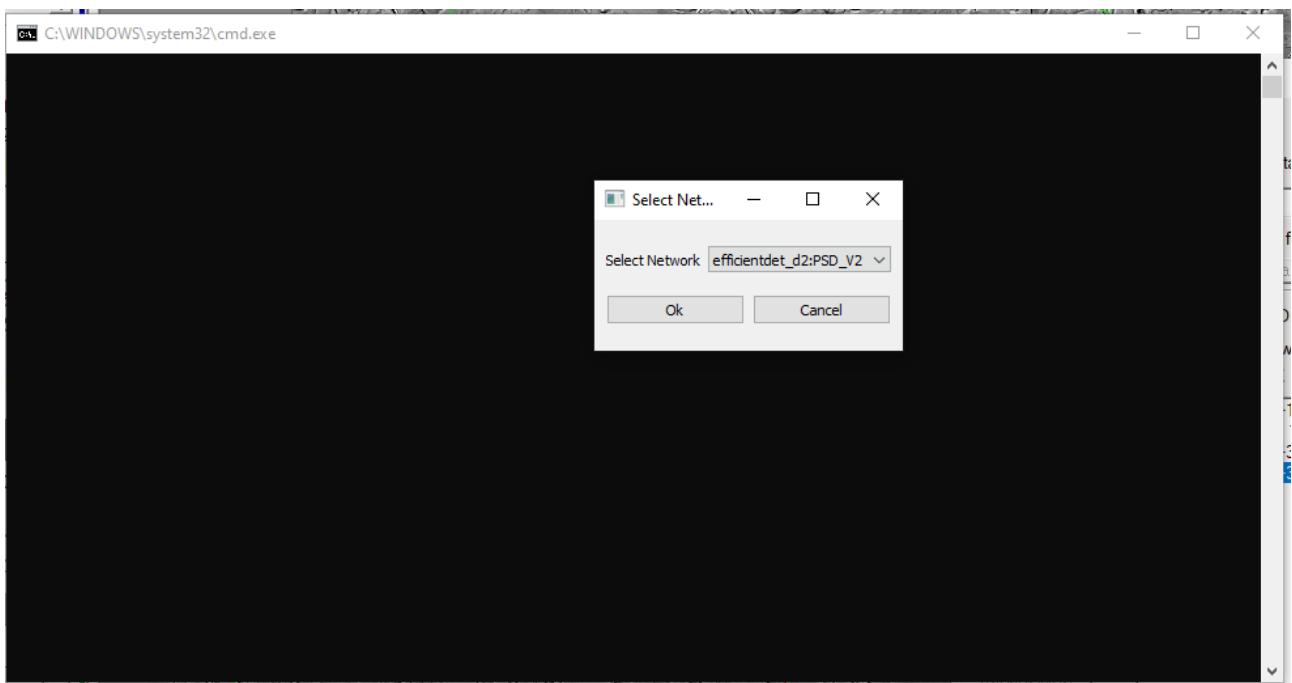
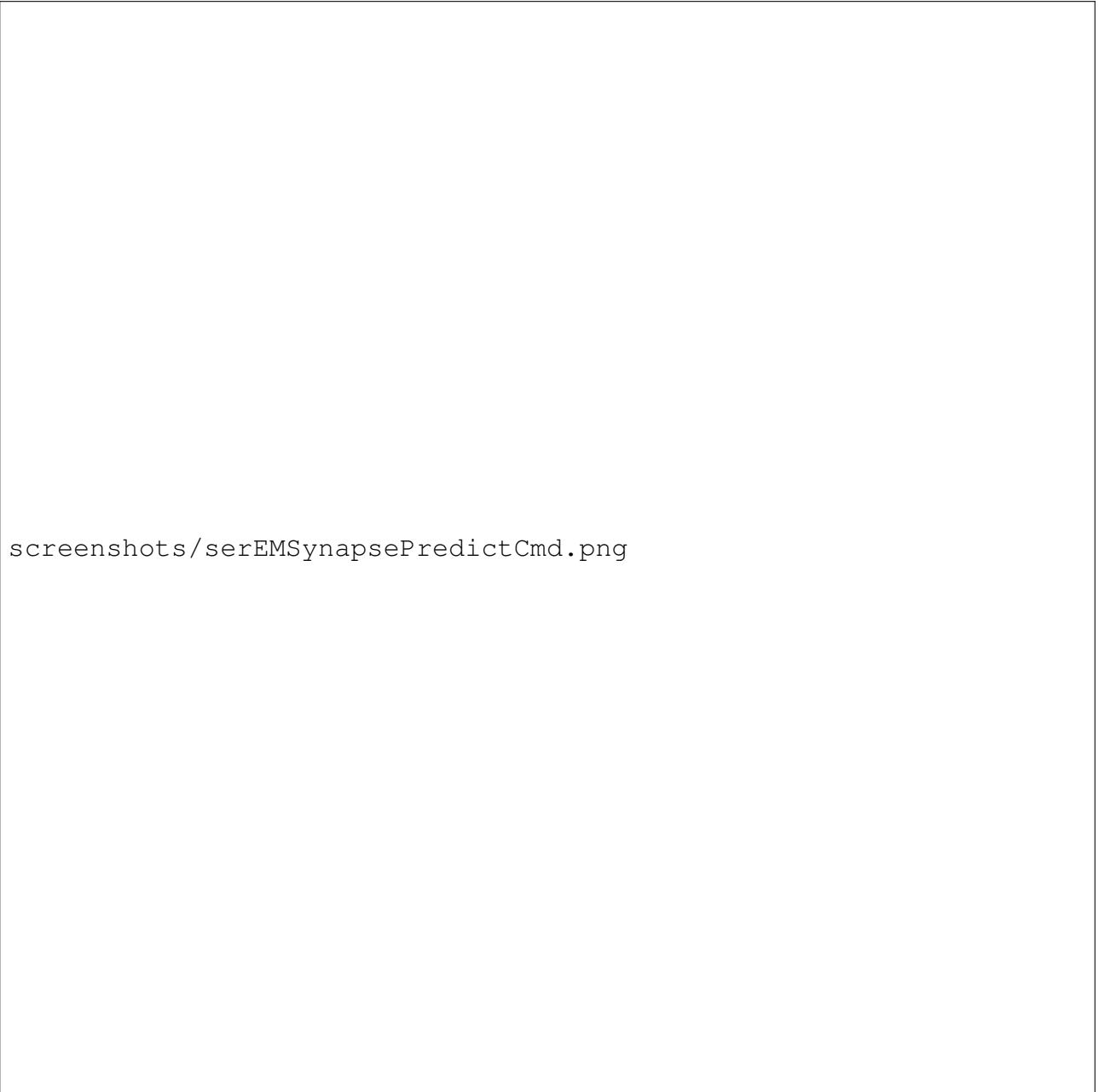


