Wishes and Realities for Specimen Handling

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Our facilities

- The Biological Sciences Imaging Resource (BSIR)
  - University core facility
  - Houses TEMs, SEM, and light microscopes
- The Southeastern Consortium for Microscopy of Macromolecular Machines ($SECM^4$)
  - Supported by U24
  - Uses half of our Titan time

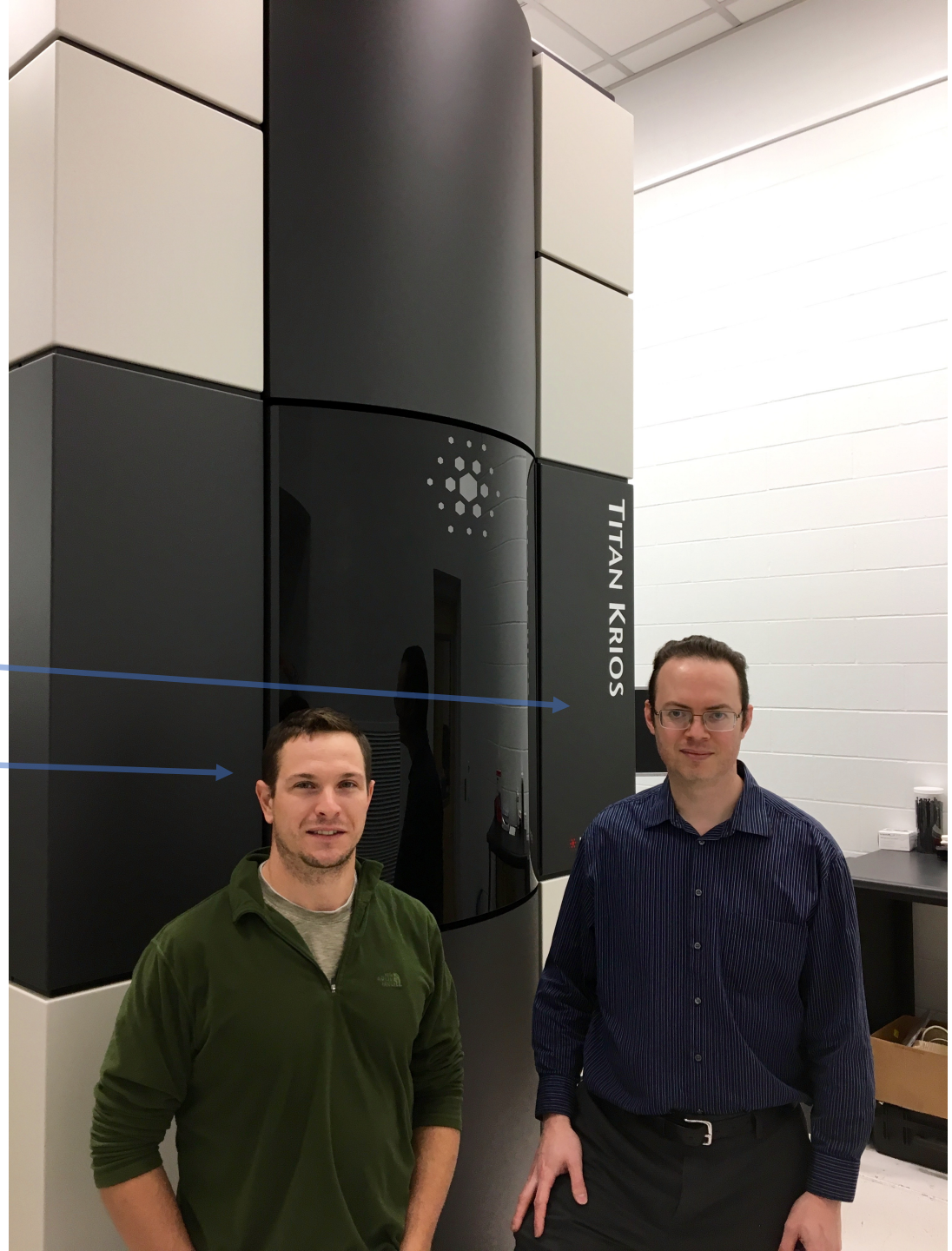
Specimen handling in a service facility

- Every grid manipulation increases the chances of degradation (warming, contamination, transfer ice, bending grid, etc)
- Considerations
  - Shipping specimen
  - Clipping grids for the Titan
  - Storing clipped grids
  - Mounting clipped grids in the cassette
  - Shipping unused grids back to user

