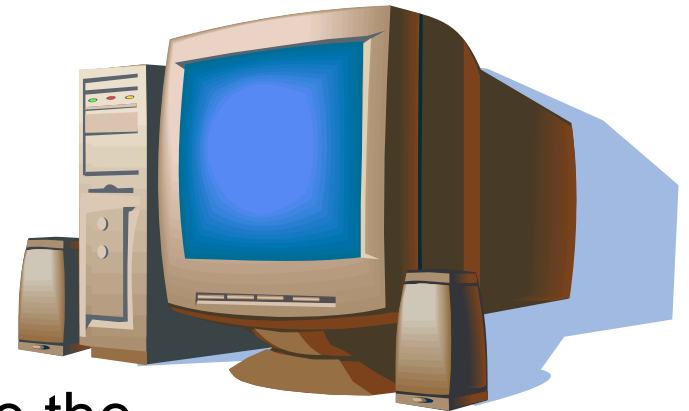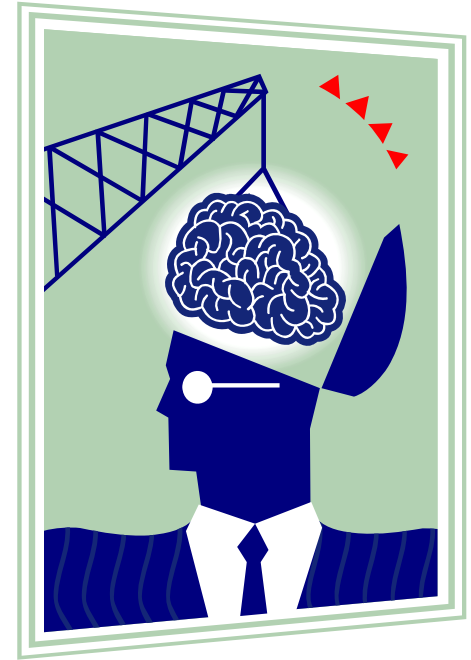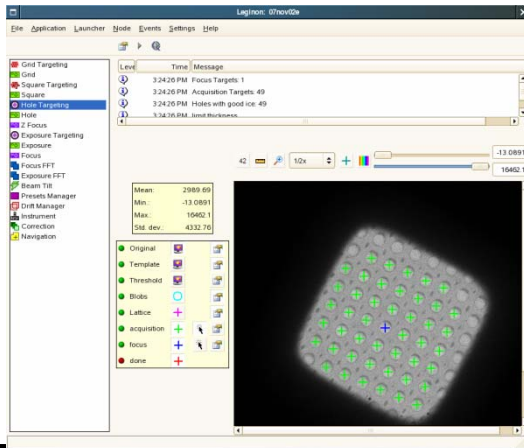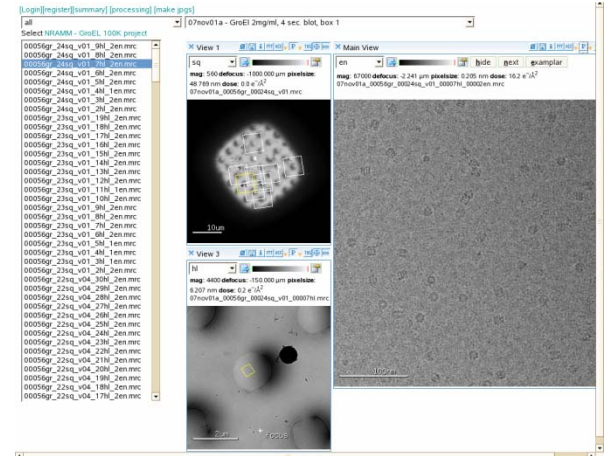# Challenges in automation