Figure 20: Text

7 Automatically find the features of interest - Using another PC with access to the same network drive

This can be done at the same time as the microscope is imaging

7.1 Windows



screenshots/serEMSSynapsePredictCmd.png

Figure 21: Text

screenshots/serEMSSynapseFinderMenu.png

Figure 22: Text

8 Add detections to the map

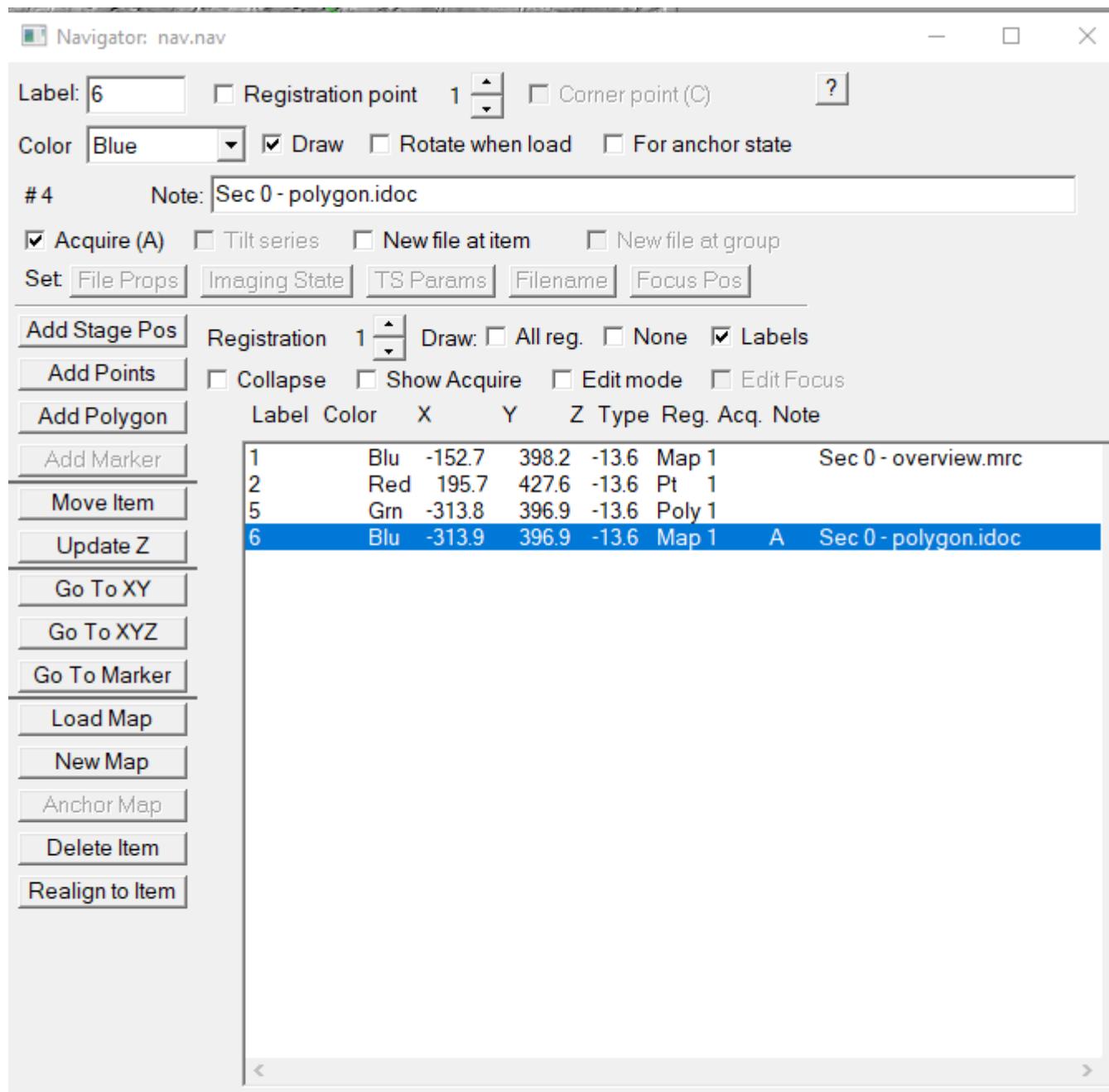


Figure 23: Text

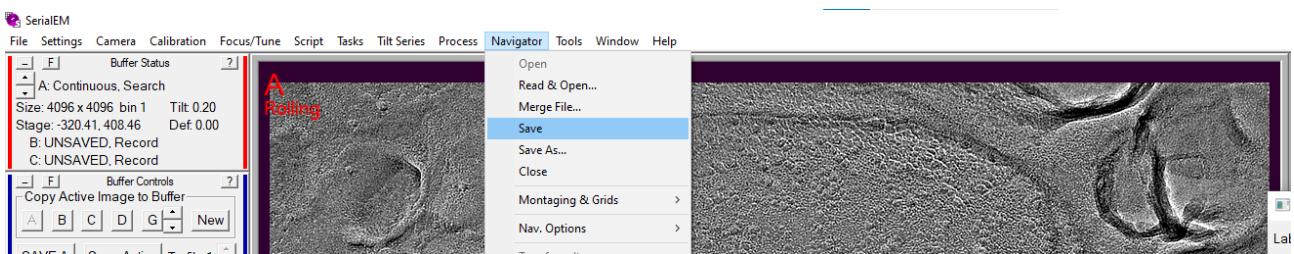


Figure 24: Text

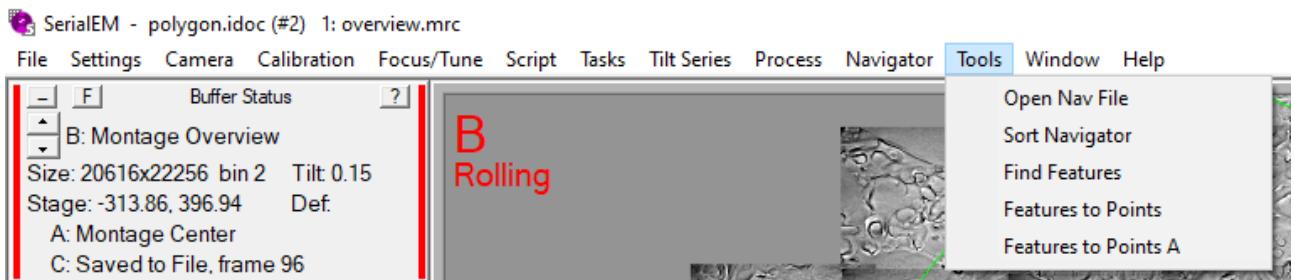


Figure 25: Text

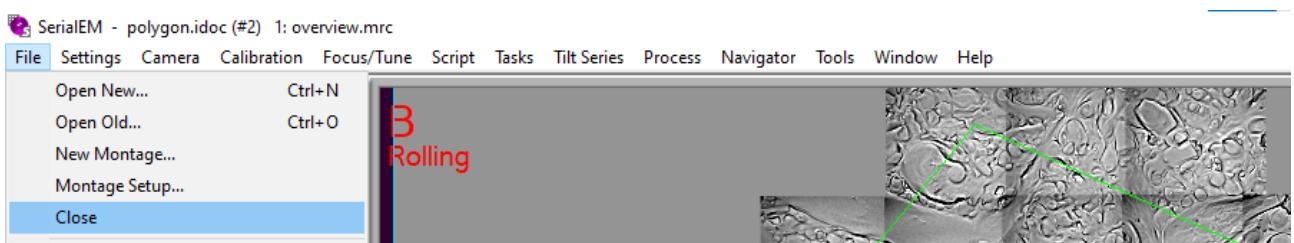


Figure 26: Text

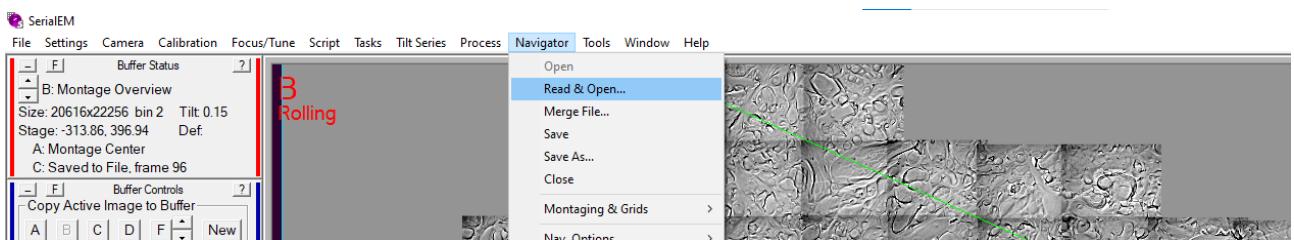


Figure 27: Text

