The essentials of a cryoEM lab

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Outline

[What do you need? What can you borrow? How do you validate your equipment? How do you service your equipment? How do you assess a new specimen?]

Equipment

- Our setup
- Morgagni/Gatan 2K CCD
- T12/Gatan 4K US4000 CCD
- T20/K2
- Krios/GIF+K2 (space considerations)
- Cryo-holder + pumping station
- Carbon evaporator
- Vitrobot/CP3/Leica/Spotiton/Manual plunger
- Cluster/GPU boxes
    - Scheduler
- Storage space

Equipment

- Freezers
- FPLC
- Incubators/shakers
- Centrifuges
- Thermocycler
- Spectrophotometer
- …

Funding

- Your startup package should cover:
    - 2-3 people for 2-3 years
    - Your salary for 2-3 years
    - Purchase of the equipment you need
    - Access to microscopes for 2-3 years
        - Some places cover for microscope time (rare in the US)

Staff

- Take your time
- Grad students eager to join new labs
    - Rotations very useful (microscope time)
- Postdocs
- EM training not mandatory
    - Could bring new knowledge to the lab
- Facility manager?
    - Probably needed for large-scale training

Projects

- Your own projects (chalk talk)
- Be open to collaborate
    - Situation different if you are establishing single particle EM in a University/city
- Learn how to prioritize and to say no when needed
    - You have limited resources and you need to prioritize your own projects

How do you validate your equipment?

- Test specimen
  - Apoferitin
  - T20S
  - Aldolase
  - …

How do you assess a new specimen?

- Negative stain!!!
    - Screening
    - Data collection
    - Initial model
- CryoEM
    - Screening
    - Preliminary data collection
    - Data collection
    - Acquisition of a high-resolution data set when sample/grids ready

Acknowledgements

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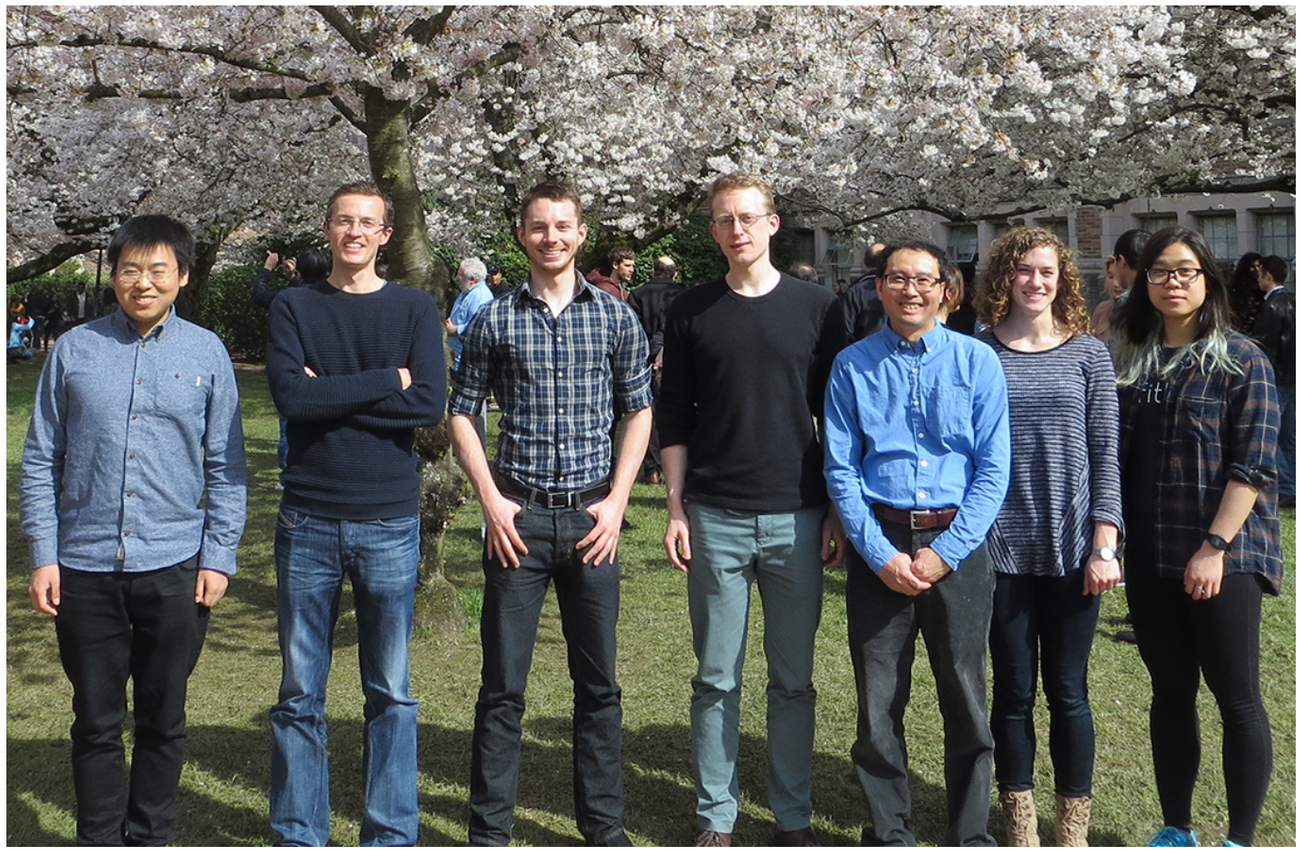

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