- 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
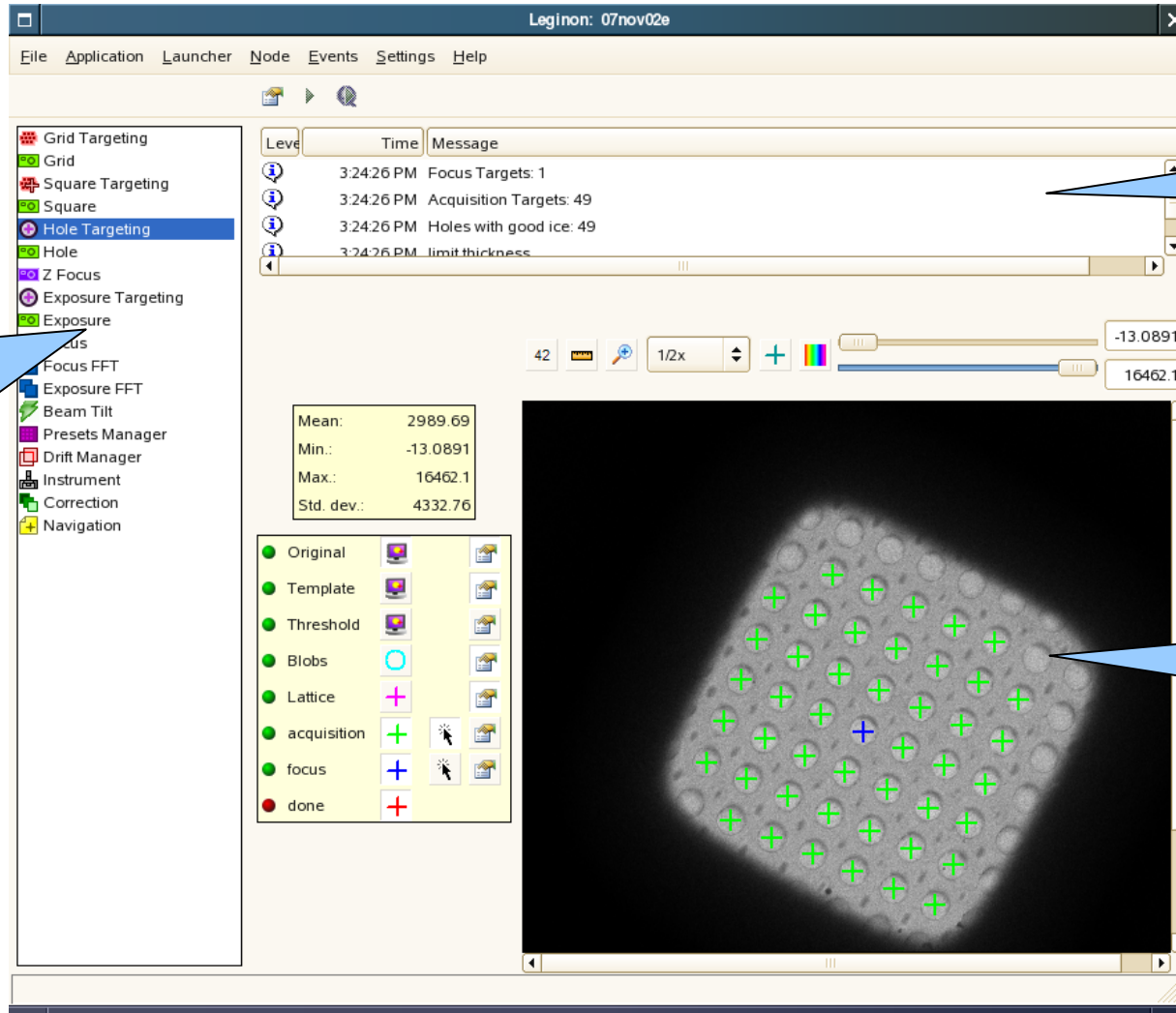
Leginon System Overview

