NRAMM Visitors Guide to Queueing
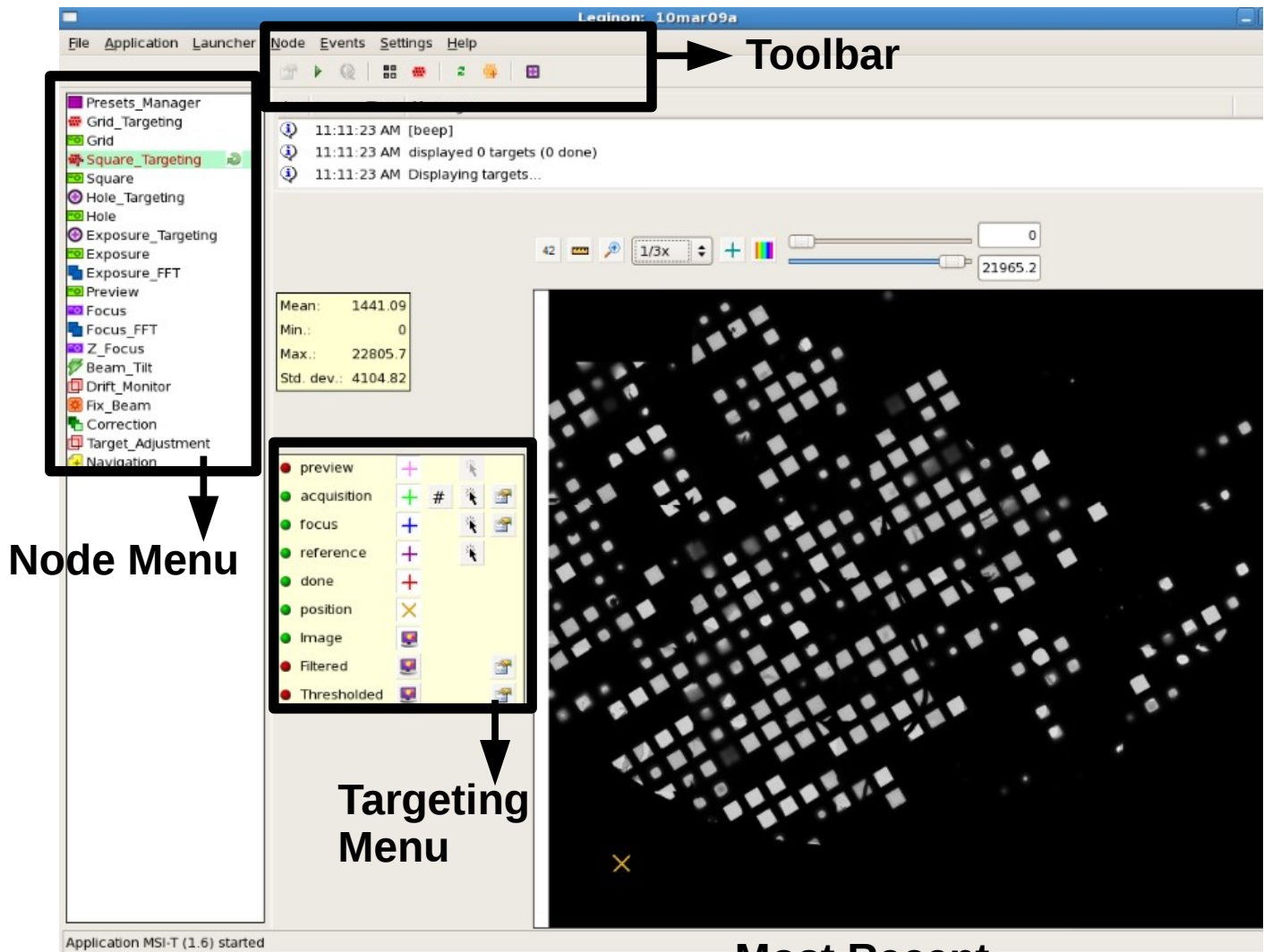
Leginon Basics

- Standard Leginon window and location of menus and functions you will be using


Toolbar

Node Menu

Targeting Menu

Most Recent Image at that nodes' level

Leginon Basics

- When working in Leginon, each selection from the left hand column performs a separate task. These are referred to as nodes.

- The nodes available may vary between different leginon applications based on the different tasks necessary to perform different types of data collection, but the nodes of matching name will perform the same task in the same way across leginon applications