The word from the trenches

Duncan Sousa – BSIR manager

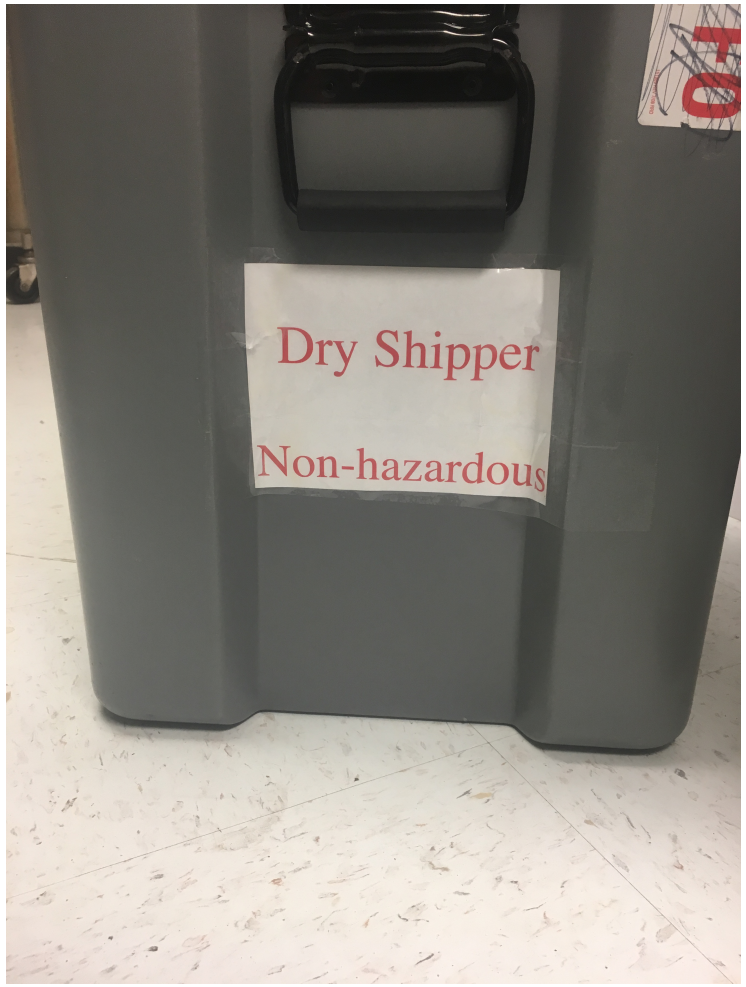
John Spear – SECM4 EM specialist

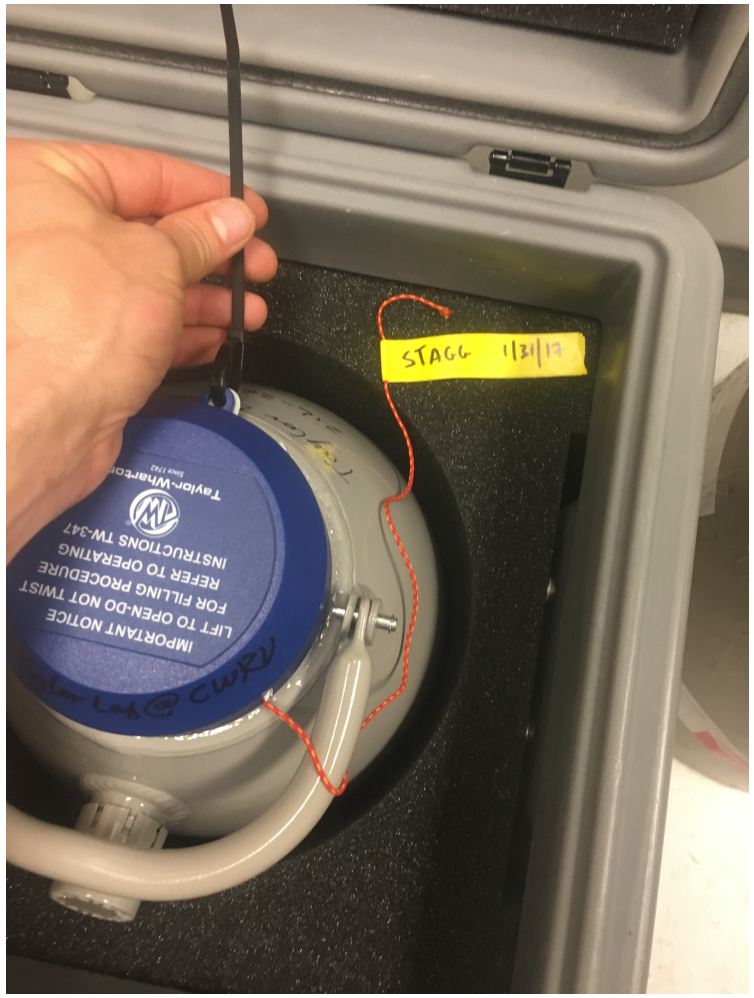


Receiving shipped grids

Stuff John likes

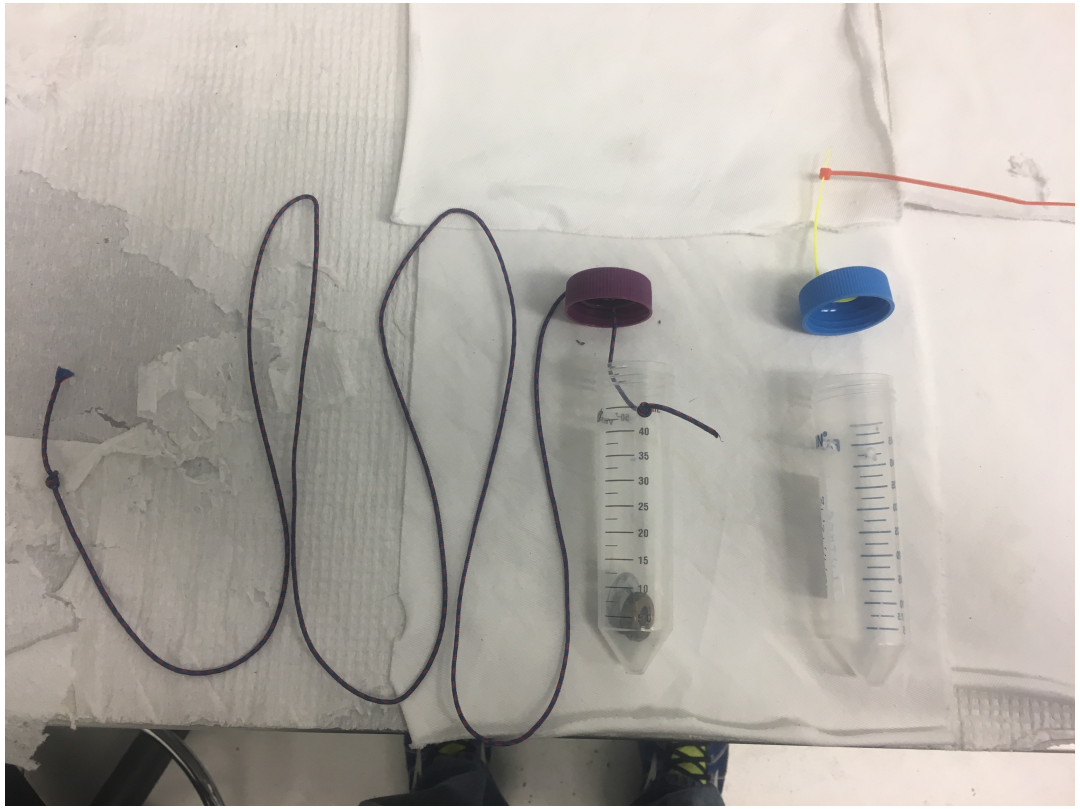
- Communication
  - All stages, shipping, receiving, screening, etc
- Abundant labels
  - With a key to the label meanings
- Secure closures
- Long retrieval cords
  - Paracord – slick, strong
- Round grid boxes with notches

