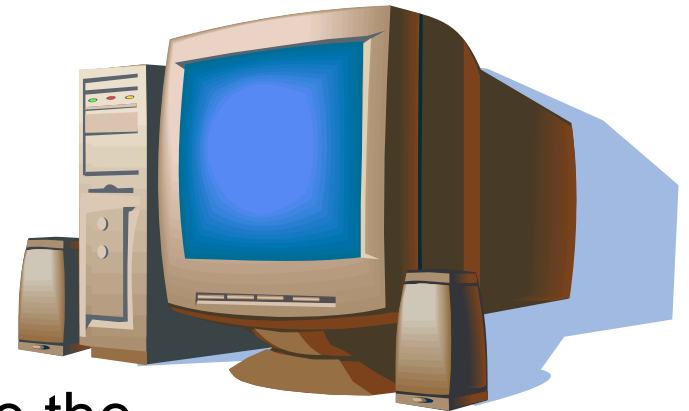
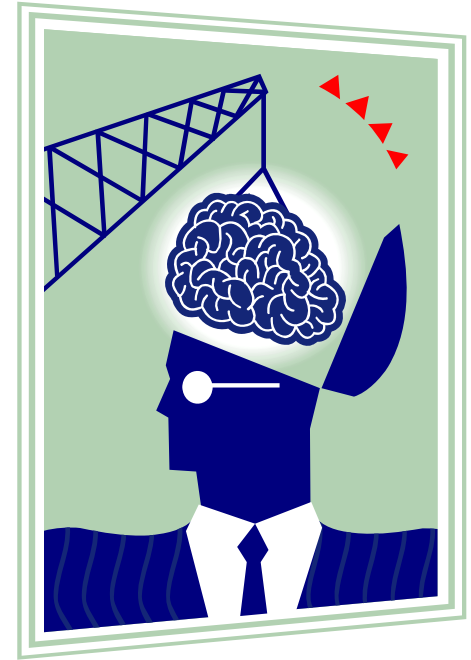
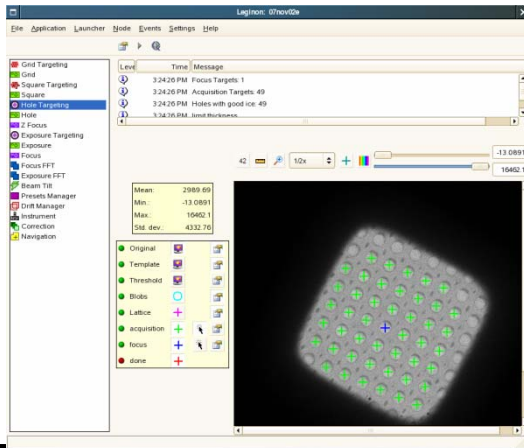


Challenges in automation

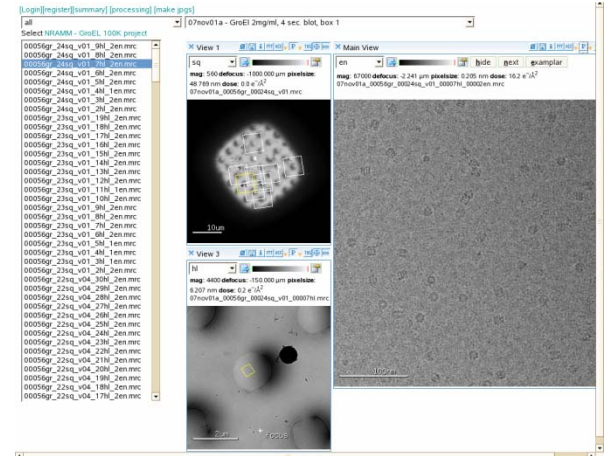
- Hysteresis of the lenses/deflector
- Extensive calibrations
- High alignment requirement for calibration stability
- Multiple users/diverse protocols
- Targets are not necessary at where the calibrations indicate