- You will only be using a few of these nodes for target queueing (nodes marked *)
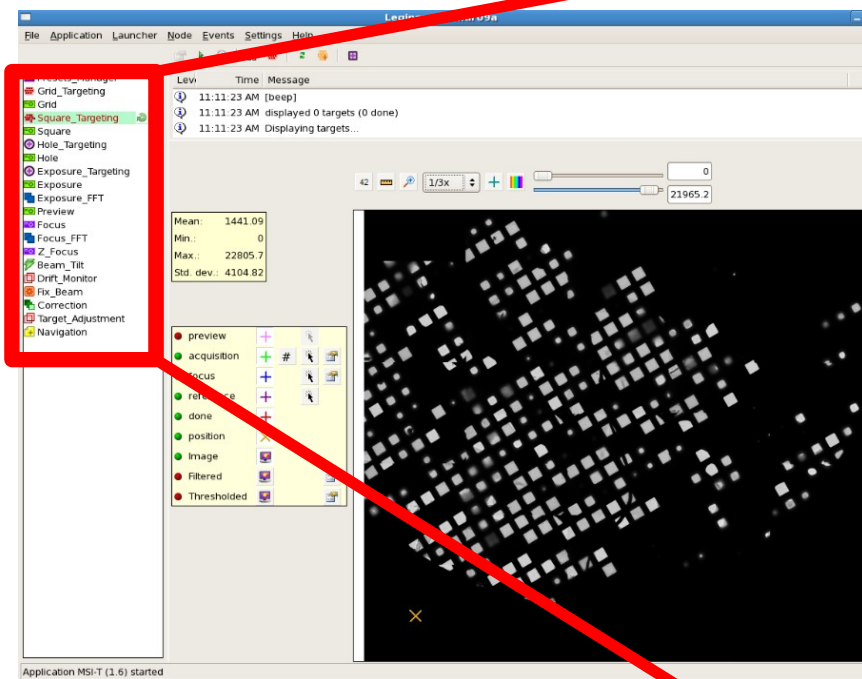
Leginon working screen
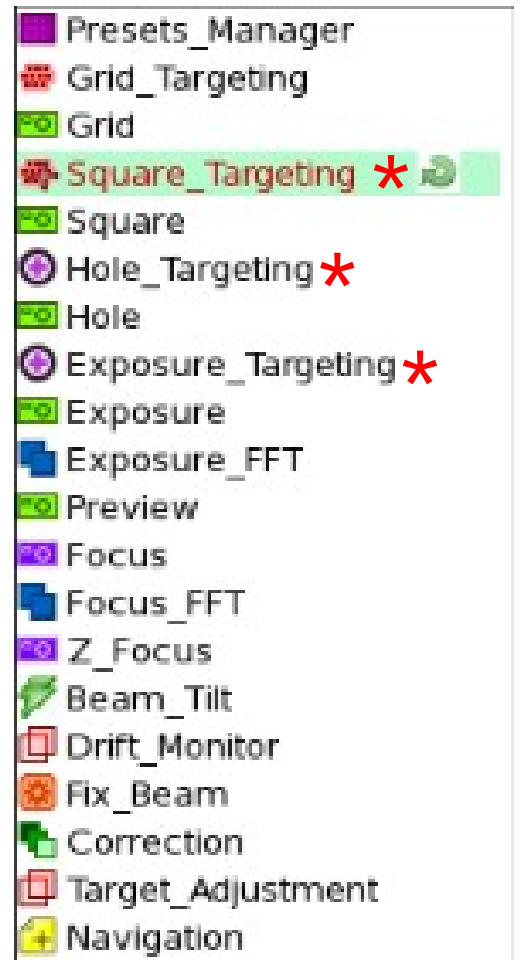
Node Menu

Leginon Basics

- When leginon is doing a task, green arrows will spin next to the nodes that are involved, as shown in the top node menu

- When leginon is waiting for you to make a selection, it will display a question mark (as shown in the bottom node menu) next to the node you need to make your selection in
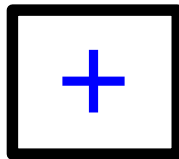
  - A question mark next to Hole targeting means you need to select holes on the next square

  - A question mark next to Exposure Targeting means you need to select the final exposure targets for the next hole.
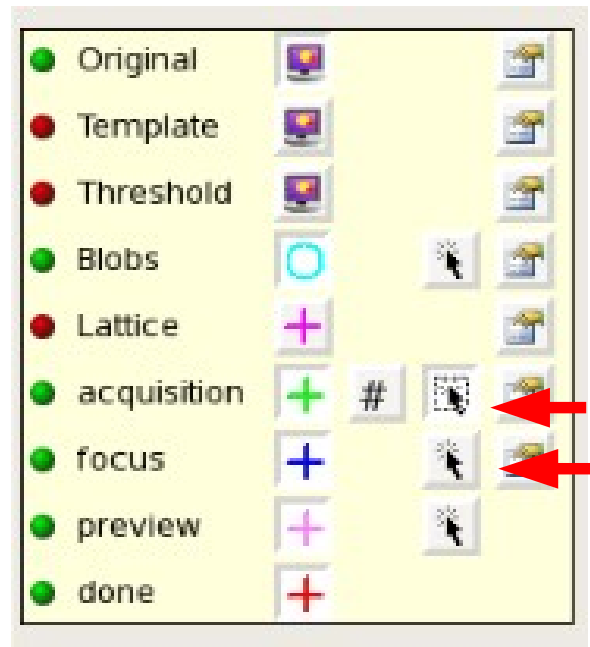
Leginon Basics

- When selecting targets:

    - Green crosses will be exposure locations ☐ (green +)
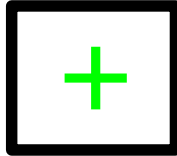
    - Blue crosses will be focus locations ☐ (blue +)

- Right clicking on the arrow icons in the targeting menu to the left of the image will change your selection between focus and exposure

- To remove a target you have selected right click on that target. You must have acquisition selected to remove an acquisition target and focus selected to remove a focus target.
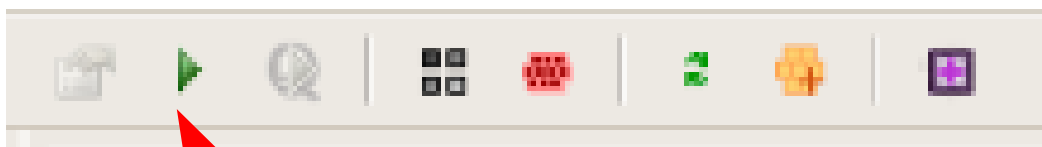
Square Targeting: How to Select Squares

- In the square targeting node, you will select squares from the atlas

    - Select Square Targeting from the node menu on the left-hand side of the screen

    - Refresh the page by left clicking the refresh icon in the toolbar; you must refresh this page every time you come back to select new targets or you will be re-submitting squares already used

Square Targeting Toolbar

Refresh Icon

- Select acquisition targeting by left clicking on the arrow next to Acquisition in the Targeting Menu (as shown in leginon basics section)

- Left click on the squares you want to select, this will leave a green cross ⊞ on the squares you have selected

- When you have selected all of the squares you want, submit them for data collection by left clicking the submit icon in the toolbar

Submit Icon   Square Targeting Toolbar

Square Targeting: Other useful Information

- In addition to the green crosses over new squares selected, the atlas image in the square targeting node will have red crosses over squares that have been previously selected and imaged +

- The atlas image will also display one orange X, this is the current position of the grid.

- Remember, you must refresh this page EVERY TIME you select new targets, or you will be re-submitting targets you have already used.

- Atlas viewing size can be changed by changing the ration in the pulldown menu at the top of the image.