Leginon Program

Web Tools

TEM, CCD

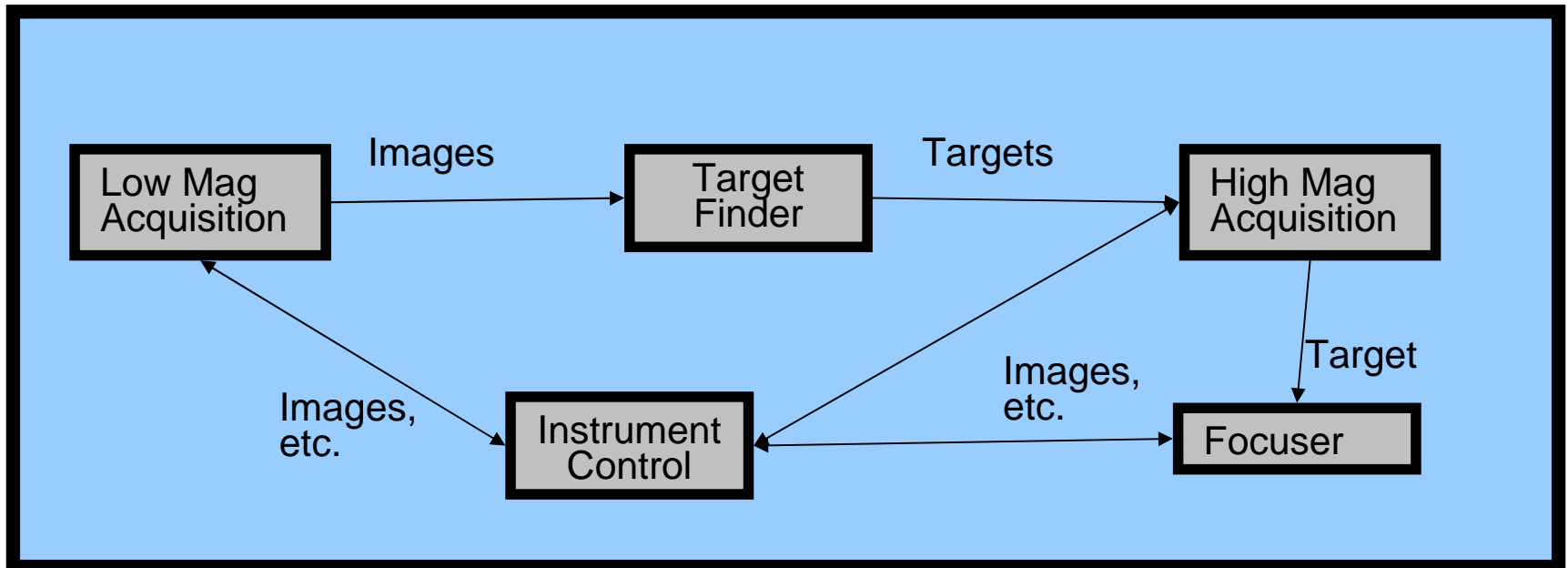Database and Image Storage

Leginon Program

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.
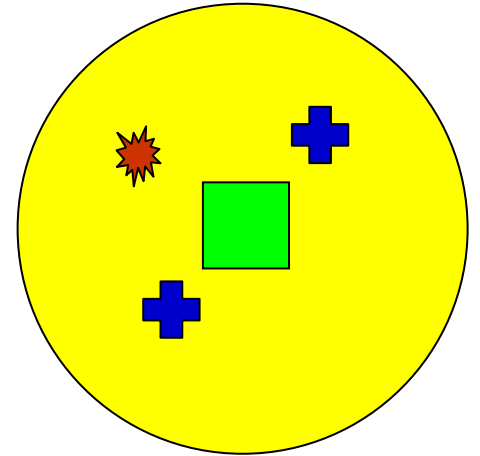
Different levels of automation

- ## 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

- 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

Square   Hole   Exposure

Hole Targeting   **Exp Targeting**
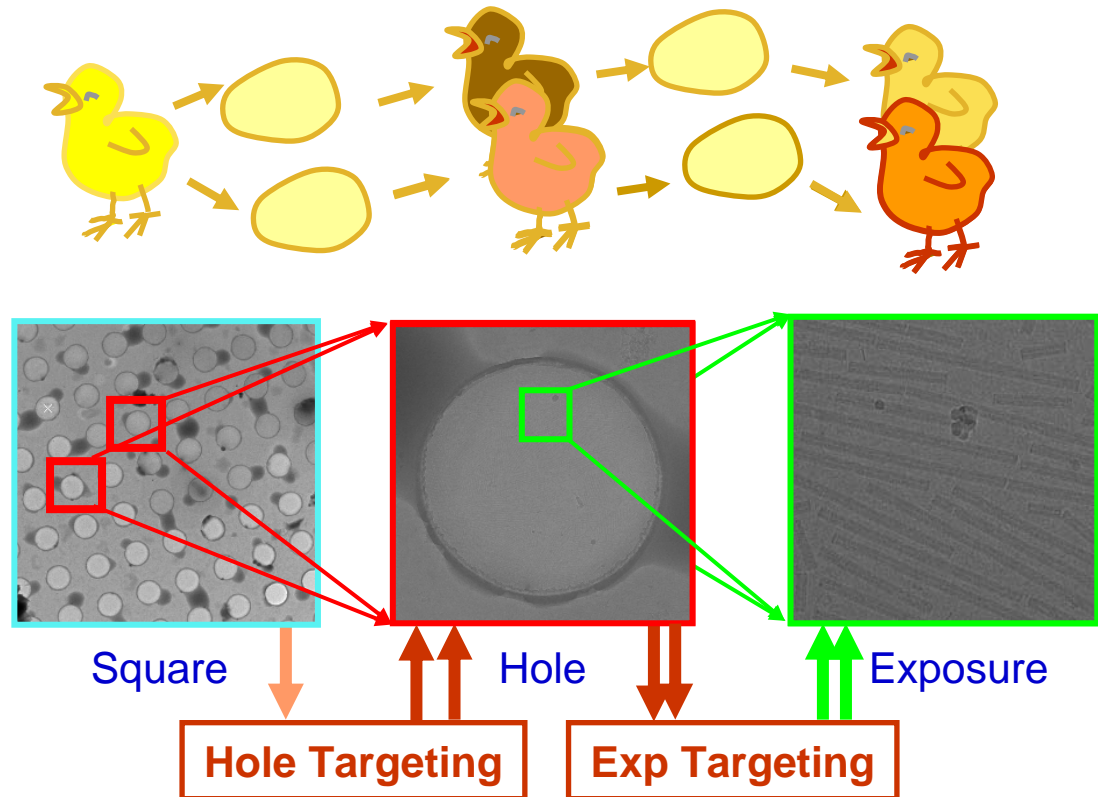
Queuing