Leginon System Overview



Leginon Program



Web Tools

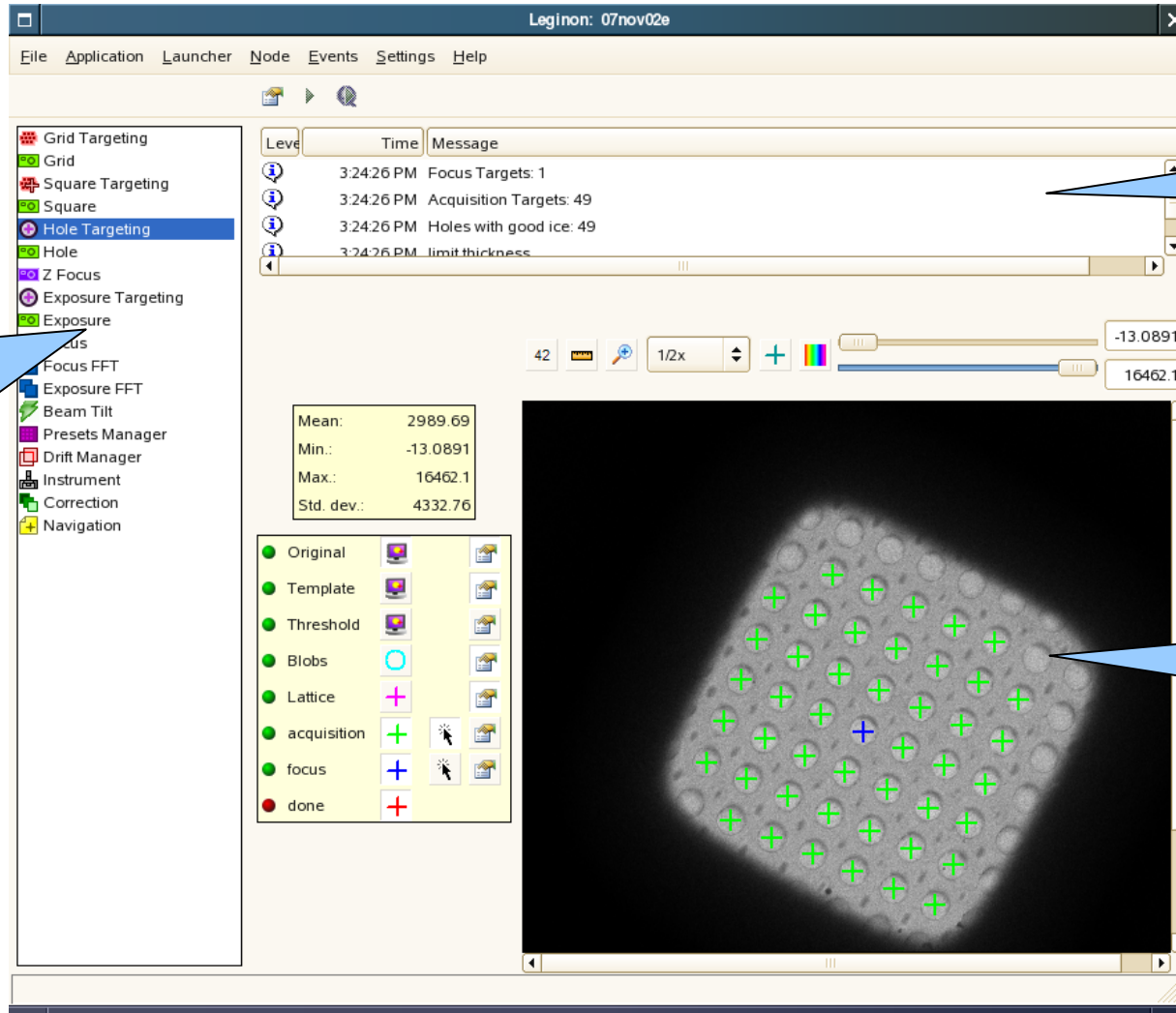
TEM, CCD



Database and
Image Storage



Leginon Program



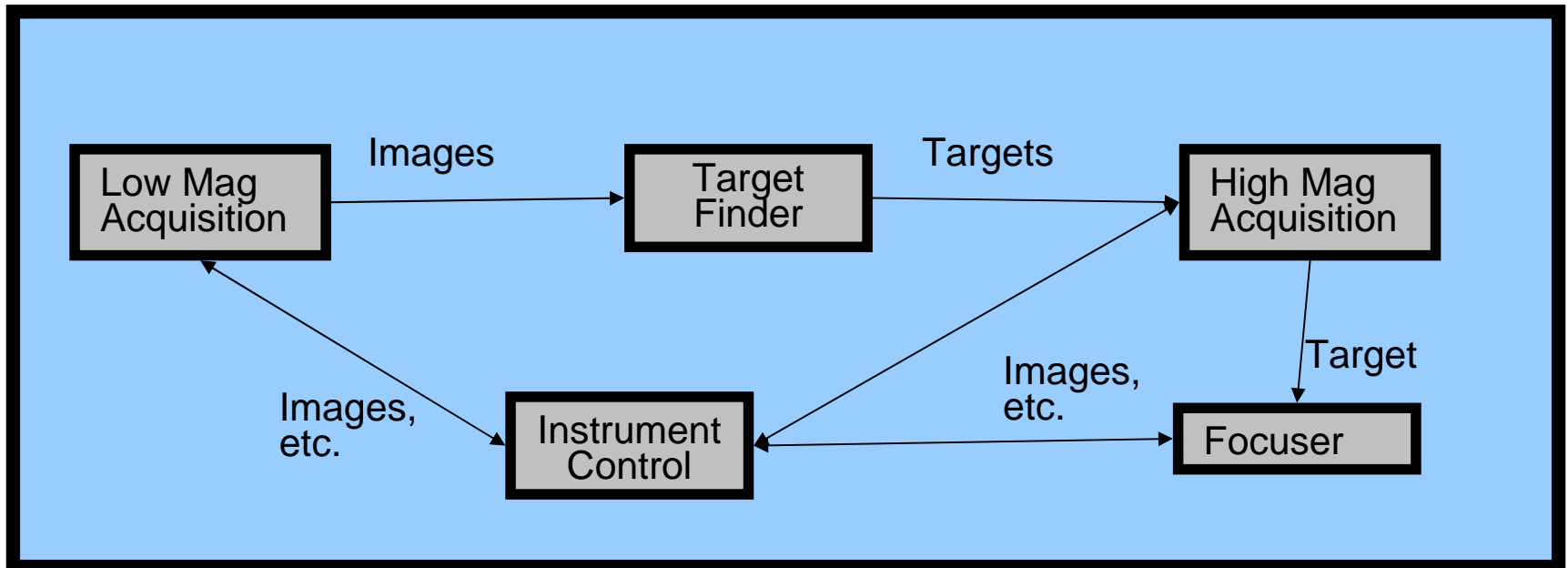
Application Nodes

Log

Main Panel

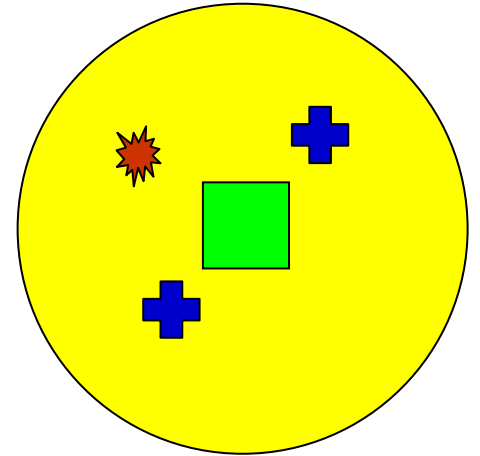
Leginon Applications

- Flexibility for different specimens, techniques
- **Nodes** define basic tasks
- **Applications** define overall behavior
- Example Application:



Using Low-Dose Kit

- Choose presets
 - search, focus, exposure
- Find the target in Search preset
- Move the target to the center
- Focus
 - by moving stage to eucentric
 - by changing defocus
- Take the image, write notes, save the file.



Different levels of automation

- Choose presets
 - search, focus, exposure

- Find the target in Search preset

- Move the target to the center

- Focus **Simple Application**

- by moving stage to eucentric
- by changing defocus

- Take the image, write notes, save the file.