- If some squares still have green crosses after refreshing, this means these squares have not been imaged yet and are still waiting in the queue. Do not re-select them. You also cannot un-select them by removing the cross- they have already been submitted.

- If you decide you do not want to use a square after submitting, you must wait until it comes up in the next step of queueing and submit it with no targets selected. This is the ONLY way to abort or delete submitted selections.

- If you decide you want to use a square that was not selected, you must wait until the next time you submit squares to add this selection.

Square Targeting: Typical Square Targeting Screen
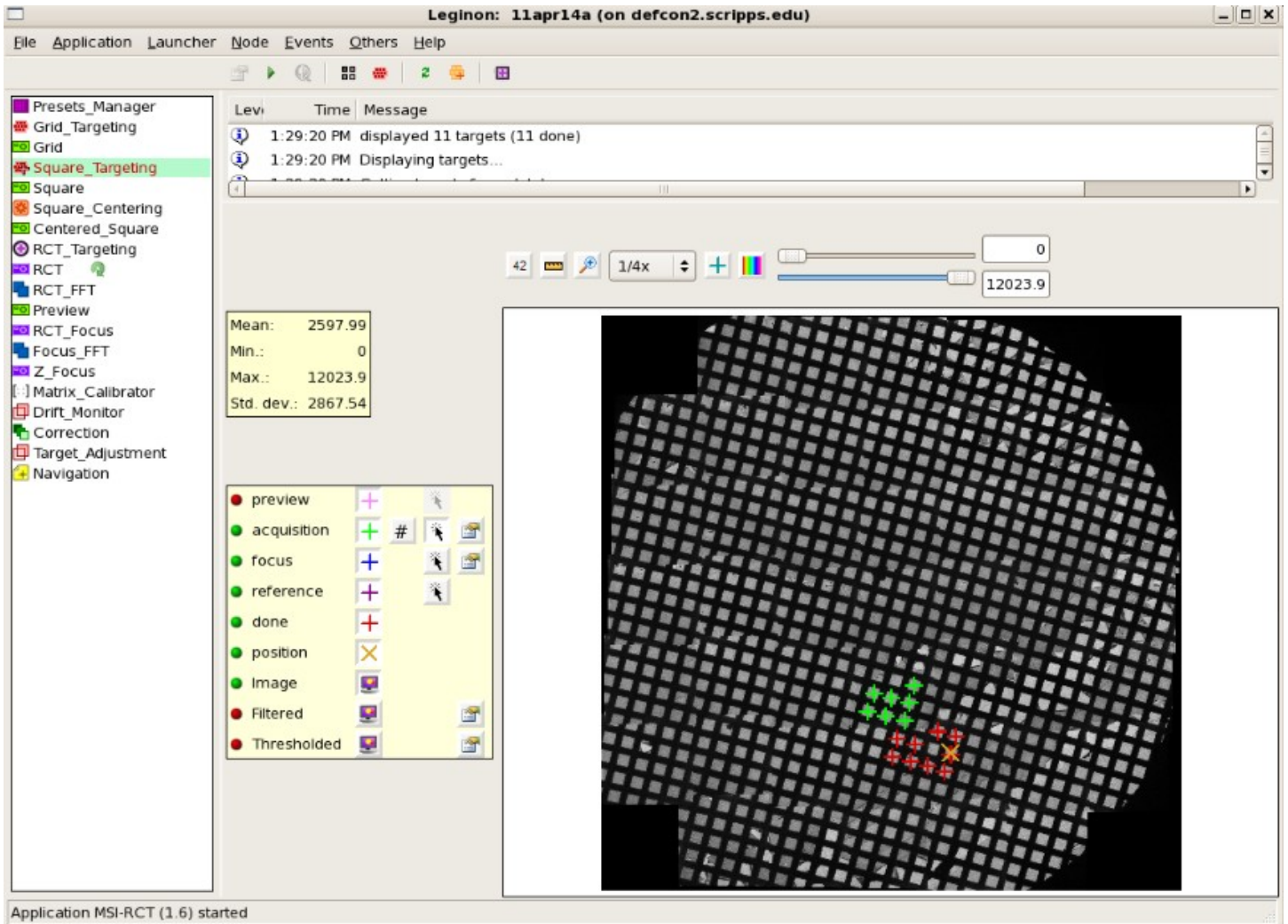
- On the Atlas image:
  - Red: Previously used squares
  - Orange: Current stage position
  - Green: Newly selected squares

Hole Targeting
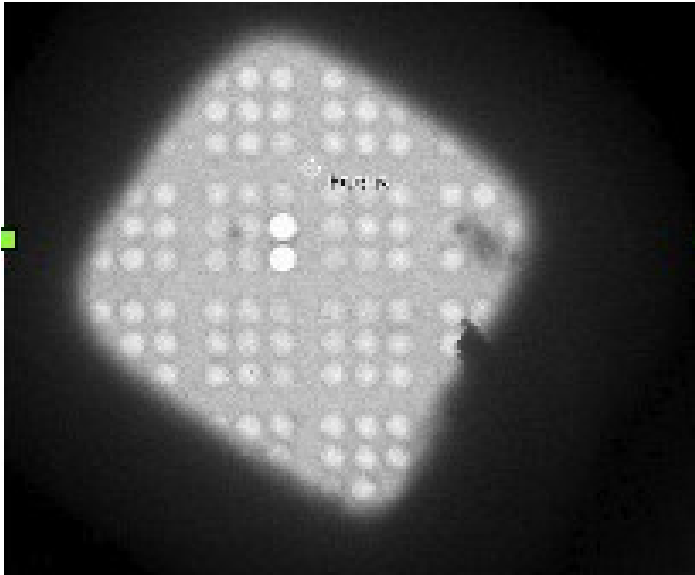
3X3 Hole Pattern
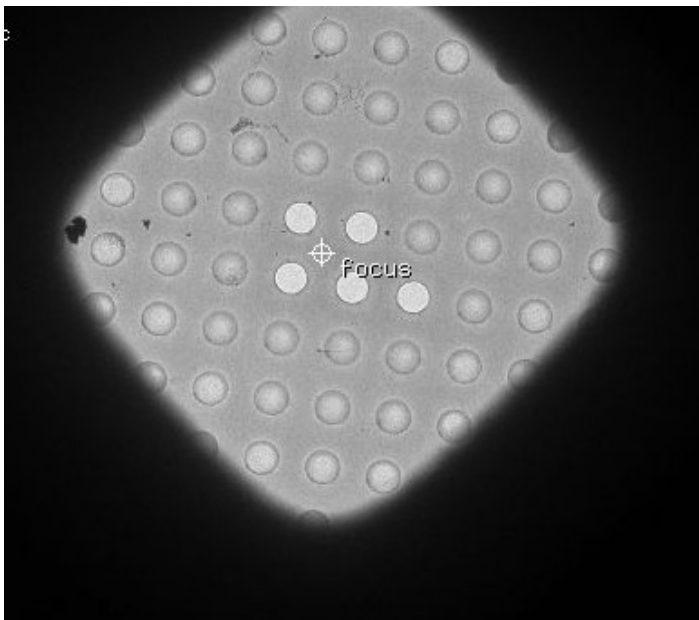
2/2 Hole Pattern

