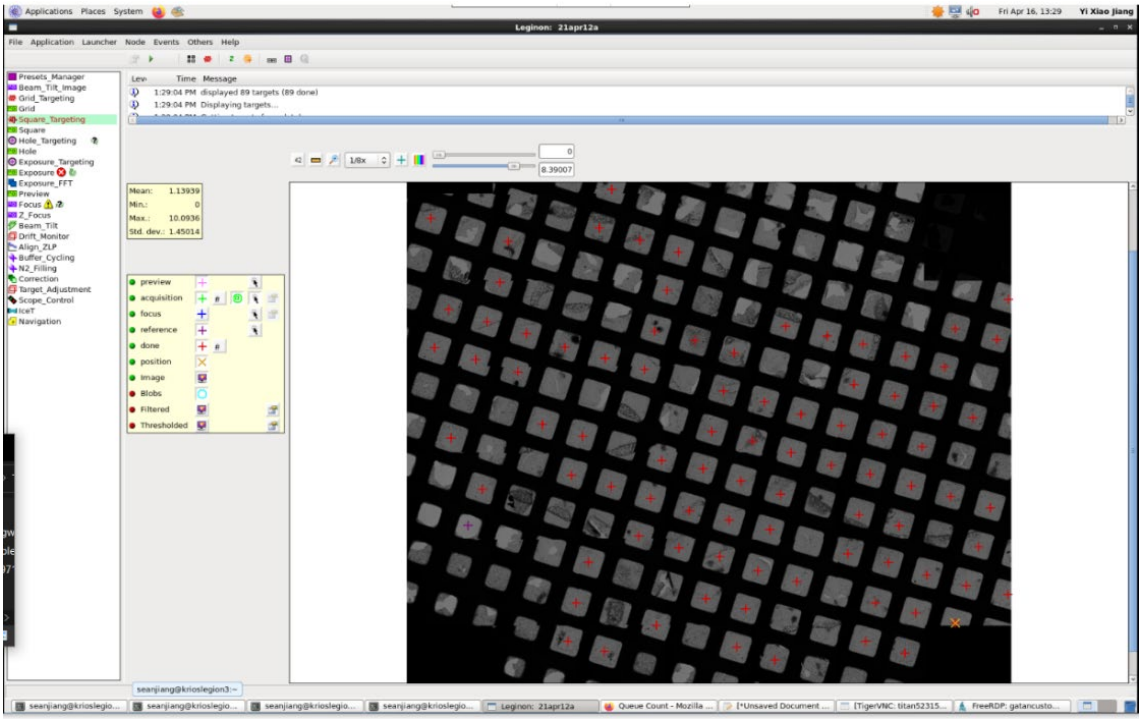
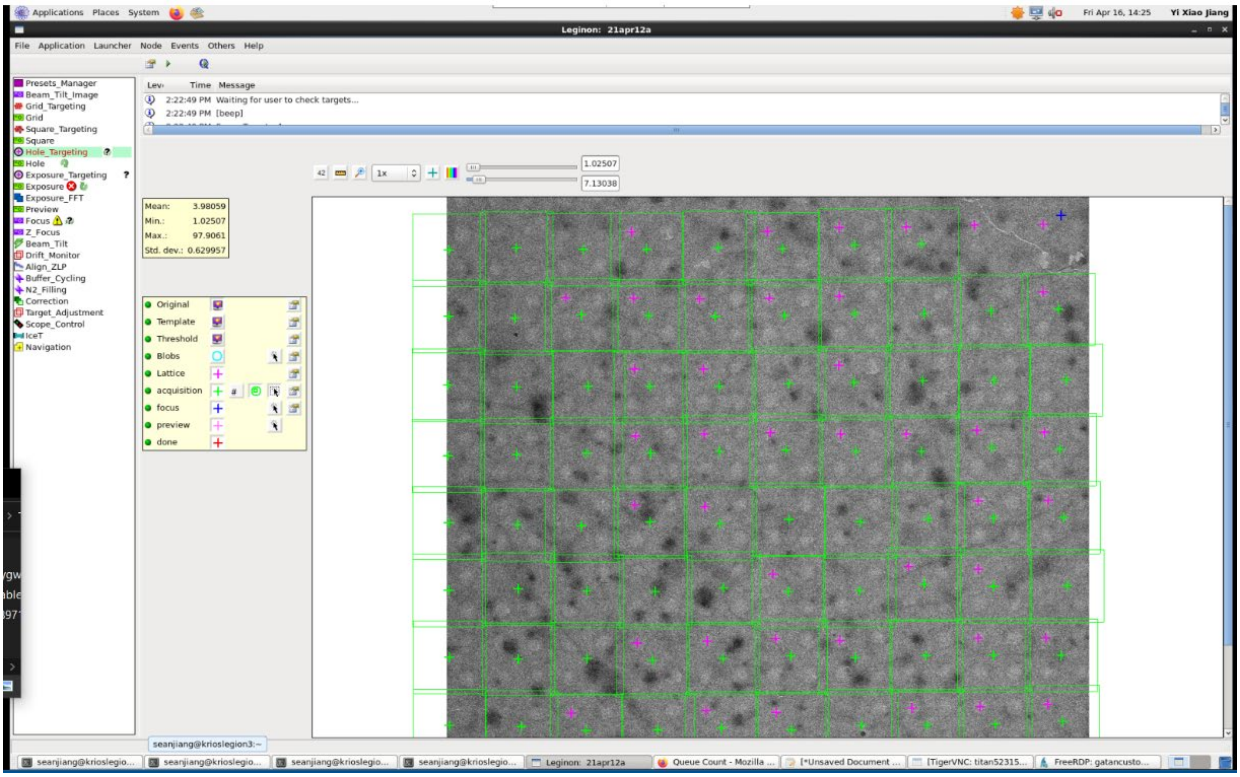
Our samples are extracted from disease patient brain tissues and thus are very sparse and precious. With these limited tissues, the grids we prepared have lower sample concentration than what is normally optimal for automated data collection. We estimate that ~10-20% of the holes contain our fibril particles. To increase the efficiency of data collection, we implement a target selection strategy using Leginon. First, we take low dose "pre-exposure" images to see our particles in the holes. Then we use these images to manually select exposure targets to be submitted into a queue. Below we show screenshots of our data collection using Leginon, to give an idea of our procedure.