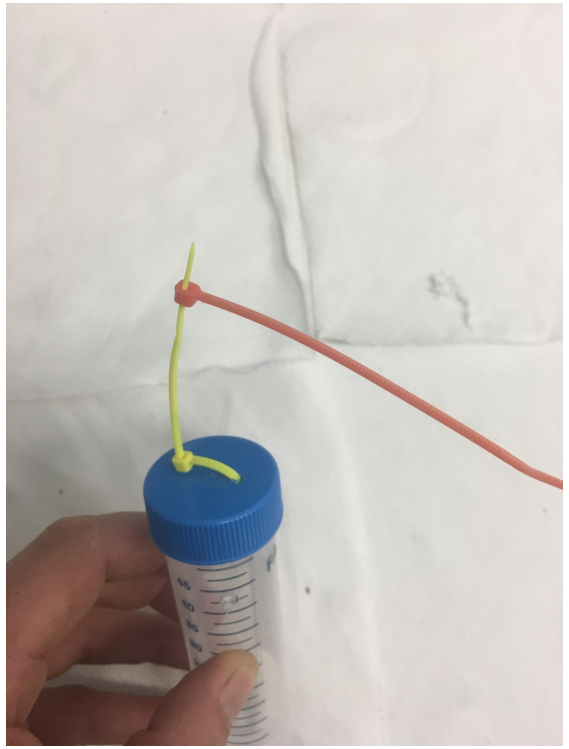




Unintended consequences

- Importance of good string
  - It should be easy to get the transfer conical out of the dry shipper