Manual Application (61%) 7421 images

MSI Application, queuing (18%) 44085 final images

MSI Application, automated rastering or hole finding (13%)

Manual Application

The screenshot displays the Leginon Manual Application interface. At the top, the window title is "Leginon: 07oct30anchitest". The menu bar includes "File", "Application", "Launcher", "Node", "Events", "Settings", and "Help". Below the menu bar is a toolbar with icons for file operations and a "MF" button. On the left, a sidebar shows "Instrument" with "Manual" selected. The main area is a log window with columns "L.", "Time", and "Message". The log entries are:

L.	Time	Message
?	2:02:56 PM	Image acquired.
?	2:02:56 PM	Image acquisition complete

Below the log window is an "image viewer - SeaMonkey" window. The address bar shows the URL "http://cronus3.scripps.edu/appionweb/imageviewer.php". The browser interface includes a search bar with "all" selected and a dropdown menu for "07oct30anchitest - test". Below the search bar is a list of files under "Select NRAMM - Test project":

- 00008ma_1.mrc
- 00007ma_1.mrc
- 00006ma_1.mrc
- 00005ma_1.mrc
- 00008ma_1.mrc
- 00007ma_1.mrc
- 00006ma_1.mrc
- 00005ma_1.mrc
- 00004ma_1.mrc
- 00003ma_1.mrc
- 00002ma_1.mrc
- 00001ma_2.mrc
- 00001ma_1.mrc
- 00002ma_2.mrc
- 00002ma_1.mrc
- 00001ma_2.mrc
- 00001ma_1.mrc

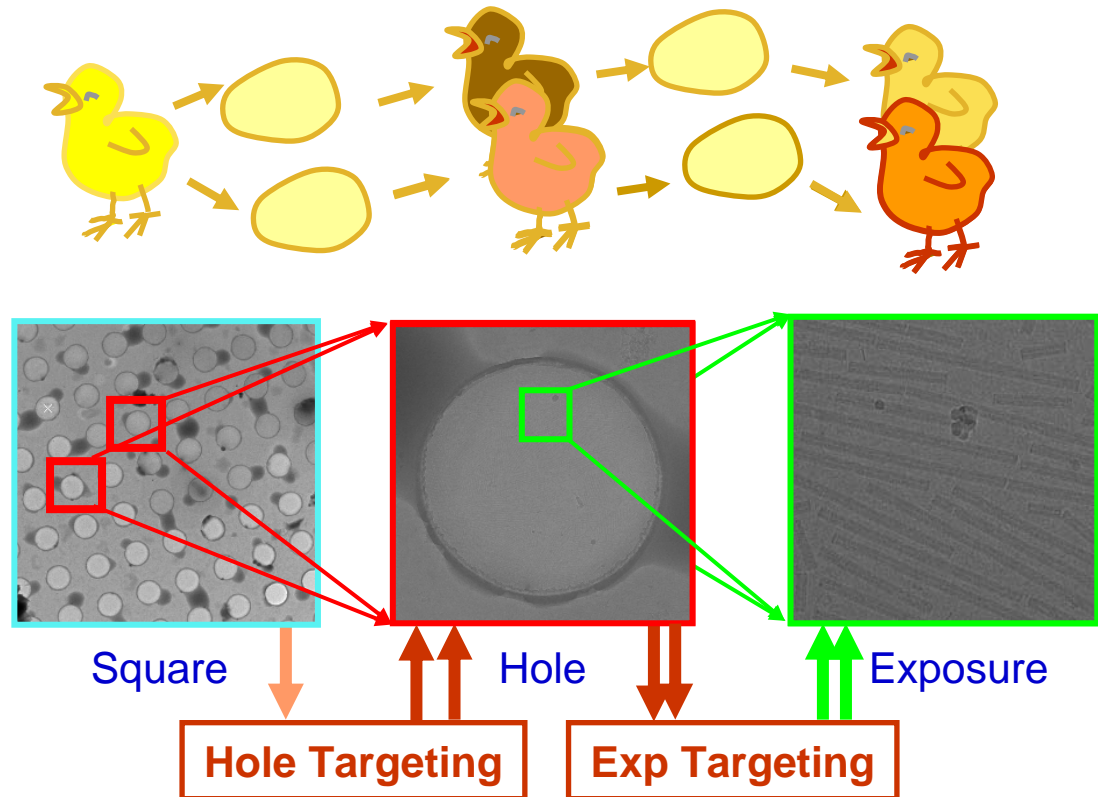
The "Main View" window shows the selected file "07oct30anchitest_00007ma_1.mrc" and a text input field containing "test image". Below the text field is a grayscale image of a biological specimen, likely a micrograph, showing a grid of circular features.