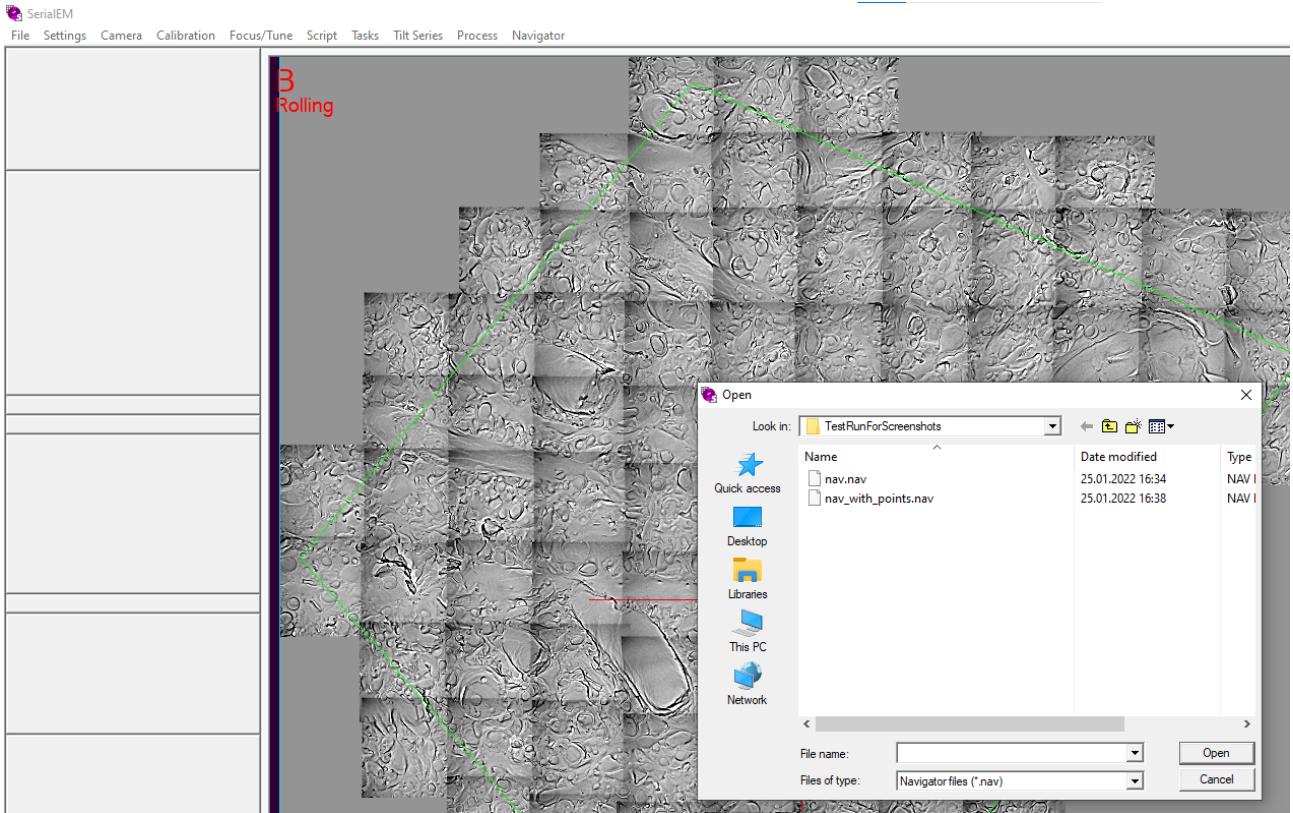


Figure 28: Text

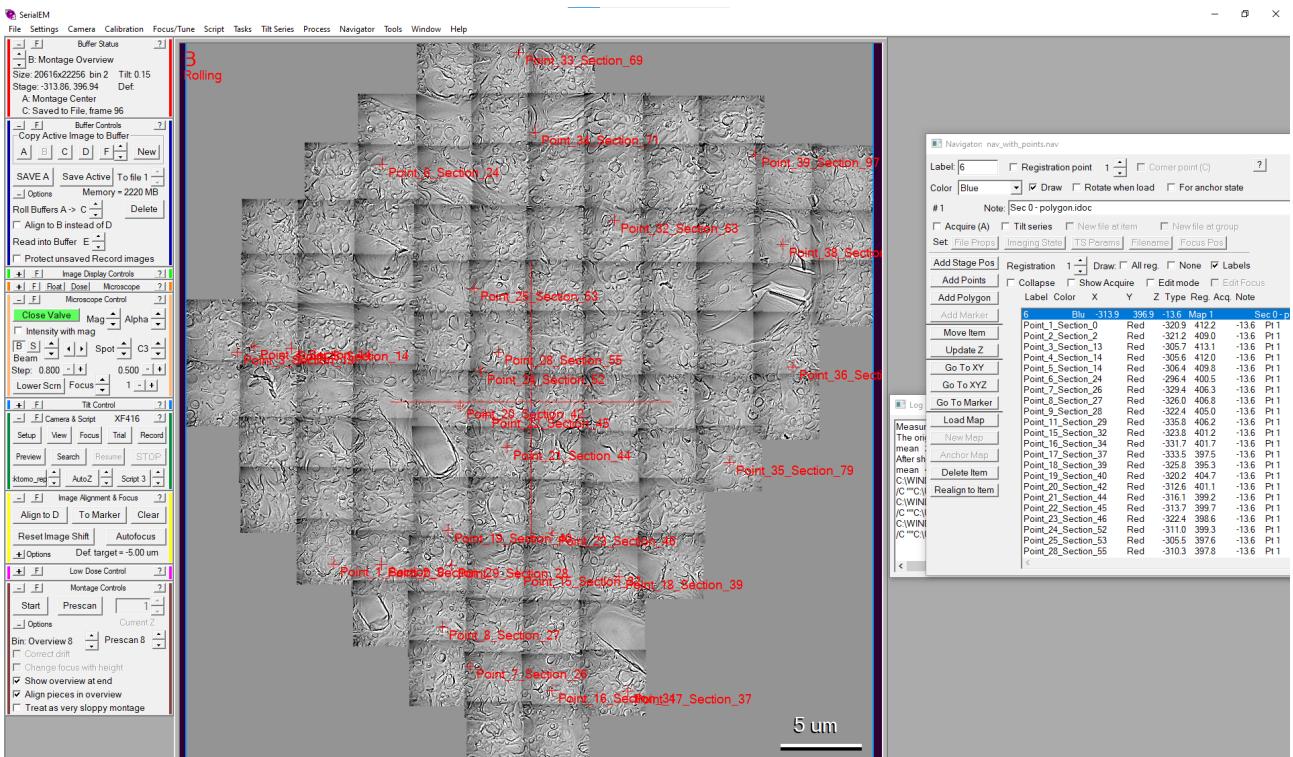


Figure 29: Text

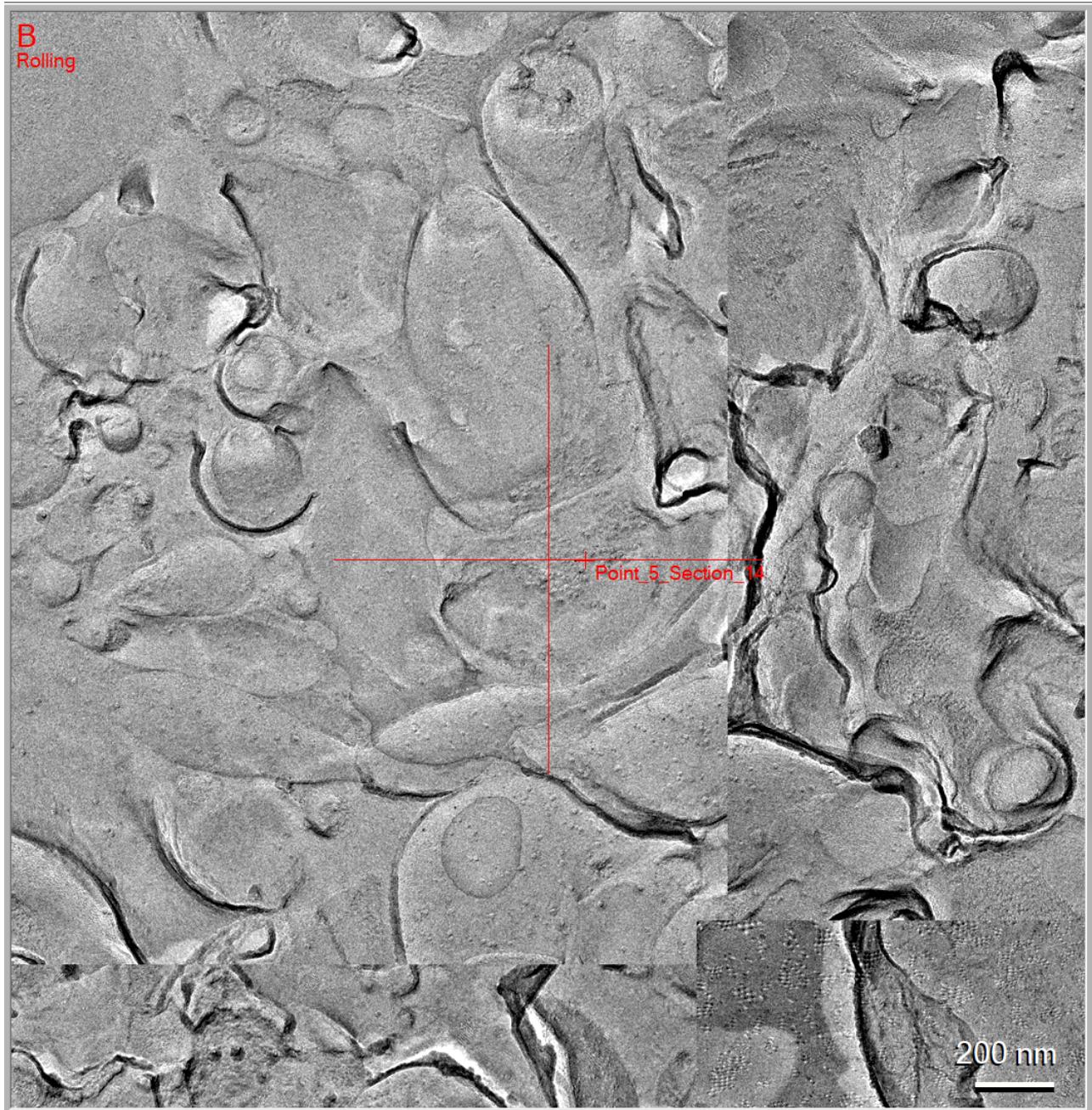


Figure 30: Text

9 Tilt Series of the Synapse

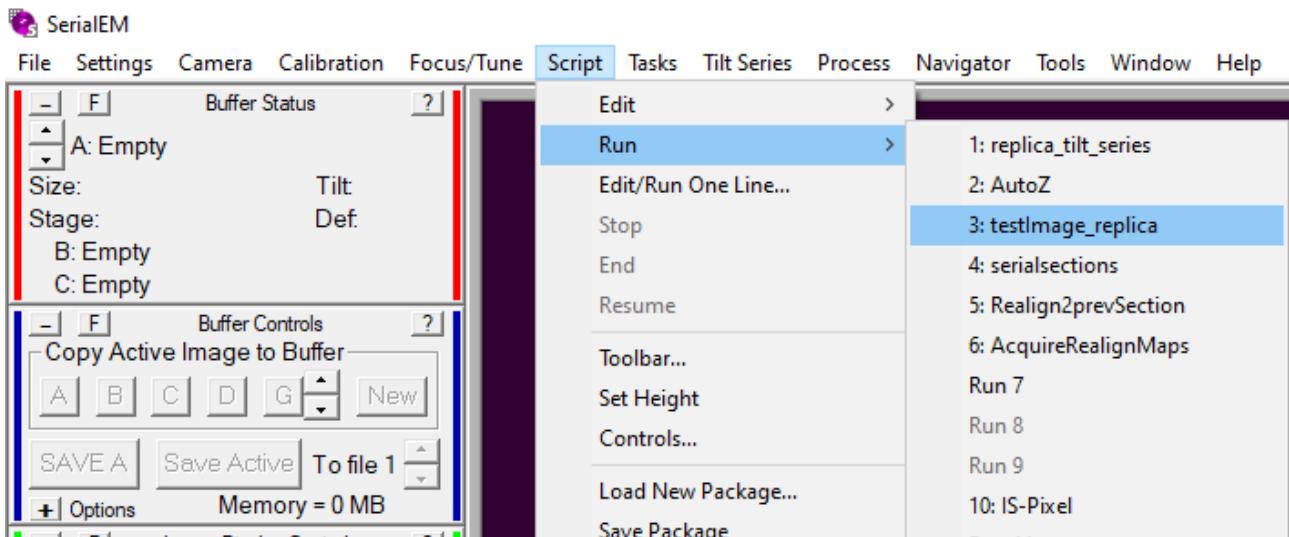


Figure 31: Text

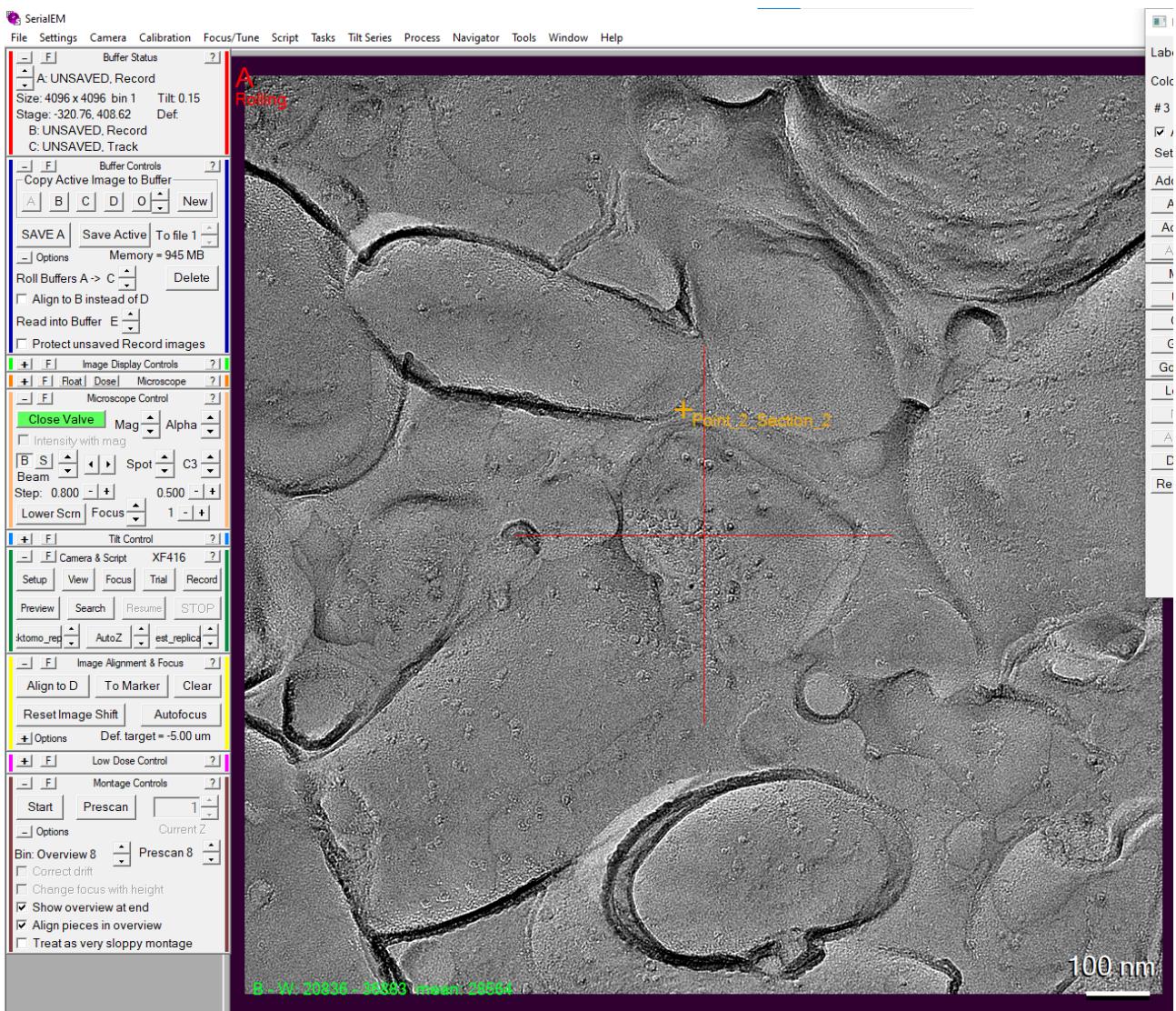


Figure 32: Text