- If your sample is frozen on grids at NRAMM, it will probably be done on one of our 2 most commonly used grid designs

  - The 3X3 hole pattern has clusters of 9 two-micron holes. Each cluster is picked as 1 hole, in the middle of the useable area

  - The 2/2 hole pattern has individual 2 micron holes, evenly spaced 2 microns apart across the whole square. Each 2 micron hole is picked as 1 hole.

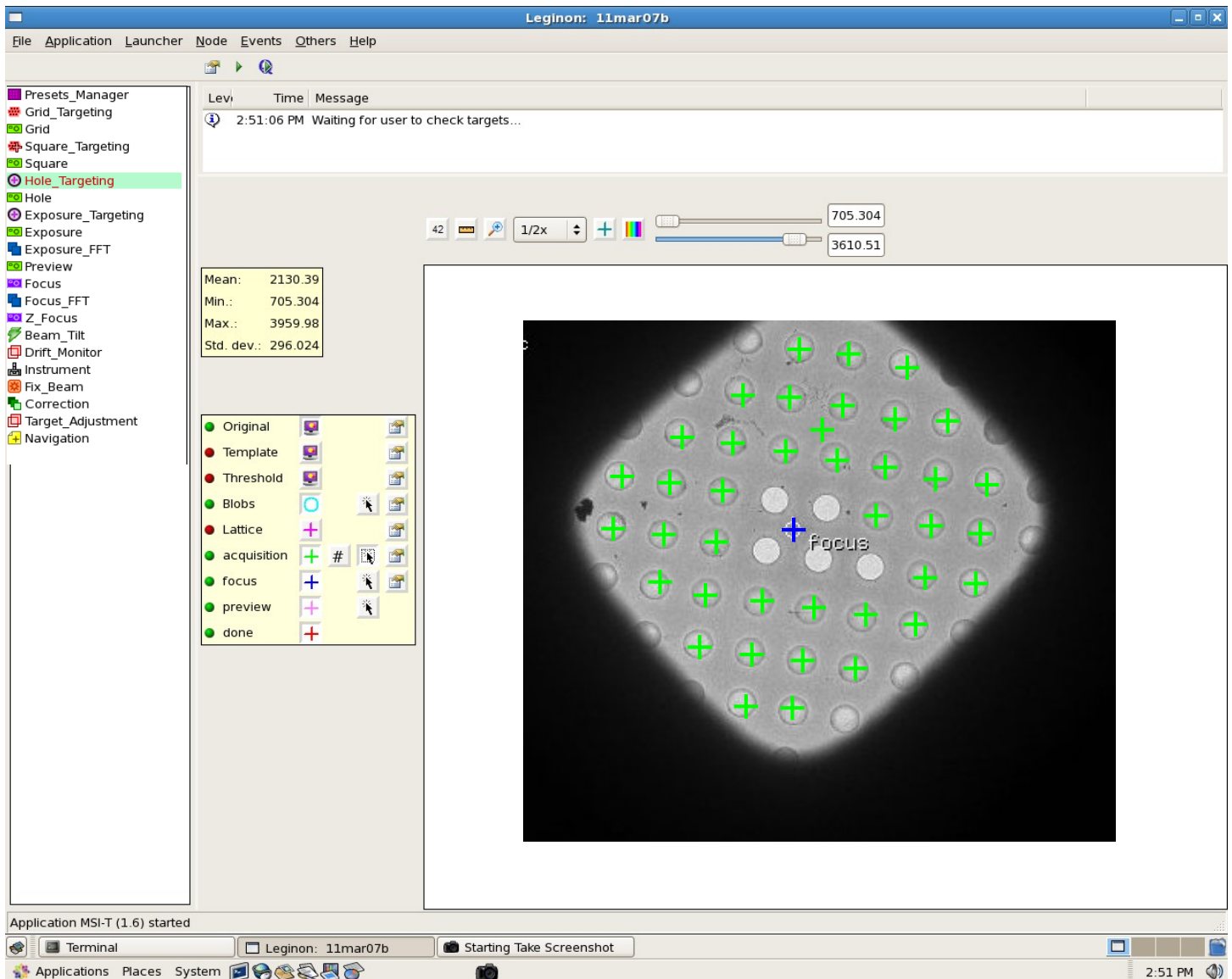# Hole Targeting: How to Select Holes

- In the Hole Targeting node, you will select holes from the square

  - Select Hole Targeting from the node menu on the left-hand side of the screen.