- Operate in low-dose kit
- Live-fft manual focusing
- Commenting
- Save automatically and display in Leginon Web Viewers

MSI Application

Depth-First Targeting/Imaging Path

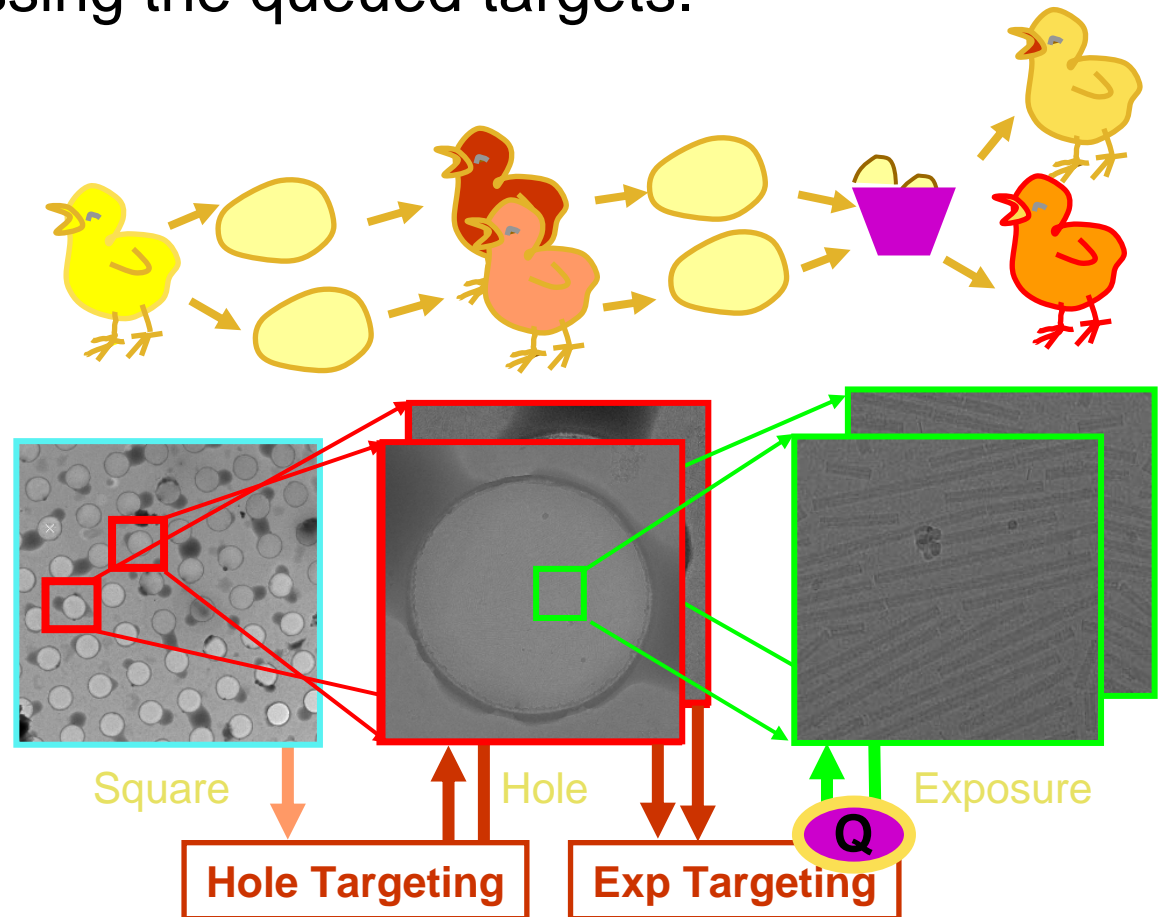
- Finish final exposure before processing next hole target on the square



