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Title: Strategies for Optimization of Cryogenic Electron Tomography Data Acquisition

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group?

   ☒ Interview Statements are read by JoVE's voiceover talent.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 30
Number of Shots: 37

Introduction

1. Introductory Interview Statements

NOTE to VO Talent: Please record Interview and Conclusion statements

REQUIRED:

1.1. The set-up of a large-scale data collection session is a time-consuming procedure that can considerably reduce the remaining microscope time available for tilt series acquisition.

1.1.1. 8.4.3.

1.2. This method enables fast setup of large-scale automated tomography data acquisition to maximize the time-efficiency of tomography experiments that require a careful selection of acquisition targets.

1.2.1. *6.4.1*

Protocol

2. Selecting the Targets

2.1. Begin by opening a dummy SerialEM instance **[1]**.

2.1.1. SCREEN: 62383_2.1.2_mp4 00:00:00 – 00:00:14

2.2. Once the first grid square is mapped, use the SerialEM Navigator menu option **Merge** to see the montage in the dummy SerialEM instance **[1]**.

2.2.1. SCREEN: 62383_2.2.1_mp4 00:00:00 – 00:00:13

2.3. Double click on the **Navigator** window to open the grid square map **[1]**.

2.3.1. SCREEN: 62383_2.3.1_mp4 00:00:00 – 00:00:15

2.4. Search the map and use the dummy SerialEM Navigator option **Add Points** to add image acquisition points on the target of interest **[1]**.

2.4.1. SCREEN: 62383_2.4.1_mp4 00:00:00 – 00:00:11

2.5. After mapping the new squares, save the **Navigator** file and merge the Navigator again. Continue until all grid squares are mapped **[1]**.

2.5.1. SCREEN: 62383_2.5.1_mp4 00:00:00 – 00:00:29

3. Generate Virtual Maps

3.1. Again, merge the navigator file with the dummy SerialEM instance **[1]**.

3.1.1. SCREEN: 62383_3.1.1_mp4 00:00:00 – 00:00:15:

3.2. Run the PyEM **Virtual maps** script from the dummy SerialEM menu, Select **Tools** and select **Virtual Anchor Maps**. This may take some time depending on the size and amount of the grid square maps, as well as the binning of the View and Preview maps **[1]**.

3.2.1. SCREEN: 62383_2.5.1_mp4 00:00:00 – 00:01:34

4. Microscope Tuning

4.1. To ensure proper microscope performance, use the same magnification and beam size setup for data acquisition, in the following order **[1]**. Run SeriaLEM **Coma-free alignment by CTF [2]**.

4.1.1. SCREEN: 62383_4.1.1_mp4 00:00:00 – 00:00:27

4.1.2. SCREEN: 62383_4.1.2_mp4 00:00:00 – 00:00:22

4.2. Insert and center an objective aperture **[1]**. Run SerialEM **Correct Astigmatism by CTF [2]** and **GIF Quick Tune [3]**.

4.2.1. SCREEN: 62383_4.2.1_mp4 00:00:00 – 00:00:20

4.2.2. SCREEN: 62383_4.2.2_mp4 00:00:00 – 00:00:20

4.2.3. SCREEN: 62383_4.2.3_mp4 00:00:28 – 00:00:30, 00:02:46 – 00:02:50.

5. Set up Navigator

5.1. In SerialEM, open the new navigator file. Deselect all **A** points, select the first View maps, select **Collapse**, click on **A** twice, and deselect **Collapse [1]**.

5.1.1. SCREEN: 62383_5.1.1_mp4 00:00:00 – 00:00:18

5.2. Select the first view map position, press **Shift plus T**, then select the very last view map position, and press **Shift plus T** again **[1]**.

5.2.1. SCREEN: 62383_5.2.x_mp4 00:00:00 – 00:00:19

5.3. Choose **Single Frame Images** in the properties of the file to open a dialog **[1]**.

5.3.1. SCREEN: 62383_5.2.x_mp4 00:00:19 – 00:00:23

5.4. In the next File Properties dialog, select the desired parameters according to the imaging needs and the instrument setup **[1]**.

5.4.1. SCREEN: 62383_5.2.x_mp4 00:00:23 – 00:00:29

5.5. When prompted, give a name with a number and click on **Save [1]**.

5.5.1. SCREEN: 62383_5.2.x_mp4 00:00:29 – 00:00:39

5.6. Set up the tilt series controller for the first TS position. When done, click **OK** to set these parameters for all tilt series after this acquisition item. All Preview maps are now selected as **TS** with a numbered file name **[1]**.