- 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 Leginon to start processing the queued targets.

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

Square

Hole

Exposure

Hole Targeting

Exp Targeting

Q
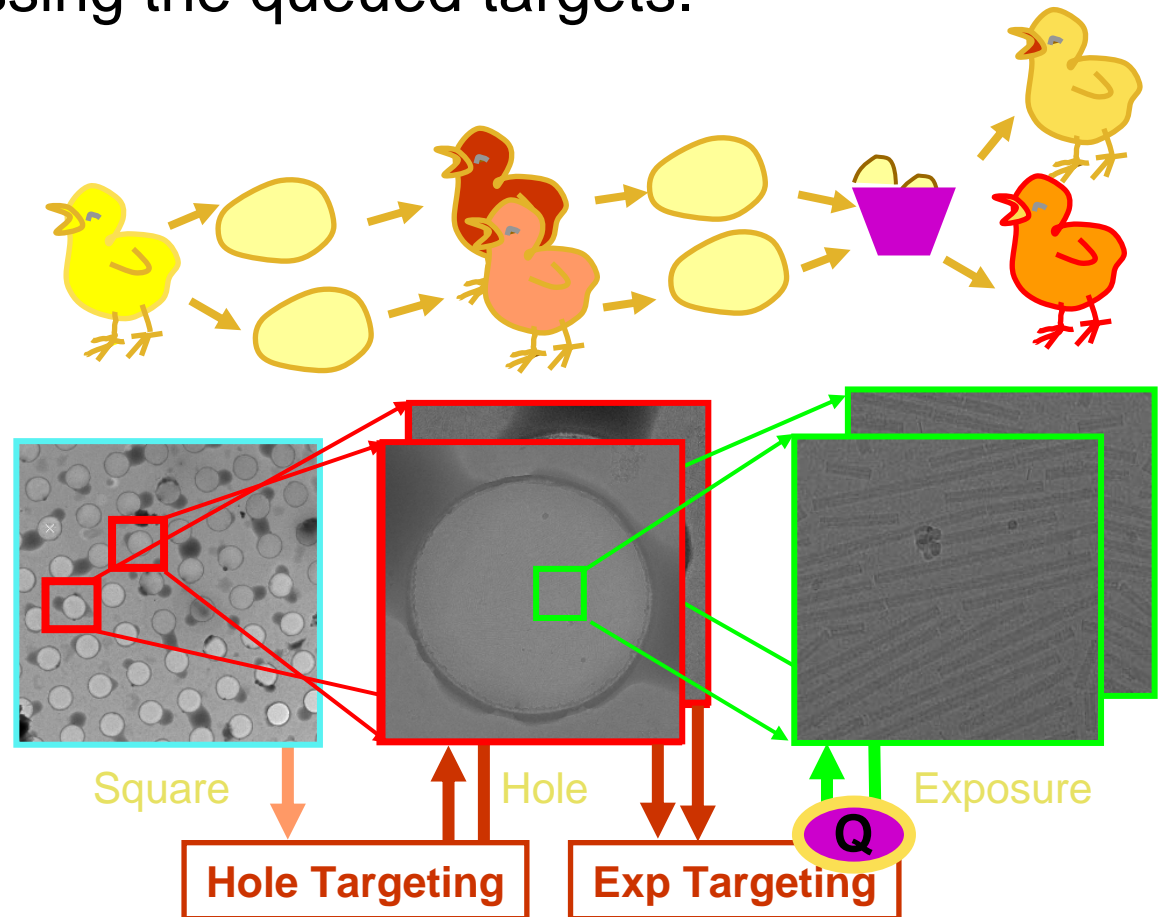