  - When Leginon is ready for you to pick holes there will be a question mark next to Hole Targeting in the Node Menu

  - Select acquisition targeting by left clicking on the arrow next to Acquisition in the Targeting Menu (as shown in leginon basics section)

  - Left click on the holes you want to select. This will leave a green cross [+] on the holes you have selected

- When you have selected all of the holes you want, select one focus point for Leginon to determine the eucentric height of the square

  - Select focus targeting by left clicking on the arrow next to Focus in the Targeting Menu (as shown in leginon basics section)

  - Left click on one spot you want to select. This can be between holes or over a part of the square you will not be using. This will leave a blue cross [+] on the spot you have selected

- See the following examples of each hole pattern with all necessary selections made, ready to submit

Hole Targeting: 3X3 Hole Pattern Example Square
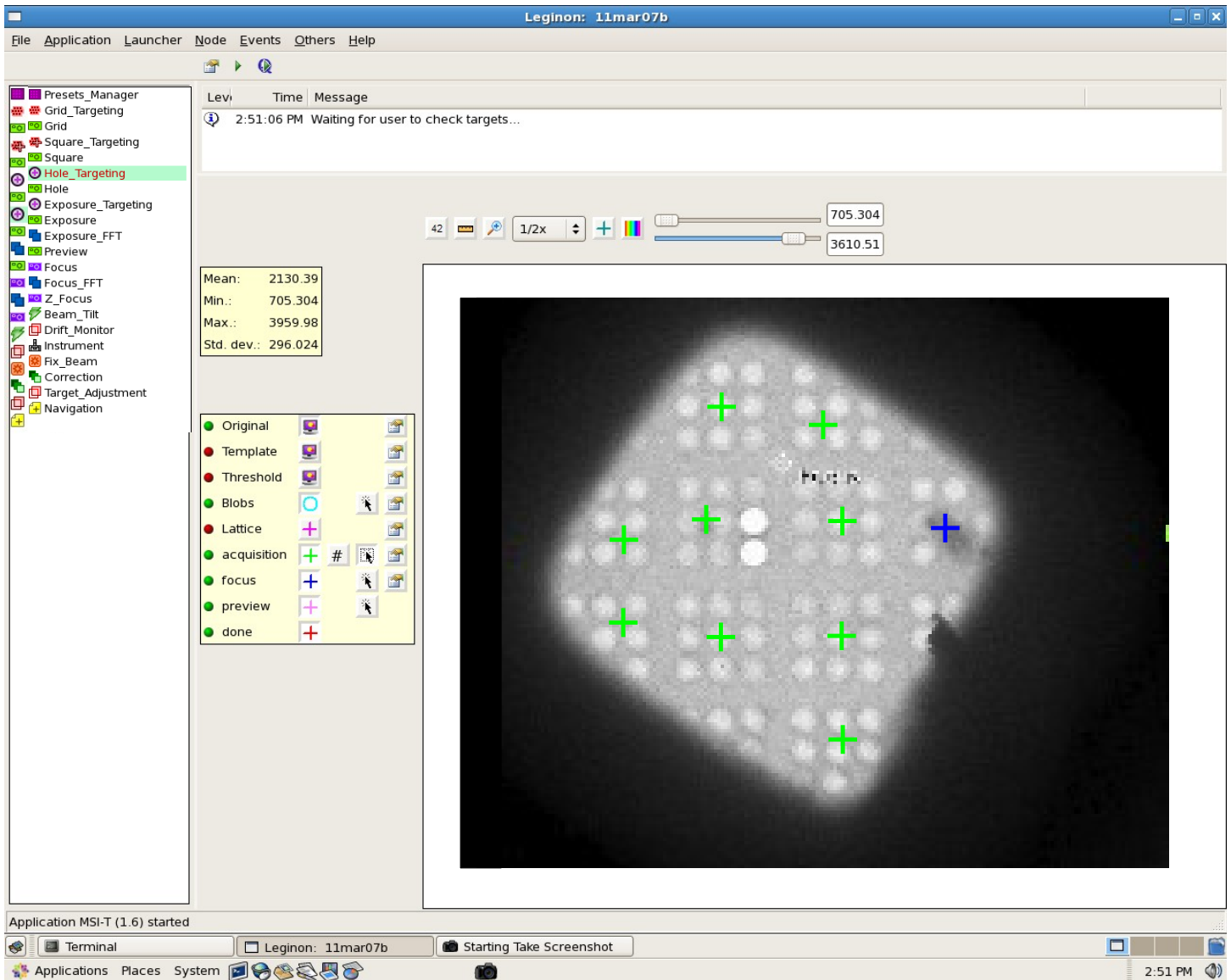
- Green crosses indicate Hole Targets

  - Note that for this hole pattern, the hole target is selected in the middle of the area you want to use. If all 9 holes are useable, then it goes directly in the middle, but if only a portion of the 3X3 array is useable, you want to place your target in the middle of this useable area.

- Blue cross indicates Eucentric Focus Target

Hole Targeting: 2/2 Hole Pattern Example Square

- Green crosses indicate Hole Targets

  - Note that for this hole pattern, the hole that you want to use is selected as an individual target.

- Blue cross indicates Eucentric Focus Target

Hole Targeting: How to Select Holes

- After selecting all the holes you want to use on the square AND 1 focus target, submit the holes

  - Right click on the submit button ▶ in the toolbar

- There will be a delay while Leginon determines the eucentric height. You will see spinning arrows in the Z-focus node, but you do not need to do anything.

- After performing the Z-focus steps, Leginon will take an image of your first hole. When it is ready you will see a question mark next to the Exposure Targeting node in the node menu.

Hole Targeting: Other useful Information

- Square image viewing size can be changed by changing the ratio in the pulldown menu at the top of the image.

- If you selected a square you do not want to use, simply submit it in Hole Targeting with NO targets selected.

- If you decide you do not want to use a selected hole after submitting, you must wait until it comes up in the next step of queueing (Exposure Targeting) and submit it with no targets selected. This is the ONLY way to abort or delete submitted selections.

- If you forget to select a focus point at this level, submit all holes with NO targets and re-select square in you next round of square targeting. Proceeding without a z-focus process will result in all final images from that square being improperly focused

- DO NOT attempt to use the abort or abort queue buttons on the toolbar of any node to remove targets at ANY level.