5.6.1. SCREEN: 62383_5.2.x_mp4 00:00:39 – 00:01:11

6. Set the Focus/Track Positions

6.1. If needed, set the focus/track distance for each target **[1]**.

6.1.1. SCREEN: 62383_6.x.x_mp4 00:00:00 – 00:00:05

6.2. Double click on the **View Map** to load it, select the **Preview Map** in the Navigator list, then select **Edit Focus** in the Navigator window **[1]**.

6.2.1. SCREEN: 62383_6.x.x_mp4 00:00:00 – 00:00:05

6.3. In the **Low Dose Control** panel, deselect **Rotate inter-area axis** to position **Trial** and **Focus** along the stage tilt axis **[1]**.

6.3.1. SCREEN: 62383_6.x.x_mp4 00:00:05 – 00:00:12

6.4. Click on the desired region in the loaded view map to set the focus-trial position for this tilt series and ensure that the Navigator item has **TSP** set. Repeat the procedure for all items **[1]**.

6.4.1. SCREEN: 62383_6.x.x_mp4 00:00:12 – 00:00:30

7. Set up Additional Scripts

7.1. Edit the focus range in the script **pretomo**, which runs before each tilt series **[1].**

7.1.1. SCREEN: 62383_7.1.1_mp4 00:00:00 – 00:00:23

7.2. In the SerialEM Tilt Series menu, check **Run Script in TS** and select the script number of the **duringtomo** script during each tilt **[1]**.

7.2.1. SCREEN: 62383_7.2.1_mp4 00:00:00 – 00:00:18

8. Run

8.1. Check the nitrogen tank level and whether the Autoloader turbo off is selected **[1]**.

8.1.1. SCREEN: 62383_8.1.2_mp4 00:00:00 – 00:00:10

8.2. Check the data storage free space. In the SerialEM file menu, deselect continuous saving for the log file. Each tilt series will get its own log file **[1]**.

8.2.1. SCREEN: 62383_8.1.2_mp4 00:00:00 – 00:00:14

8.3. After clicking on **Acquire at Items** in the Navigation menu, run the script **PreTomo [1]**. Select **Primary task Acquire tilt series [2]**, then select **Run script after PostTomo [3]**.

8.3.1. SCREEN: 62383_8.x.x_mp4 00:00:00 – 00:00:07

8.3.2. SCREEN: 62383_8.x.x_mp4 00:00:07 – 00:00:08

8.3.3. SCREEN: 62383_8.x.x_mp4 00:00:08 – 00:00:09

8.4. Select **Close column valves at end [1]** and **send email at end**, then click on **GO [1]**.

8.4.1. SCREEN: 62383_8.x.x_mp4 00:00:09 – 00:00:17

Results

9. Results: Optimization of Cryogenic Electron Tomography Data Acquisition

9.1. Seventy-one suitable squares were selected on the grid map at lower magnification. Medium magnification maps were acquired with settings that allow for the direct visualization and identification of the sample of interest, coronaviruses in this case. The acquisition time was 3 minutes per square, 3 hours and 45 minutes in total **[1]**.

9.1.1. LAB MEDIA: Figure 1A

9.2. As the first square map was created, a dummy SerialEM instance was opened on a separate computer to visualize the square map and to add points on targets suitable for tilt series acquisition **[1]**.

9.2.1. LAB MEDIA: Figure 1B

9.3. SerialEM low-dose was set up and reference view and preview images were taken and saved as maps. The latter maps could then be used immediately on the dummy SerialEM instance to generate the virtual view and virtual preview from the corresponding square map images **[1]**.

9.3.1. LAB MEDIA: Figure 1C,1D

9.4. The virtual view maps were used for an initial centering of the target **[1]** followed by a final centering performed at the actual tilt series acquisition magnification **[2]** using the virtual preview map **[3]**.

9.4.1. LAB MEDIA: Figure 1C,1E

9.4.2. LAB MEDIA: Figure 1F

9.4.3. LAB MEDIA: Figure 1D

Conclusion

10. Conclusion Interview Statements

NOTE to VO Talent: Please record Conclusion statements

10.1. Users should allow themselves plenty of time to explore the sample landscape. After having searched some maps, one develops a better feel for good quality areas to collect data from.

10.1.1. *2.4.1*