Automated Target Finding

- 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
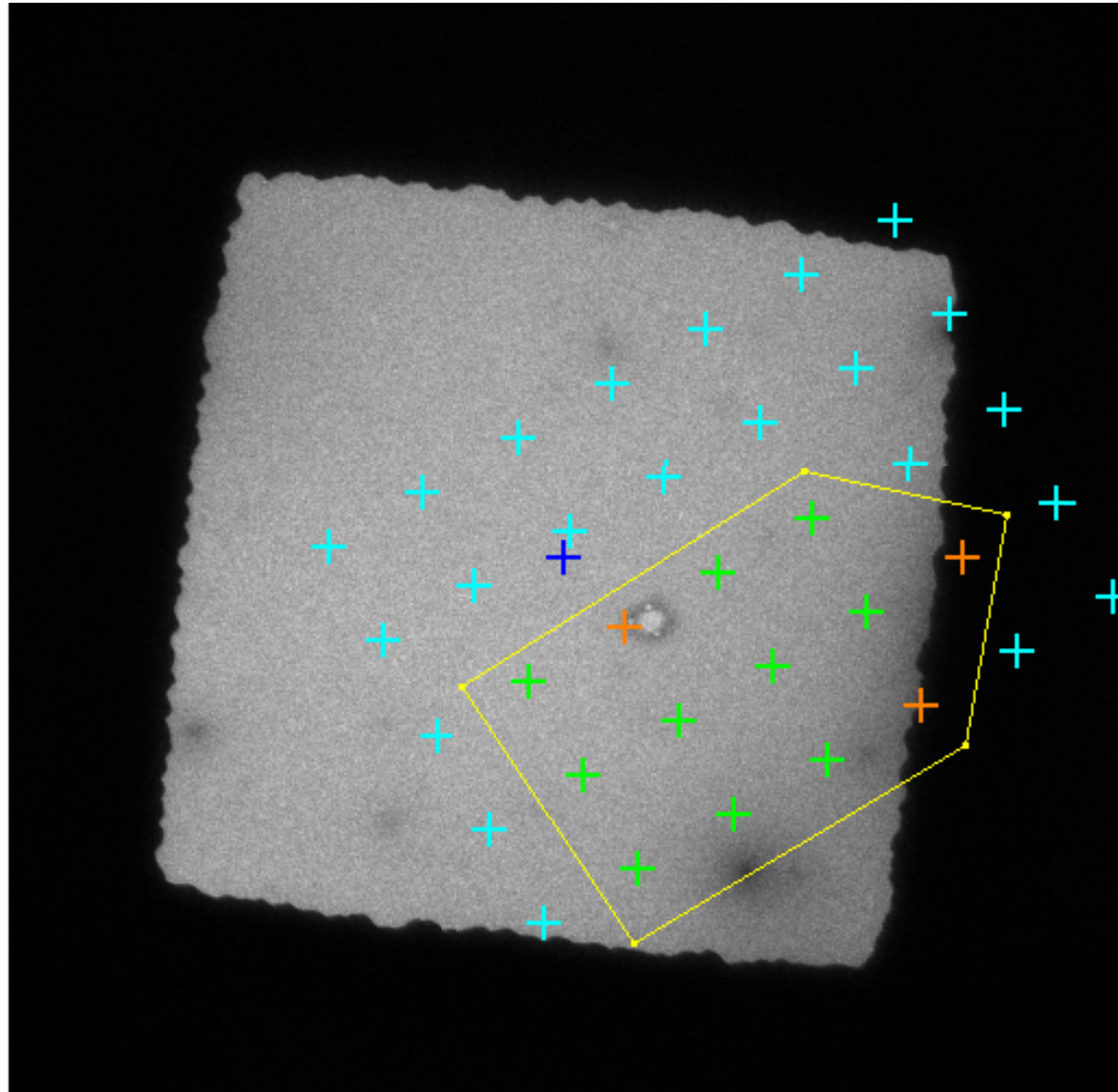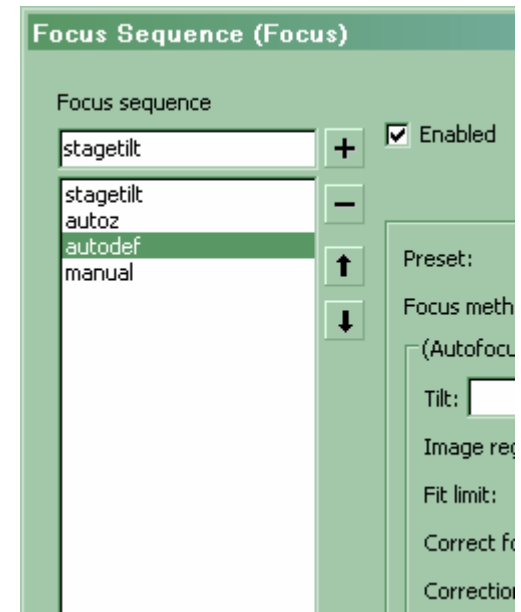 - Keep close to eucentric point
  - Image/Beam calibration