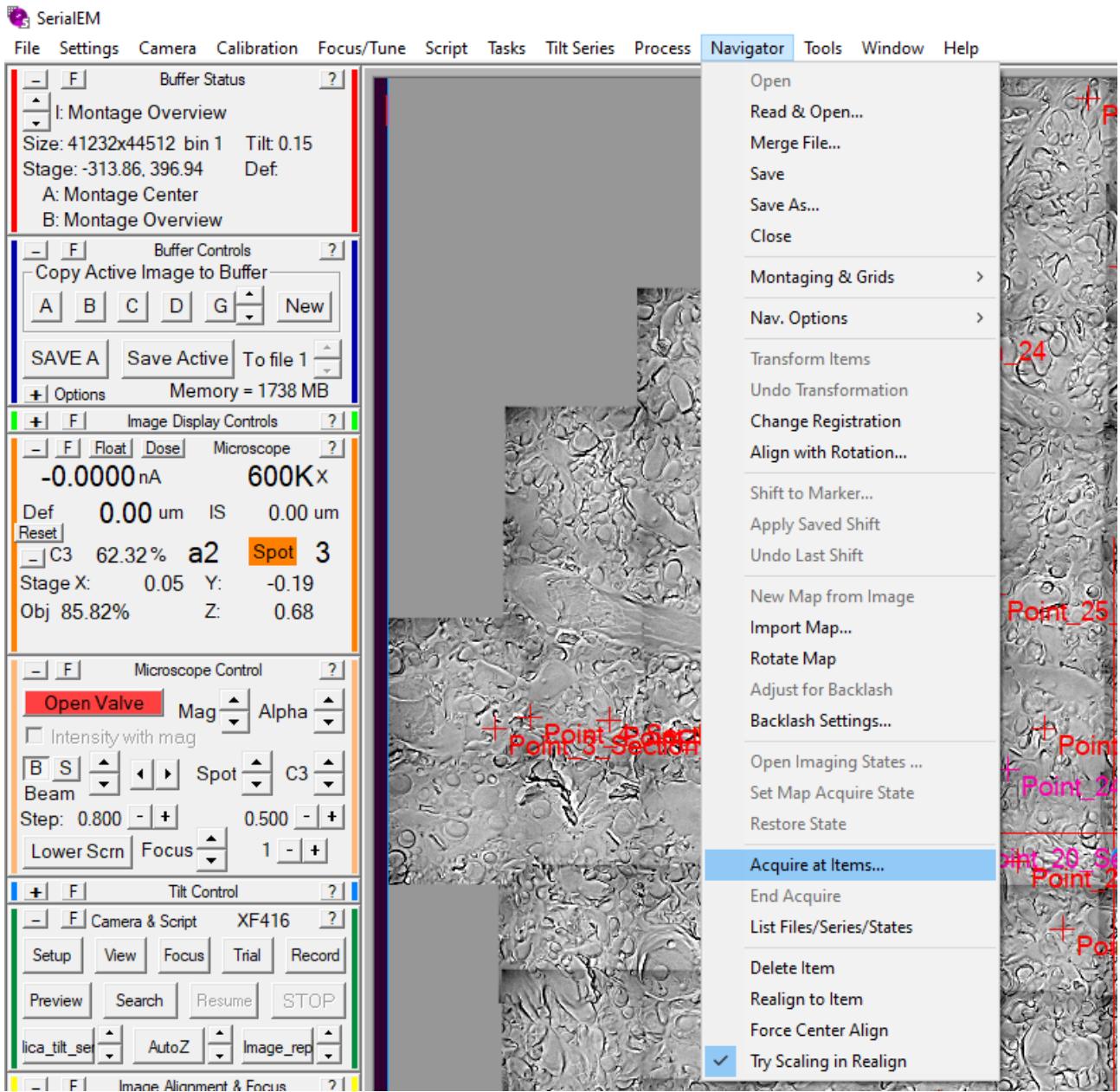


Figure 33: Text

screenshots/AcquireAtItems2.png

Figure 34: Text

10 Troubleshooting

10.1 Reopen Image



screenshots/OpenOld.png

Figure 35: Text

screenshots/Read.png

Figure 36: Text

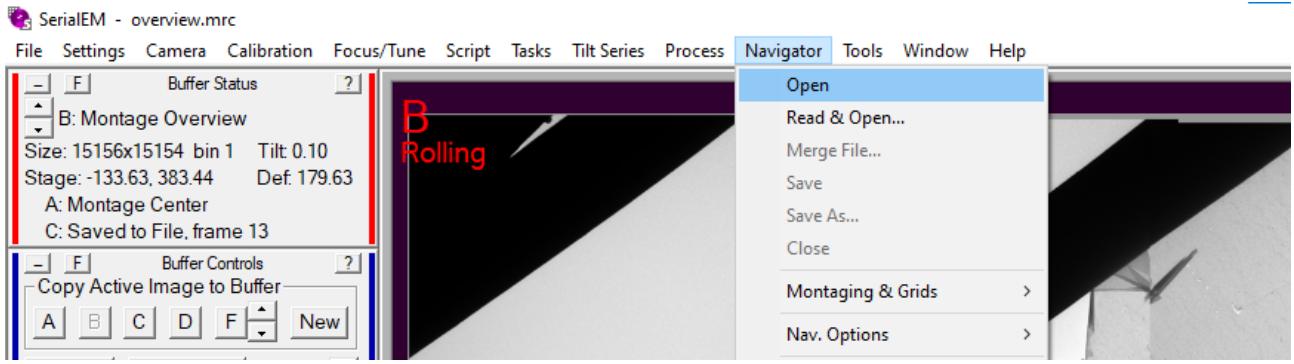


Figure 37: Text

Save and rerun tools

10.2 Synapse not in the center