Queuing

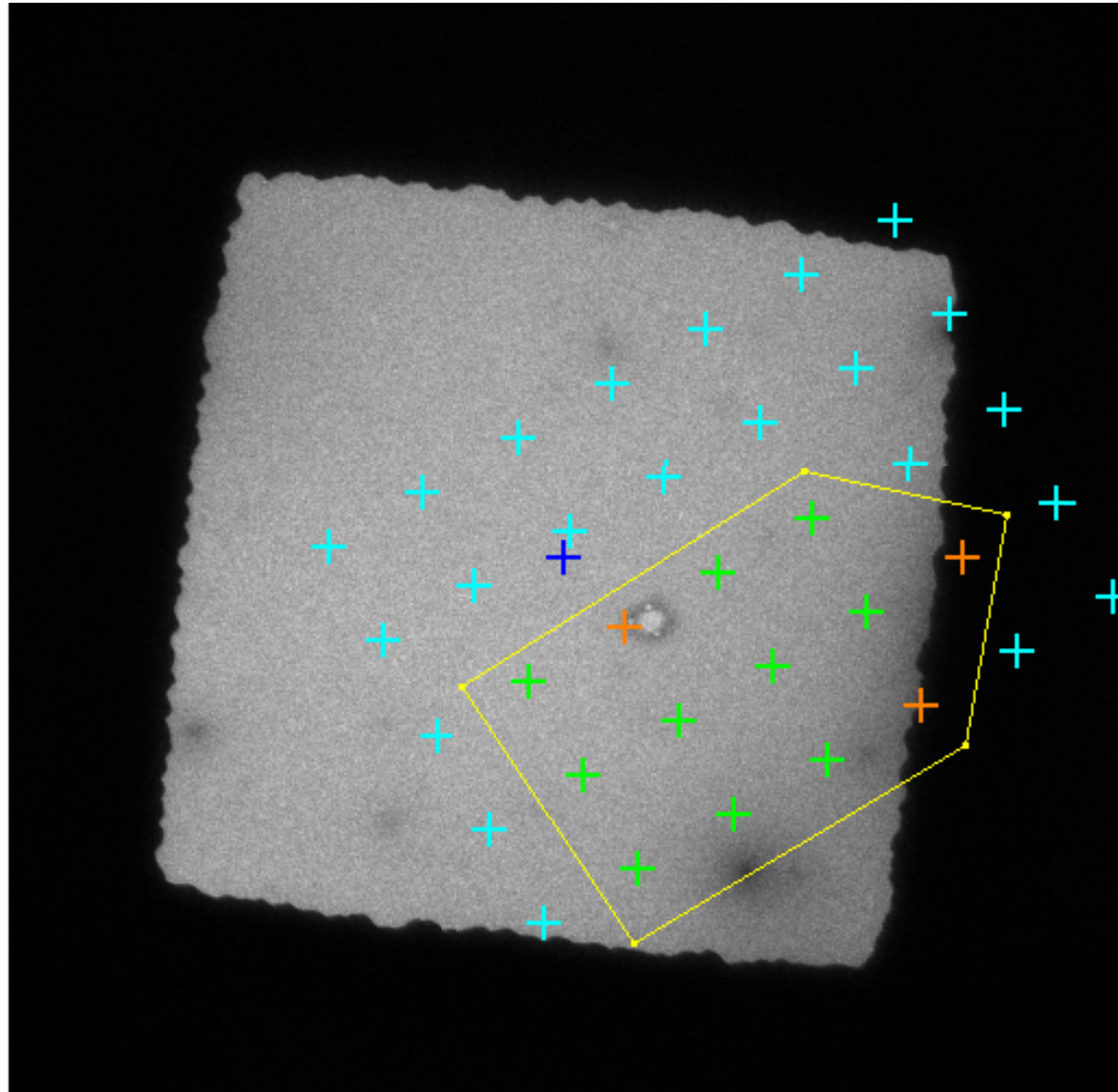
- Collect data on all targets at low magnification first and **manually** select targets on them.
- Put the targets in a queue until the user tells Legion to start processing the queued targets.