- Multiple users/diverse protocols
  - Multiple and complex applications and settings

- Targets are not necessary at where the calibrations indicate
  - Drift Management

Focus Sequence (Focus)

Focus sequence

stagetilt | + ☑ Enabled

stagetilt
autoz
autodef
manual

Preset:

Focus meth

(Autofocu

Tilt:

Image reg

Fit limit:

Correct f

Correction
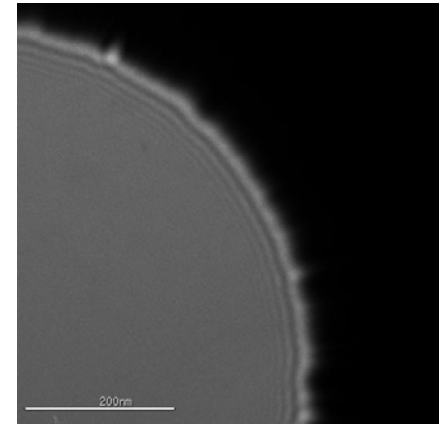
Key to routine automation-
Trusting computer to image at the target
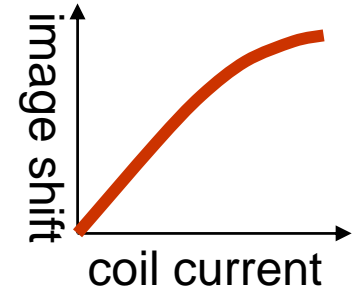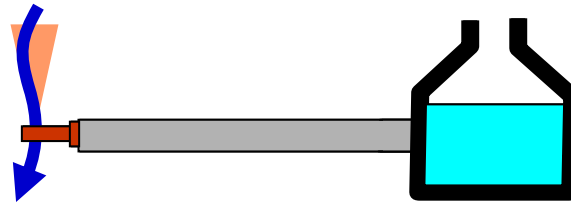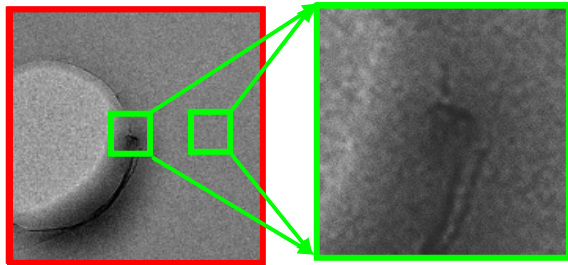
- Imaging location can be off target
  1. Gradual thermal drift
  2. Large cryo-stage thermal expansion before/after refill
  3. Changed z-axis location or stage tilt angle
  4. Inaccurate image shift/stage coordinate mapping on to the image
  5. Hysteresis in goniometer movement /image shift
  6. Position offset when robot reinserts the grid

  7. Bad relative image shift alignment between presets
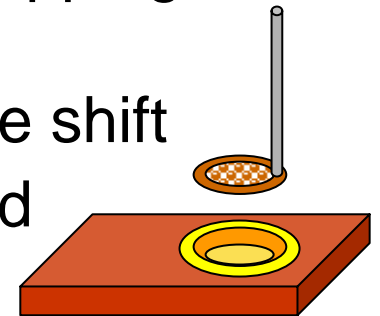
Drift Correction Sequence

Pick two targets

Acquire Low Mag Image

Move to first target,
Acquire high mag image

Reacquire low mag image
to find new target position

DRIFTING!!