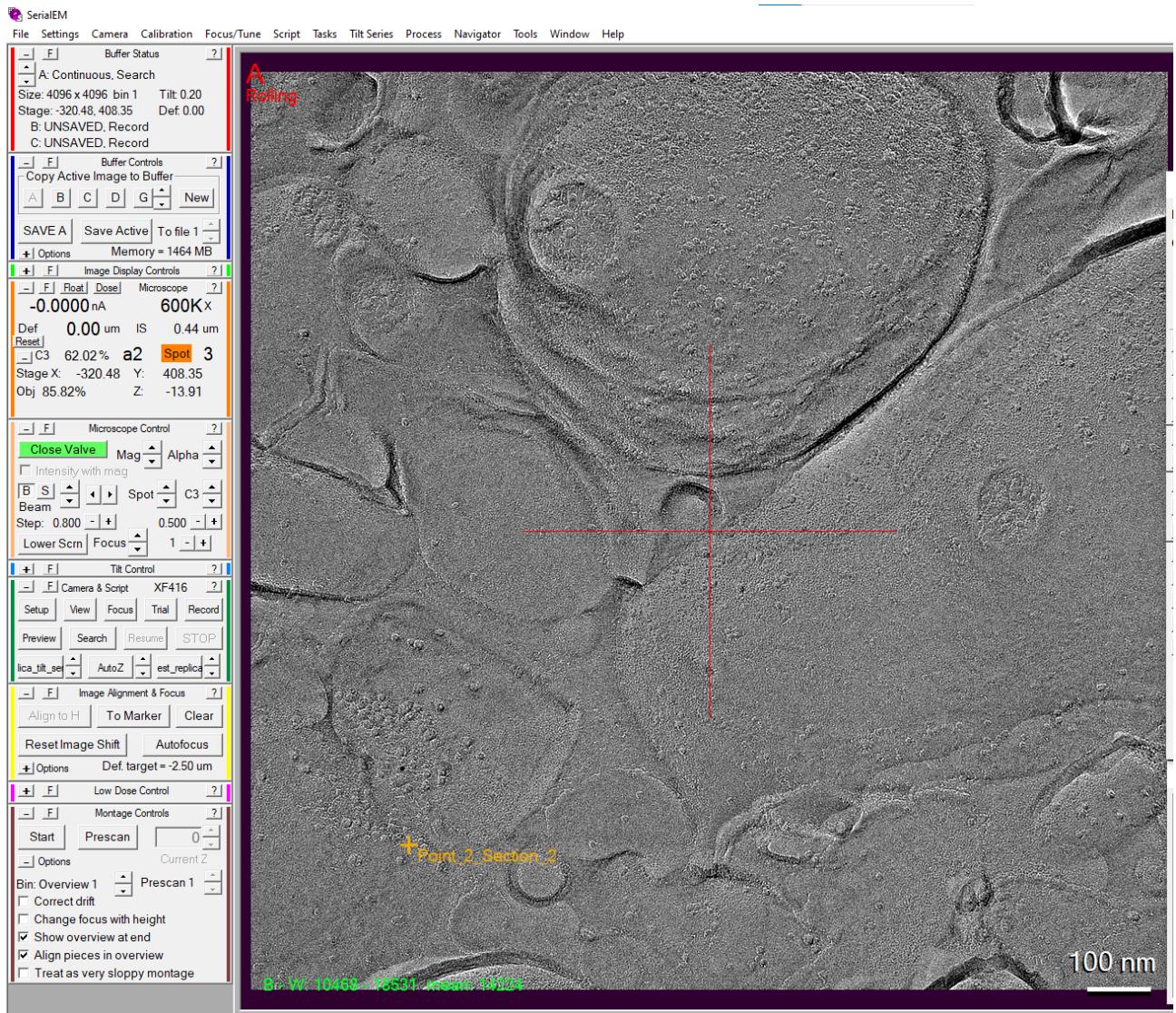


Figure 38: Text

Go to map magnification, click realign to item in navigator

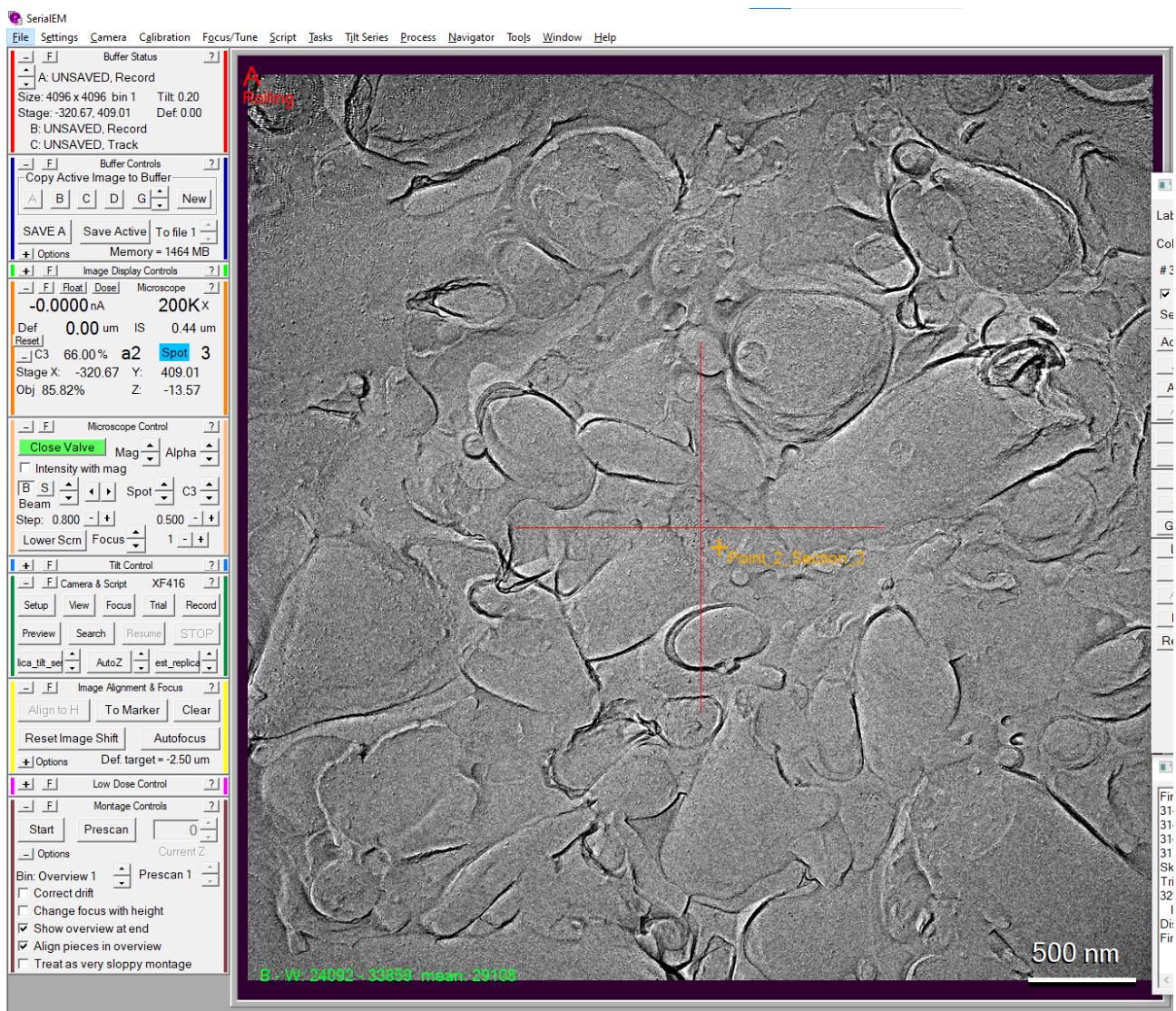


Figure 39: Text

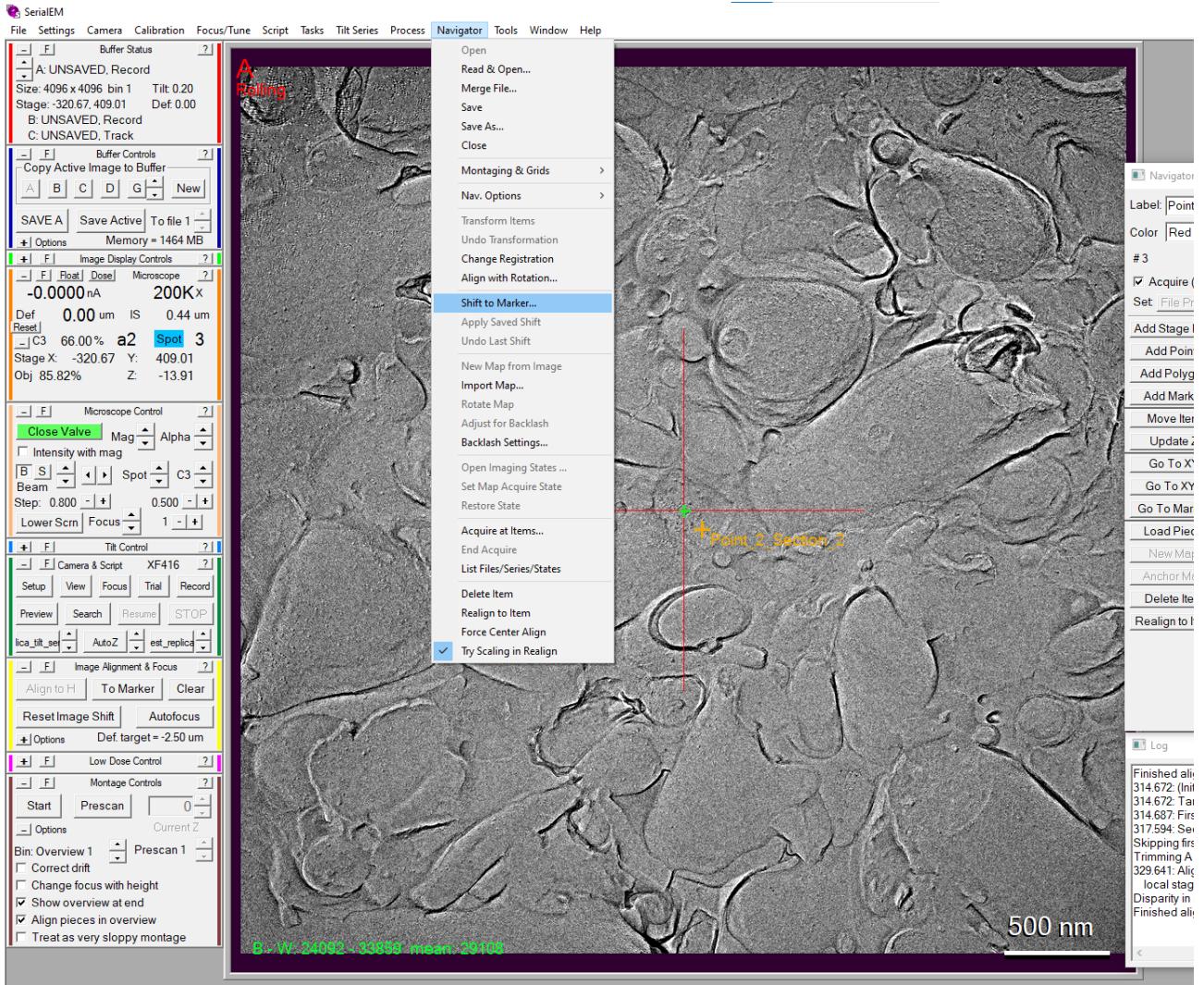


Figure 40: Text

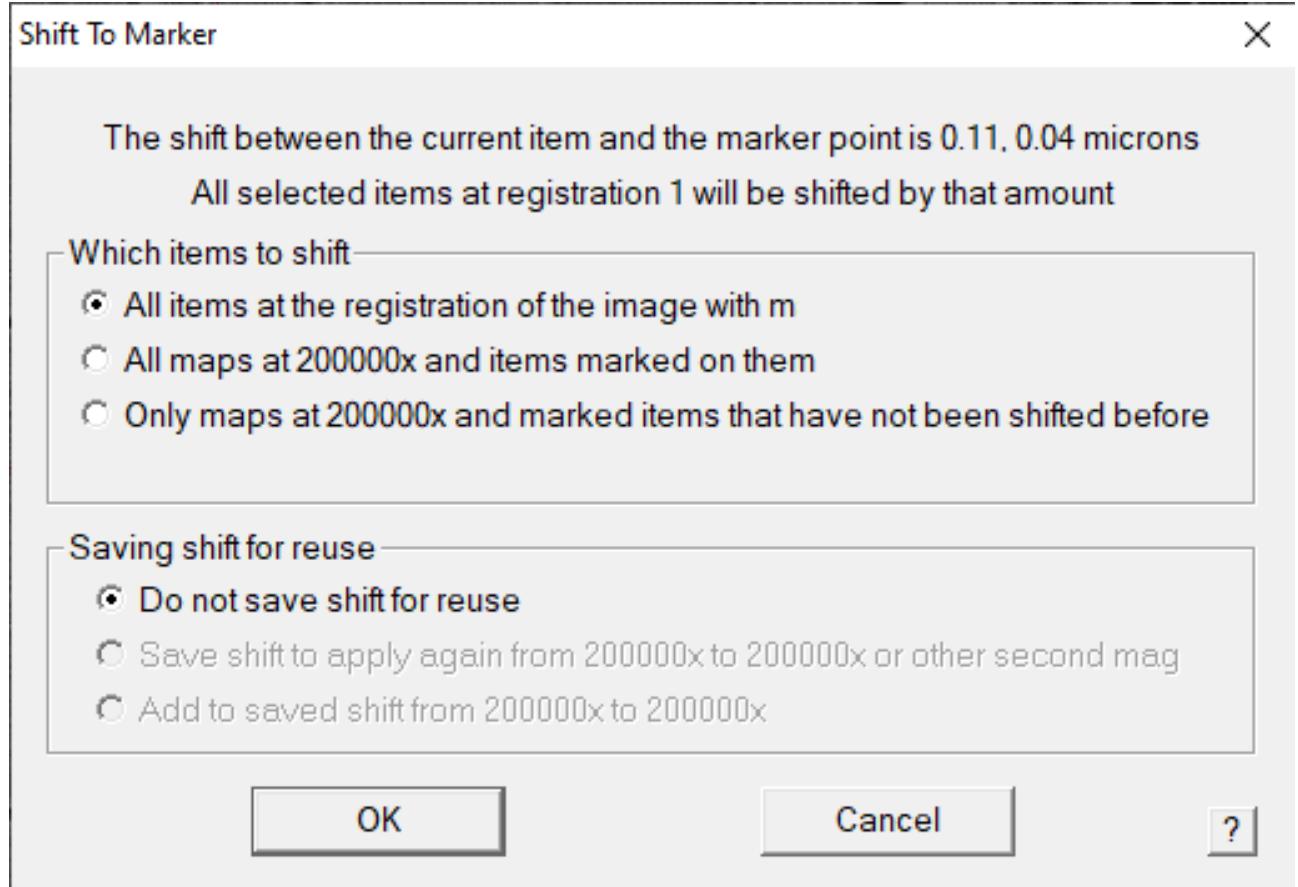


Figure 41: Text

Then go to target Mag