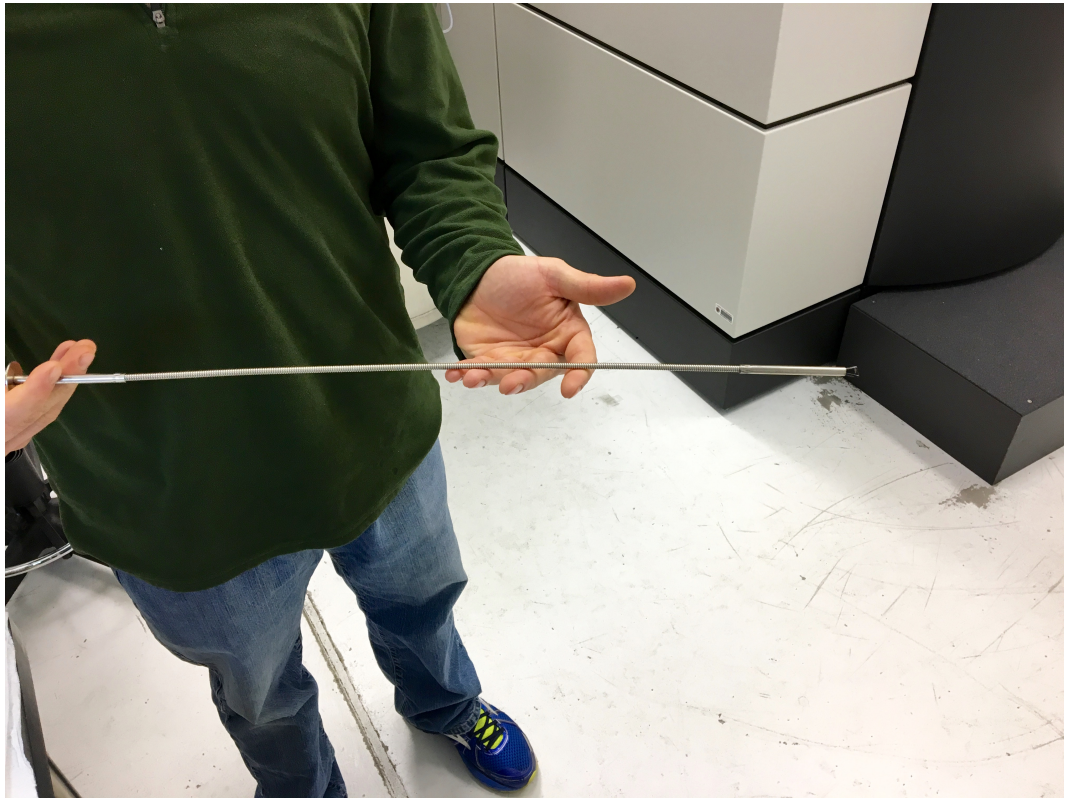




This caused lots of problems

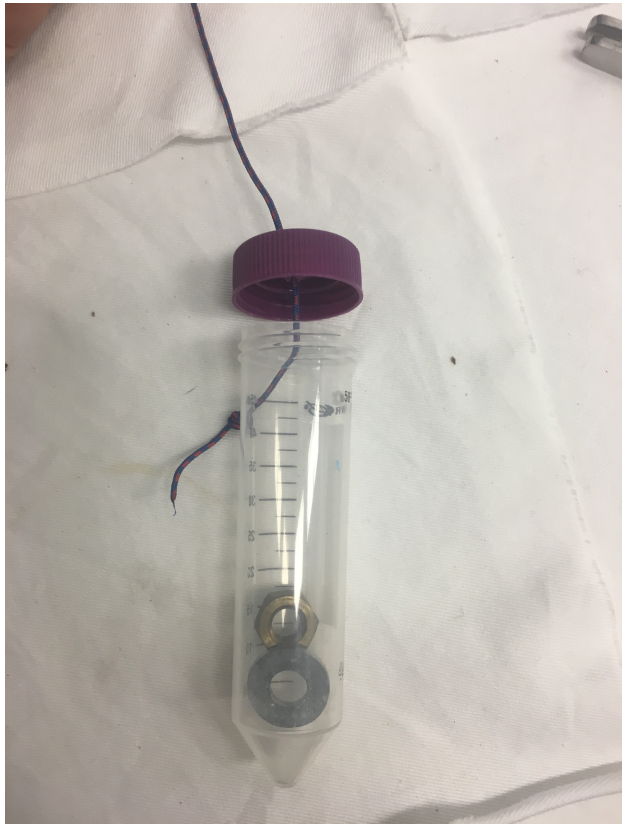
Unintended consequences

- No transfer conical
- Grid boxes fall out



Importance of transfer tubes

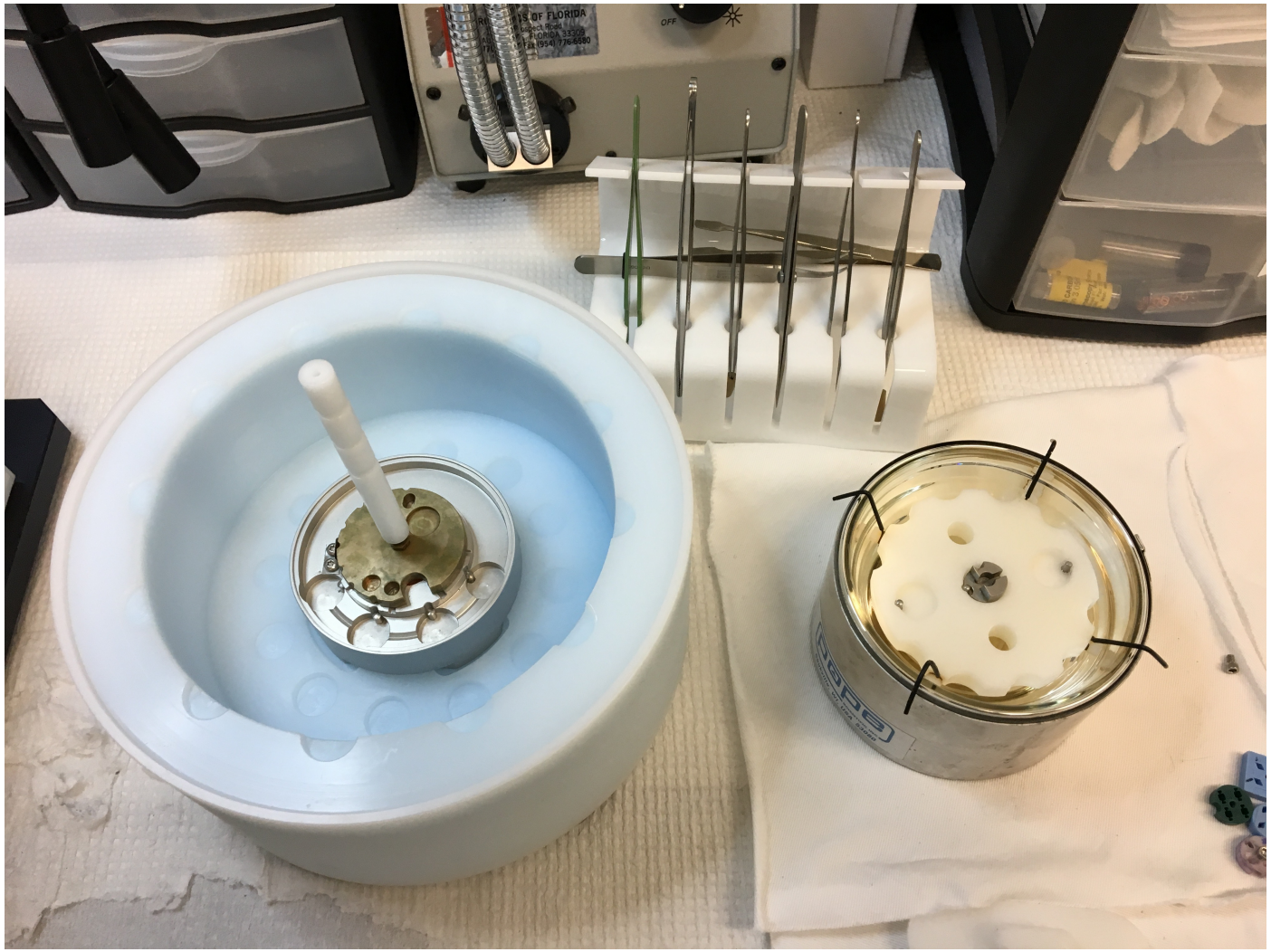
- Problems
  - Cap comes off of tube – grid boxes fall out and warm up
  - Tube floats out of nitrogen when filling up dry shipper
  - Removal string not long enough or breaks
- Solution
  - Put string through both cap and tube
  - Add weights to tube
  - Use 3’-4’ para cord for string