Move to second target,
Acquire high mag image

Time
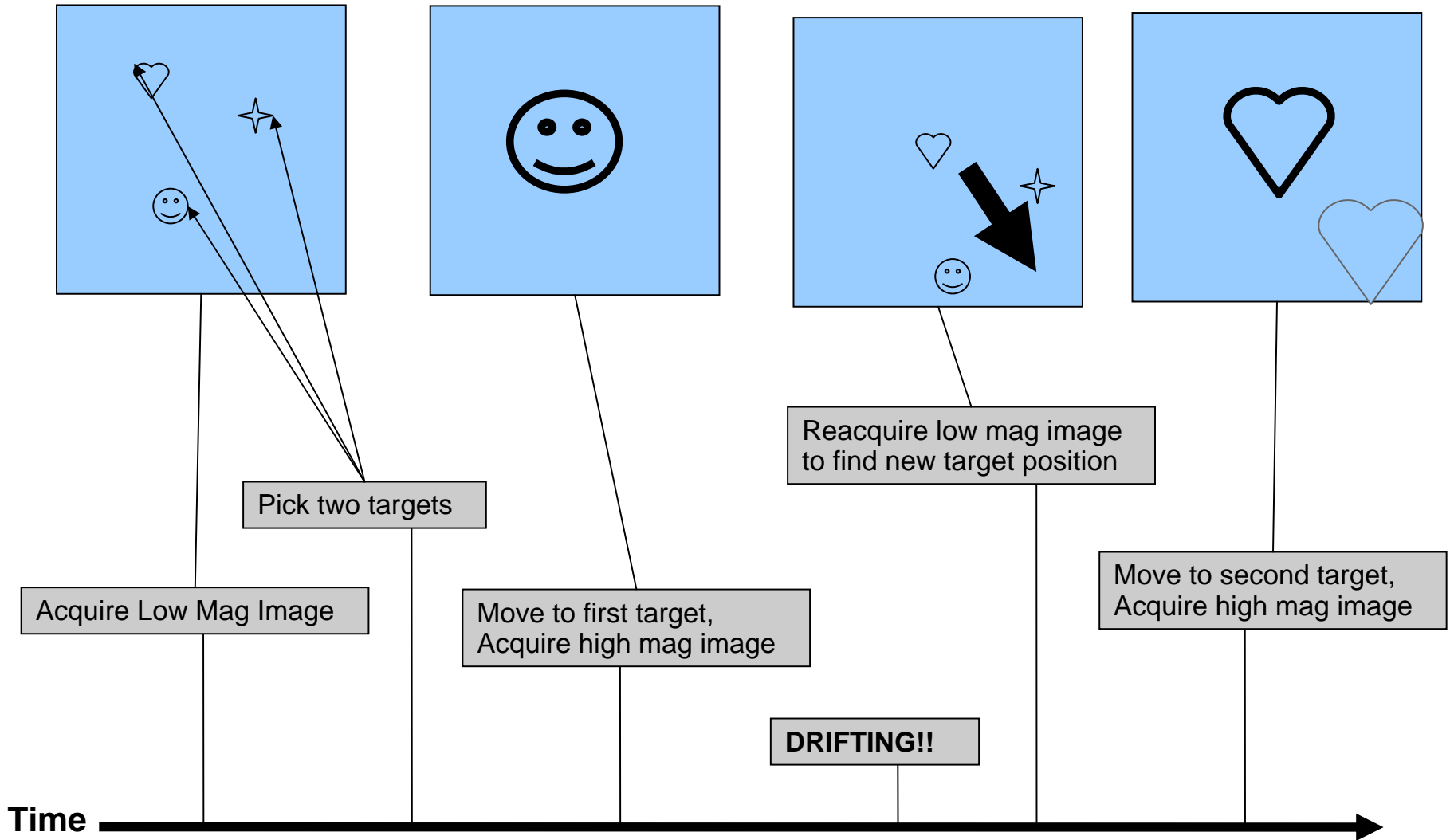
Day in the Life of a Leginon User

- 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