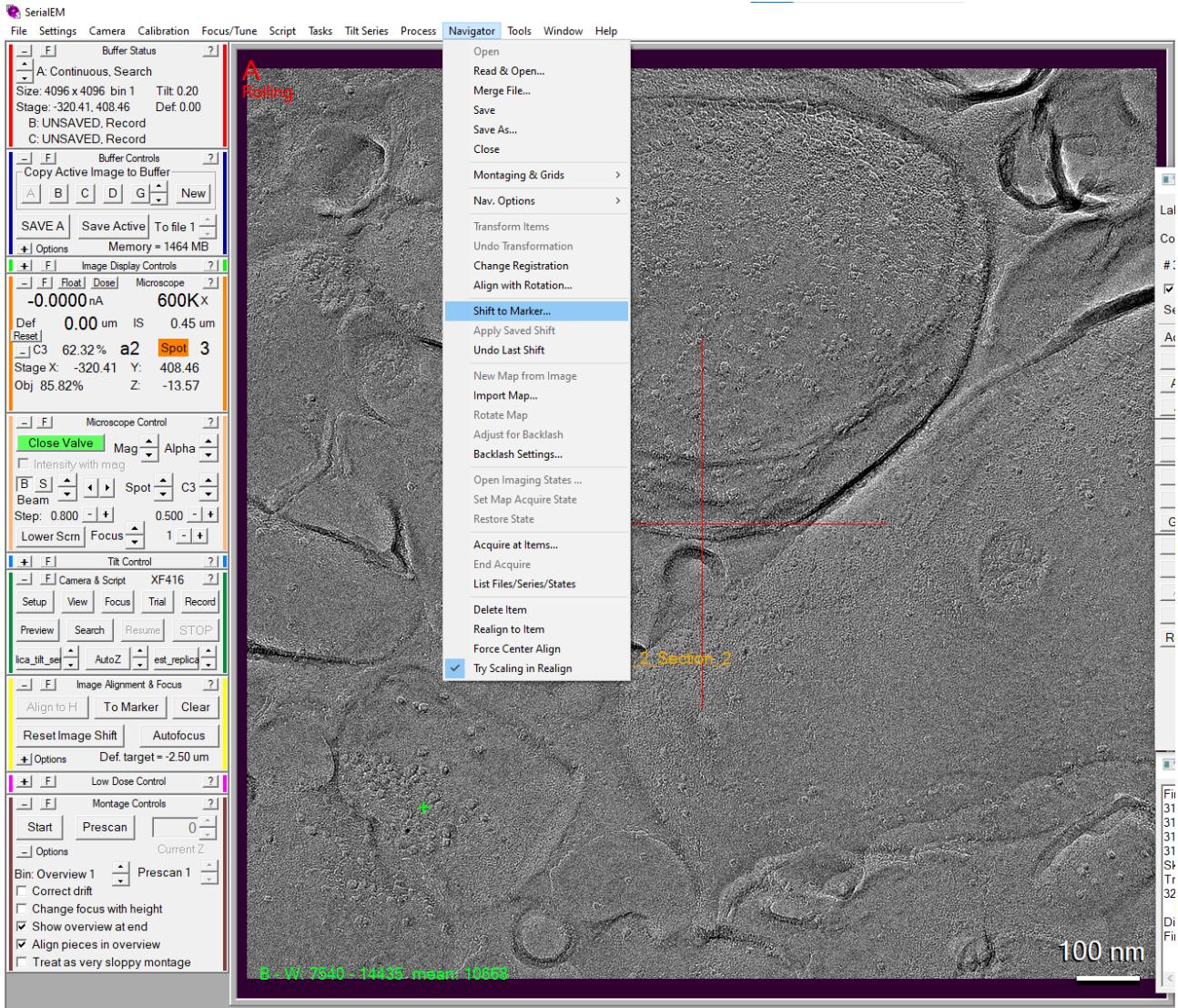


Figure 42: Text

Shift To Marker

X

The shift between the current item and the marker point is -0.16, 0.33 microns

All selected items at registration 1 will be shifted by that amount

Which items to shift

- All items at the registration of the image with m
- All maps at 200000x and items marked on them
- Only maps at 200000x and marked items that were shifted along with the map that the current item was marked on

Saving shift for reuse

- Do not save shift for reuse
- Save shift to apply again from 200000x to 600000x or other second mag
- Add to saved shift from 200000x to 600000x

OK

Cancel

?

Figure 43: Text