Pick 100's of targets and then walk away for hours



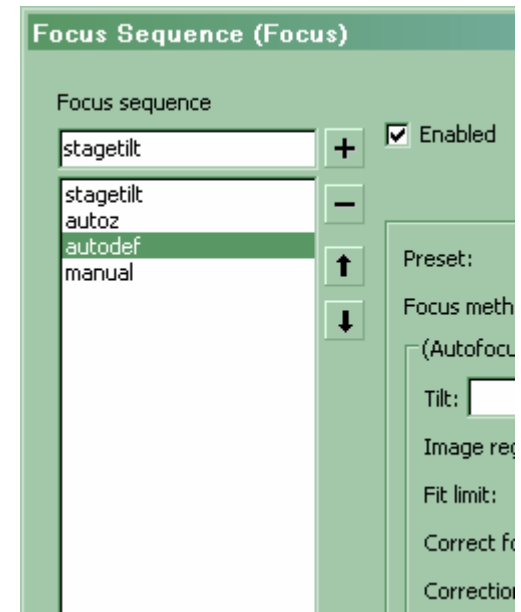
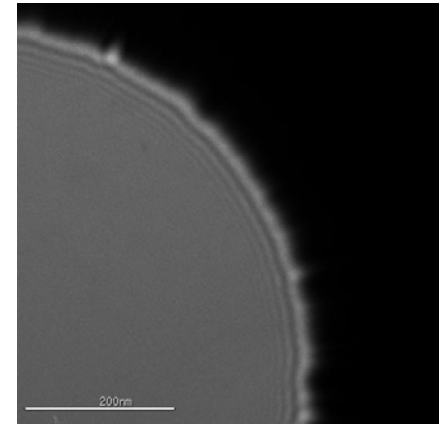
Automated Target Finding

- Template-based Hole Finding
- Regular Raster

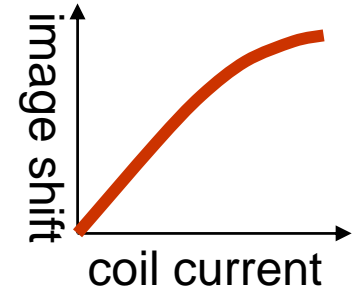
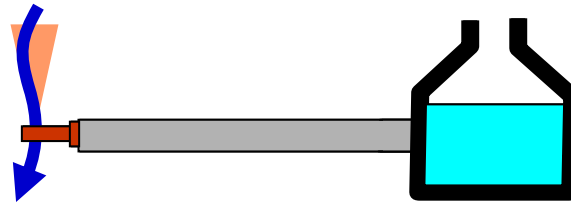
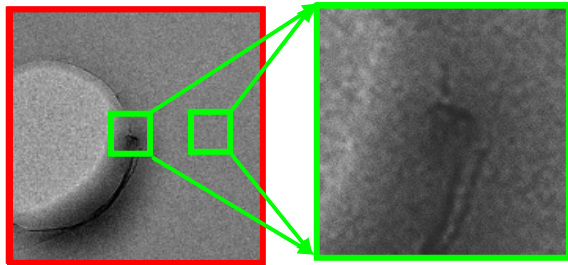


Solutions for automation

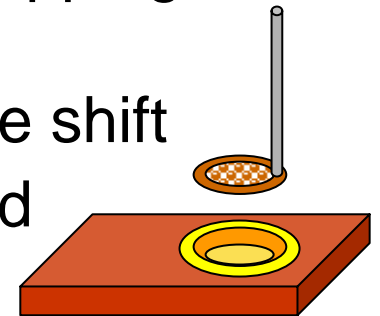
- Hysteresis of the lenses/deflector
 - Normalization/Preset Cycling
- Extensive calibrations
- High alignment requirement for calibration stability
 - Keep close to eucentric point
 - Image/Beam calibration
- Multiple users/diverse protocols
 - Multiple and complex applications and settings
- Targets are not necessary at where the calibrations indicate
 - Drift Management