Grid atlas



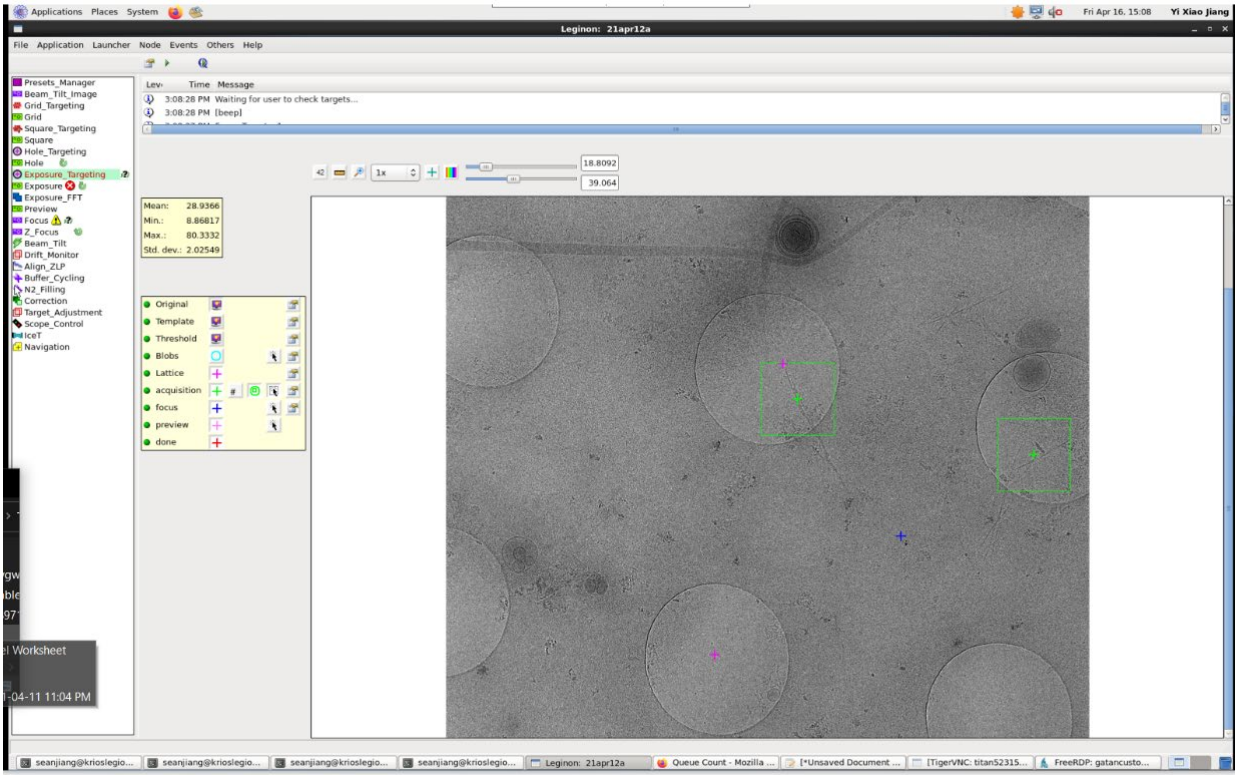
Pre-exposure at the “square level”

- **Magnification = 740x**



Pre-exposure at the “hole level”; these images allow us to see which holes have our fibril particles; then we perform manual target selection (green box) and focus selection (blue x); this pre-exposure beam is ~200x dimmer than the exposure beam and thus shouldn’t damage sample

- **Magnification = 6500x**
- Exposure time = 800msec



Exposure of selected targets

- **Magnification = 81,000x**
- C2 = 50um
- Defocus = -2.0um
- Illumination area = 1.80um
- Spot size = 5
- Dose = 12.58e-/A^2/sec
- Frame rate = 12 frames/sec
- Exposure time = 3sec
- Exposure total = 36 frames (3sec x 12frames/sec)
- Pixel size = 1.1A