Shipping - best practices

- Shipper and receiver should communicate about when dry shipper is picked up and received
  - Including tracking numbers
  - Should have secondary contacts on both sides who are also in the loop
  - John often CC’s me on emails about specimens just as a back up
- Ship on Monday through Wednesday.
  - Minimize the chances of samples sitting in a humid FedEx warehouse over the weekend

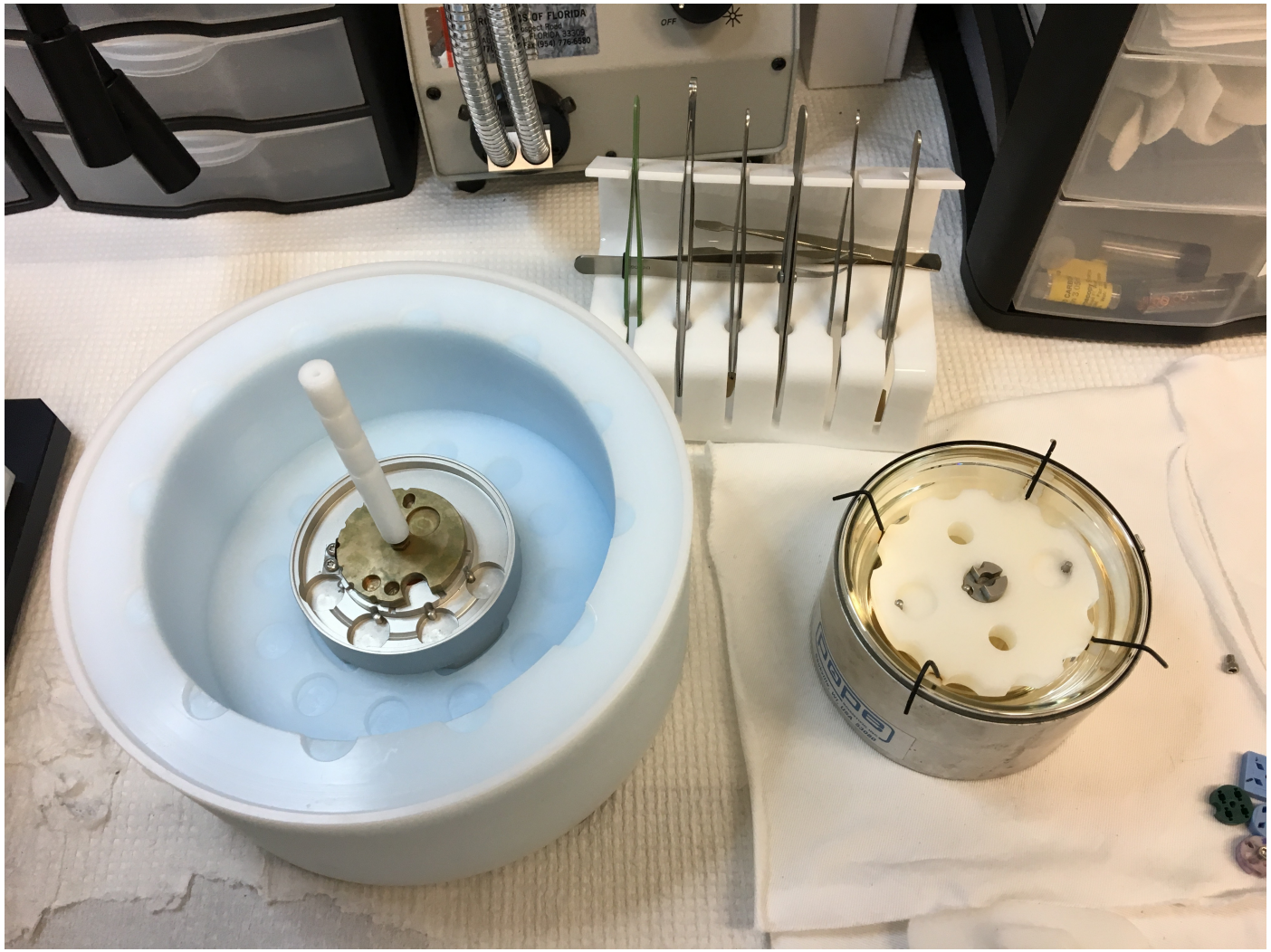
Clipping grids for the Titan



To clip or not to clip

- Our current practice is to have users send grids, and we clip them ourselves
  - Users don’t have clipping tools
  - That equipment is expensive and can be hard to budget for.
  - This will likely change as it becomes more common practice to ship grids off
- Downsides of clipping ourselves
  - If a grid gets ruined during clipping, the onus is on us
  - It would be better if users shipped preclipped grids
    - This would still require manual inspection before mounting in cassettes

Clipping grids for the Titan



Square grid boxes are problematic

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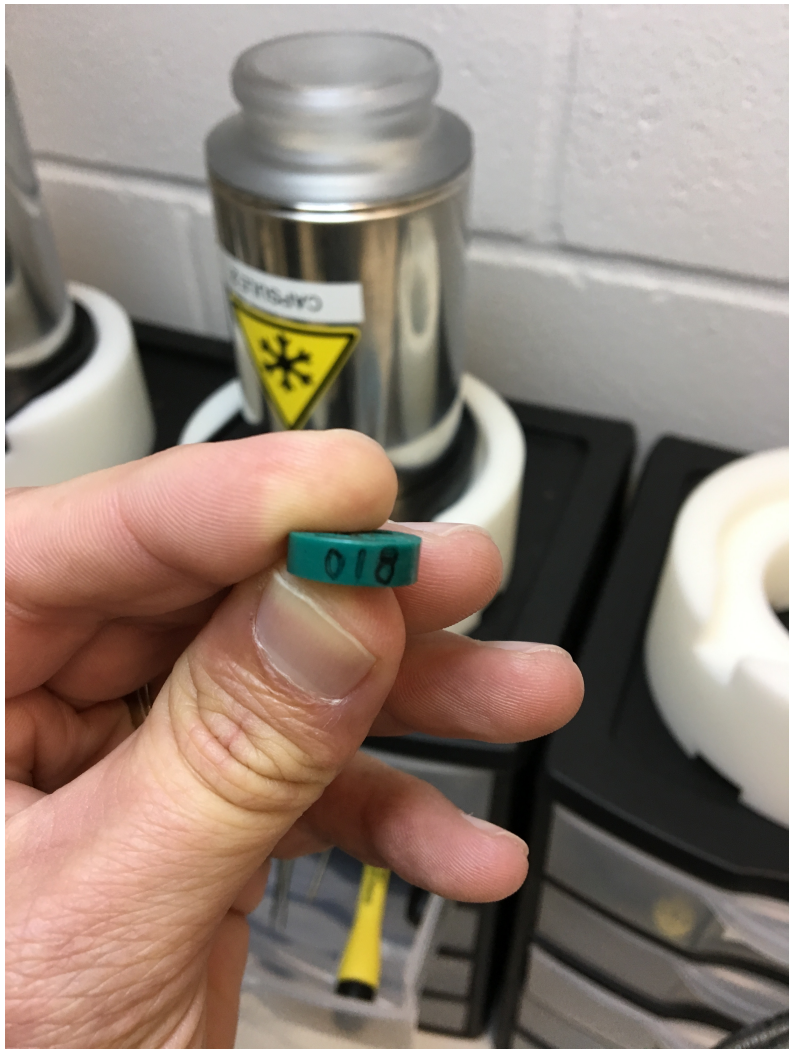
Clipping grids