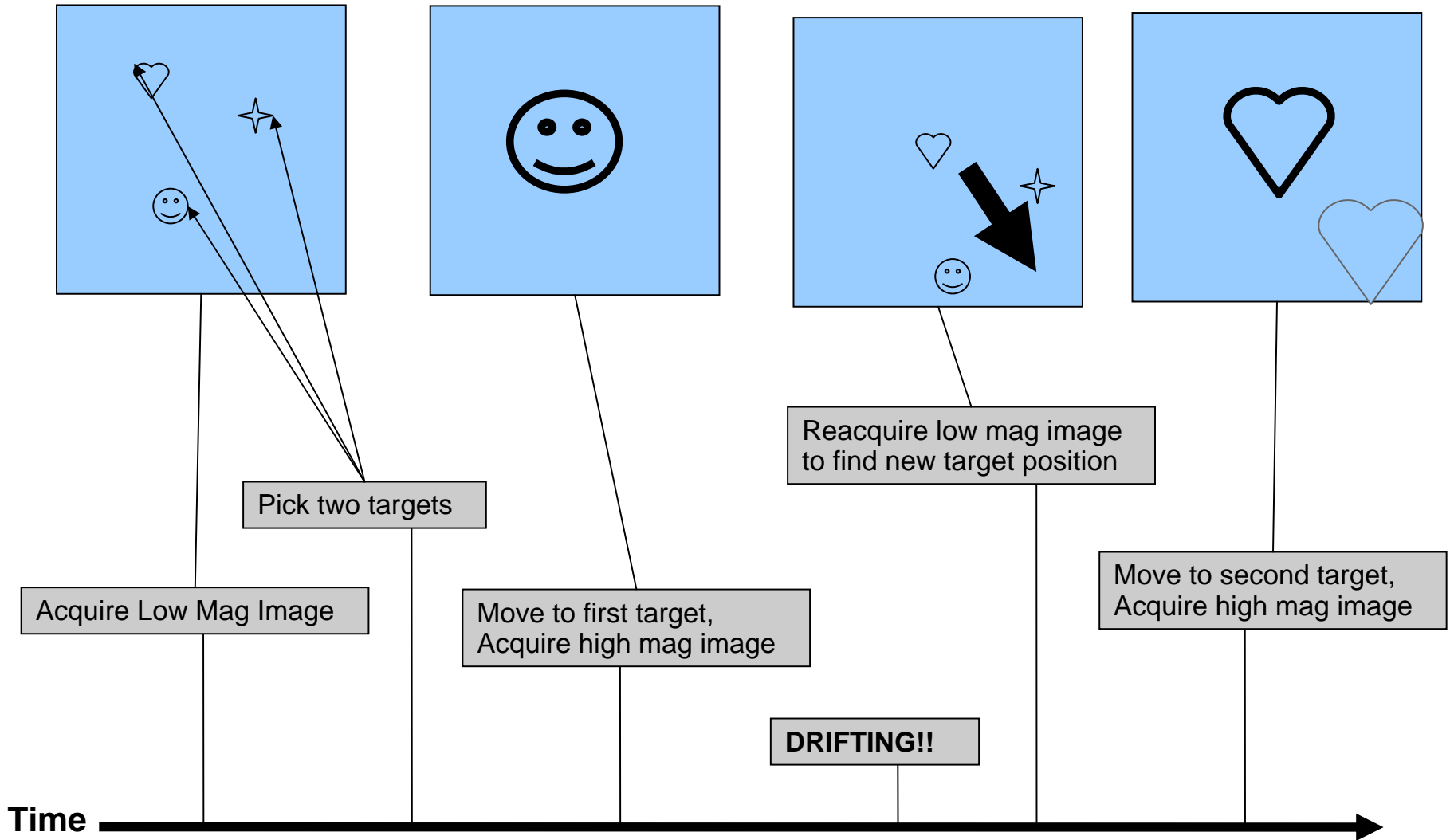
Key to routine automation- Trusting computer to image at the target



- Imaging location can be off target
 1. Gradual thermal drift
 2. Large cryo-stage thermal expansion before/after refill
 3. Changed z-axis location or stage tilt angle
 4. Inaccurate image shift/stage coordinate mapping on to the image
 5. Hysteresis in goniometer movement /image shift
 6. Position offset when robot reinserts the grid
 7. Bad relative image shift alignment between presets



Drift Correction Sequence



Day in the Life of a Legimon User

- Calibrations and Configuration
- Fire it up
- Babysit and tweak parameters
- Trust the automation
- Sit back, relax, watch DVDs
- Don't forget to top off your liquid nitrogen

Getting More Involved in Leginon

- *Everyday User*
 - Download and install it (ask us for help)
 - Leginon Training Course
- Application Designer
 - you have a new way to use Leginon
 - little or no programming experience necessary
- Programmer
 - Develop new nodes
 - contribute new features and bug fixes

For More Information...

www.leginon.org

- Downloads
- Bulletin board
- Installation/User Manual
- Publications