Hole Targeting: Other useful Information

- If you decide you want to use a hole that was not selected after the hole targets were submitted, you must select this square again at the square targeting level and start from the beginning of the queueing process. You will only need to select holes that were not selected previously, but you MUST also select another focus target. The previous Z-focus performed will not be associated with re-submission of the same square.

- Focus targets can be selected over an area of empty of un-useable holes as shown in the examples. If all holes will be used, select a point on the carbon between holes.

- Leginon will take you thru Exposure Targeting (next section) of all holes on your square before displaying the next square for hole targeting

Exposure Targeting

- Depending on the final magnification being used, you will select 1-4 final exposure targets for each individual hole (This means up to 36 targets per hole image for 3X3 pattern grids)

  - NRAMM staff will advise you of the appropriate number for final exposure targets for your data collection

- You will also select 1 focus target from each hole image

- Targets are usually selected evenly spaced around the hole, fairly close to the carbon edge

- Why select final exposure targets near the carbon edge? There are a few reasons.

  - In many cases the majority of particles are found near the edge of the whole, not in the middle

  - Having a bit of carbon in the image helps endure that the image produces a strong enough fourier transform to perform a good CTF correction

  - Having the beam touch the carbon while imaging helps prevent beam induced motion or charging, a common effect in cryo-TEM that leads to poor-quality images

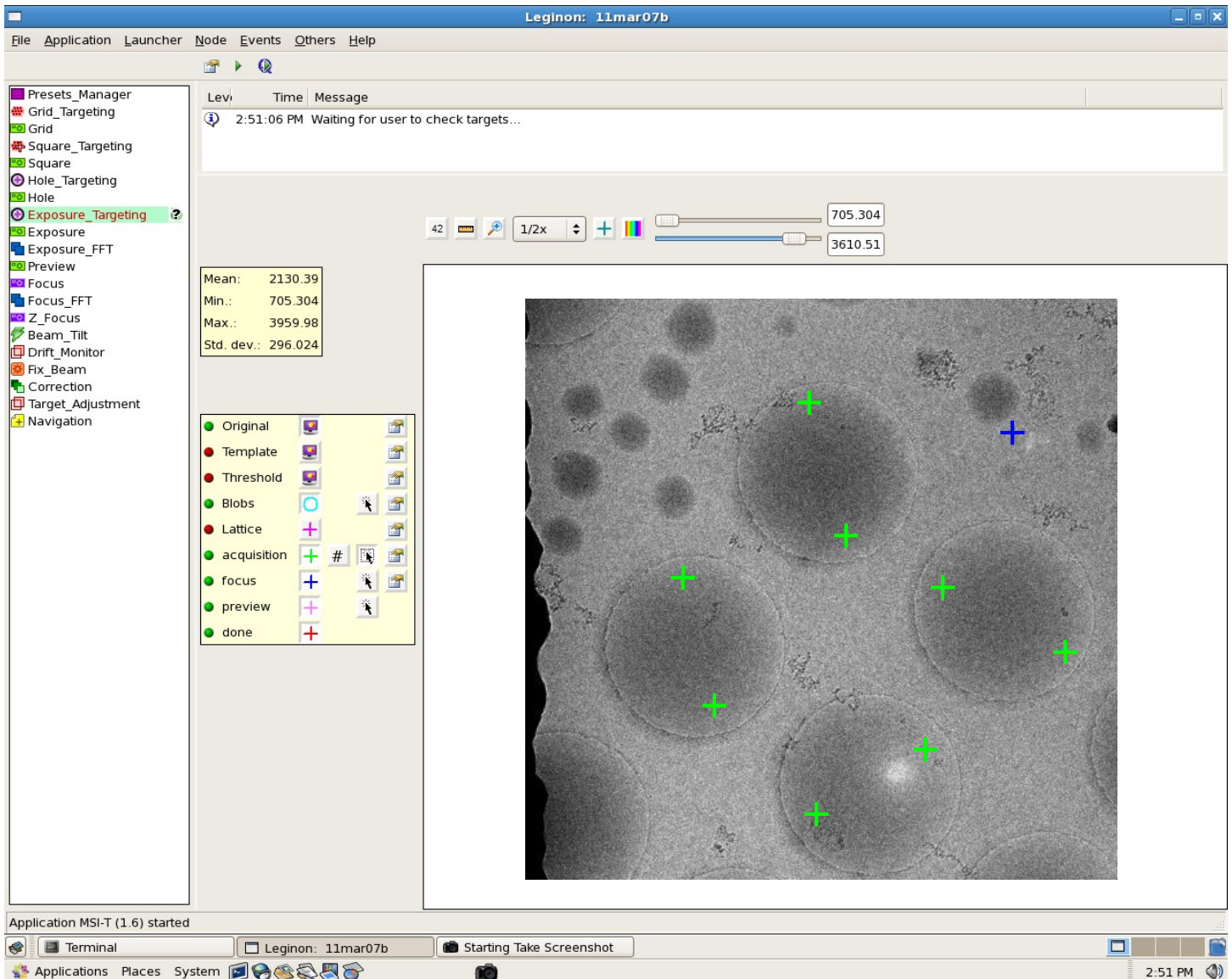# Exposure Targeting: How to Select Your Final Exposure Targets

- In the Exposure Targeting node, you will select your final exposure targets from the hole

    - Select Exposure Targeting from the node menu on the left-hand side of the screen.