Transfer from heterogeneous boxes into homogeneous boxes for storage

Storing clipped grids

Attempts to systematize specimen storage

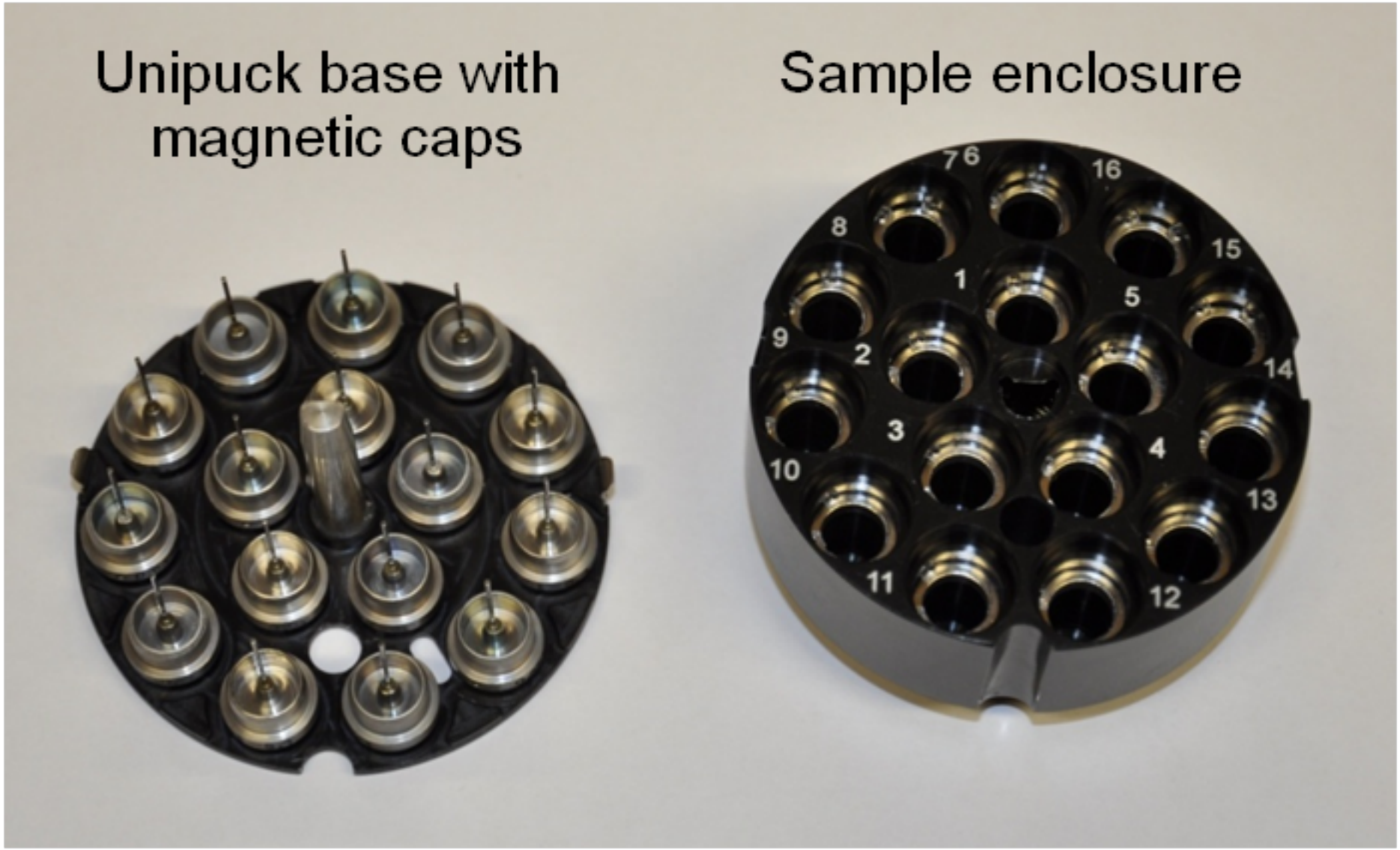
- Index all grid boxes and slots
  - Create spreadsheet record of samples and slots
- Abandoned because of the human factor
  - As soon as one person fails to follow the system or update the spreadsheet, the whole system falls apart.



Can technology help?

- Technology can either help or compound the human factor
  - Technology only helps when it makes things a lot more convenient
    - It has to be so good that EVERYBODY will use it
    - For specimen handling everybody has to be onboard or the system falls apart
  - A phone app with RFIDs?
    - Would require passive RFID tags
    - Preliminary reading suggests that they would work (Leung, *J Path Inform*, 2010)
      - It is unclear whether they would work through a Falcon tube under liquid N_2
      - Could be expensive

The synchrotron model



John’s words to live by for specimen handling

- “It is very difficult to prepare good cryo-EM samples, but very easy to destroy them”
- Treat every step of the specimen handling process as carefully as you do the steps of specimen preparation