    - When Leginon is ready for you to pick holes there will be a question mark next toExposure Targeting in the Node Menu

    - Select acquisition targeting by left clicking on the arrow next to Acquisition in the Targeting Menu (as shown in leginon basics section)

    - Left click on the points you want to select. This will leave a green cross ✚ on the points you have selected

- When you have selected all of the exposure targets you want, select one focus point for Leginon to determine the zero focus point for that area

    - Select focus targeting by left clicking on the arrow next to Focus in the Targeting Menu (as shown in leginon basics section)

    - Left click on one spot over the carbon to use for focusing. This spot should be sufficiently far from the hole to ensure you exposure area does not get burned during the focus process. This will leave a blue cross ✚ on the spot you have selected

- See the following examples of each hole pattern with selections made, ready to submit

# Exposure Targeting: 3X3 Pattern Example Hole
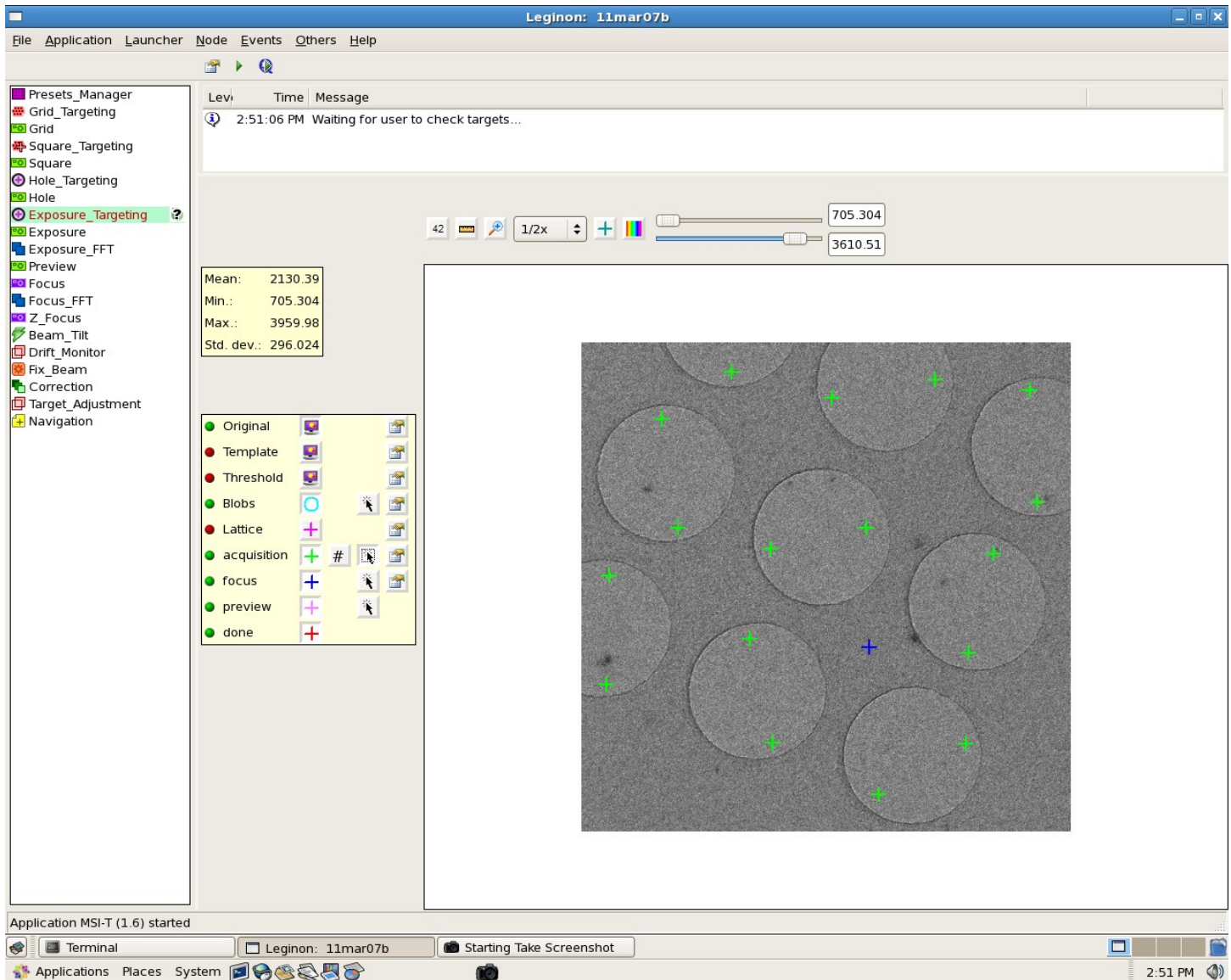
- When using the 3X3 hole pattern, targets are selected in all 9 holes on an individual hole image, as shown

- Only 1 focus target is required for the entire cluster of 9 holes

- When using all 9 holes, they will take up the entire hole image. In this case, you can place a focus target directly in the center of four holes in the 3X3 cluster, as shown in this example

Exposure Targeting: 3X3 Pattern Example Hole

- In this example, only part of the 3X3 hole cluster was used, so larger patches of carbon between the hole clusters is visible in the hole image

- In this case you can use these larger carbon areas for the focus spot

Exposure Targeting: 2/2 Pattern Example Hole
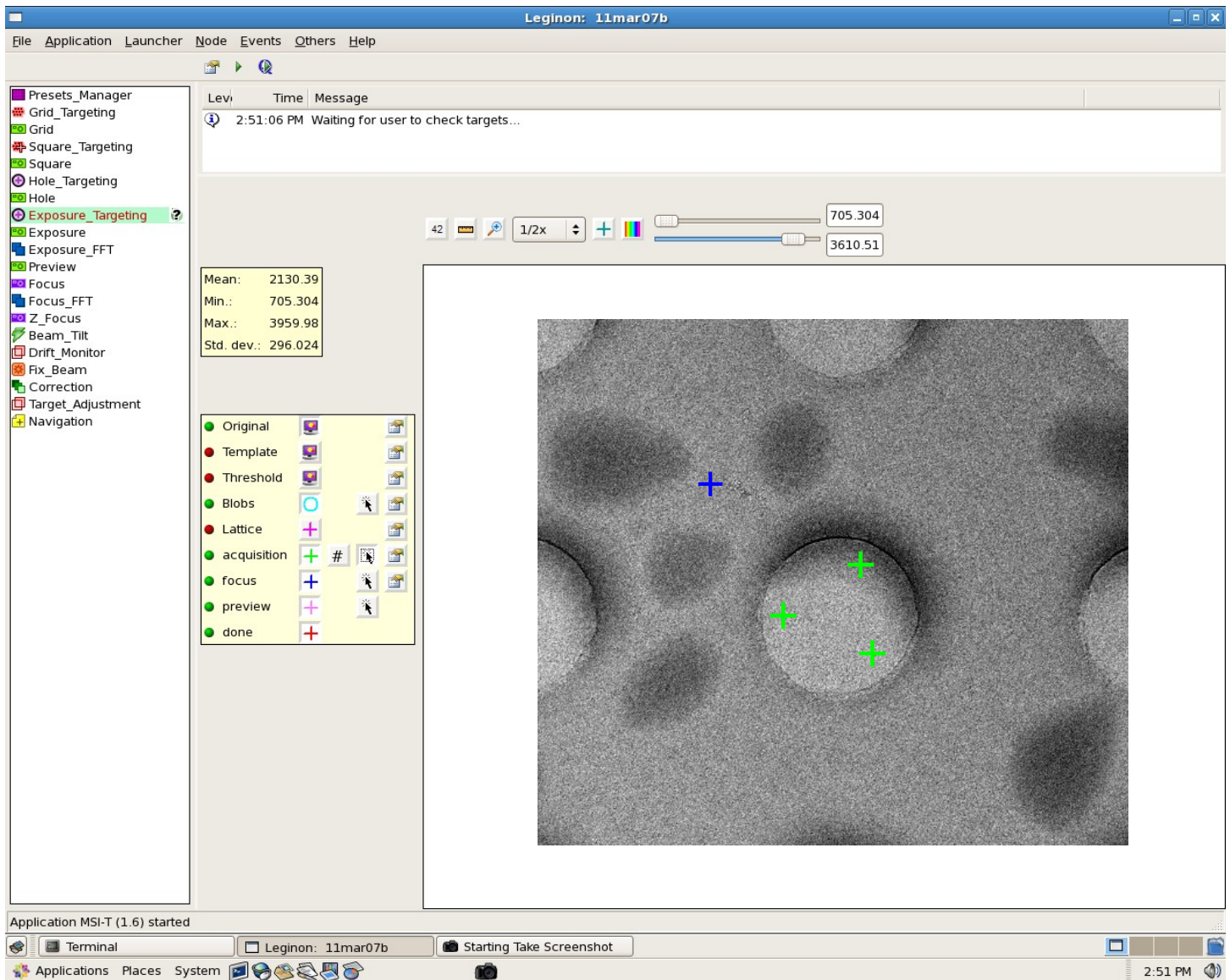
- With the 2/2 grid pattern, you select exposure targets on one individual hole

- In this example, 3 targets are selected per hole. This is a function of the magnification being used, not the grid pattern

- When using the 2/2 grid pattern, select one focus point per hole, in the carbon space between 4 holes

# Exposure Targeting: How to Select Your Final Exposure Targets

- After selecting all the exposure targets on the hole AND 1 focus target, submit the targets to the queue

    - Right click on the submit targets icon in the toolbar

Submit Targets Icon

Exposure Targeting Toolbar
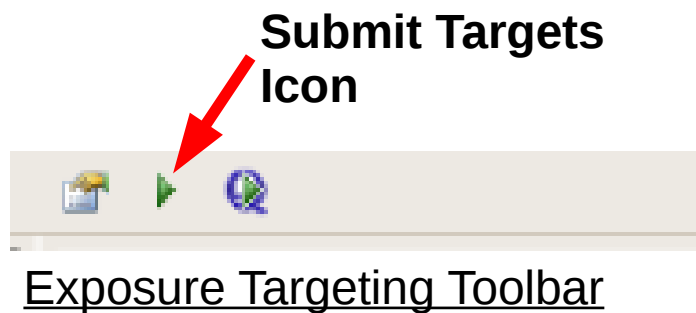
- After submitting your exposure targets to the queue, there will be a delay while Leginon moves to your next hole and takes an image for you to target. You will see spinning arrows, but you do not need to do anything.

- After taking an image of your next hole Leginon will display a question mark next to the Exposure Targeting node in the node menu, indicating it is ready for you to select the next set of exposure targets.

Exposure Targeting: How to Select Your Final Exposure Targets

- Continue this process until all holes selected have been used.

- If you selected more then one square in the square targeting node, Leginon will automatically move on to the next square when all holes have been used. In this case, it will display a question mark at the Hole Targeting Node, and you will proceed from the point of Hole Targeting.

- When Leginon has no more Holes or Squares to image, there will be no spinning arrows, no question marks, and you will not get any new Hole images in the Exposure Targeting or Hole Targeting nodes

- If you would like to add more targets to the queue, go back to the Square Targeting Node and repeat the whole process from the beginning

- If you are ready to submit the queue and start collecting data, right click on the Submit Queue icon in the Exposure Targeting Node Toolbar

Submit Queue Icon

Exposure Targeting Toolbar
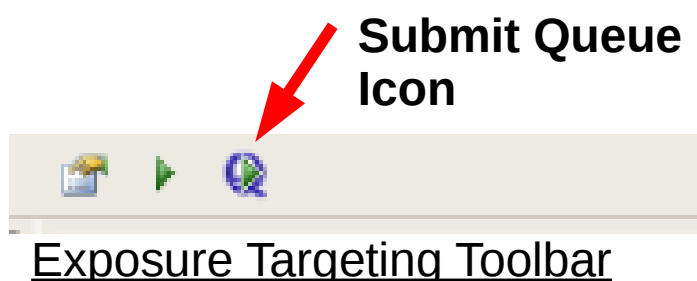
Exposure Targeting: Other useful Information

- Hole image viewing size can be changed by changing the ratio in the pulldown menu at the top of the image.

- If you selected a hole you do not want to use during hole Targeting, simply submit it in Exposure Targeting with NO targets selected.

- If you decide you do not want to use a set of selected exposures after submitting,it is TOO LATE. There is no way to remove these targets from the queue- but remember, you will be getting hundreds of images while using Leginon, so a couple bad ones is really no big deal.

- DO NOT attempt to use the abort or abort queue buttons on the toolbar of any node to remove targets at ANY level.

- Focus targets should be selected carbon, far enough away from any holes to ensure that they are not burned during the focus process.