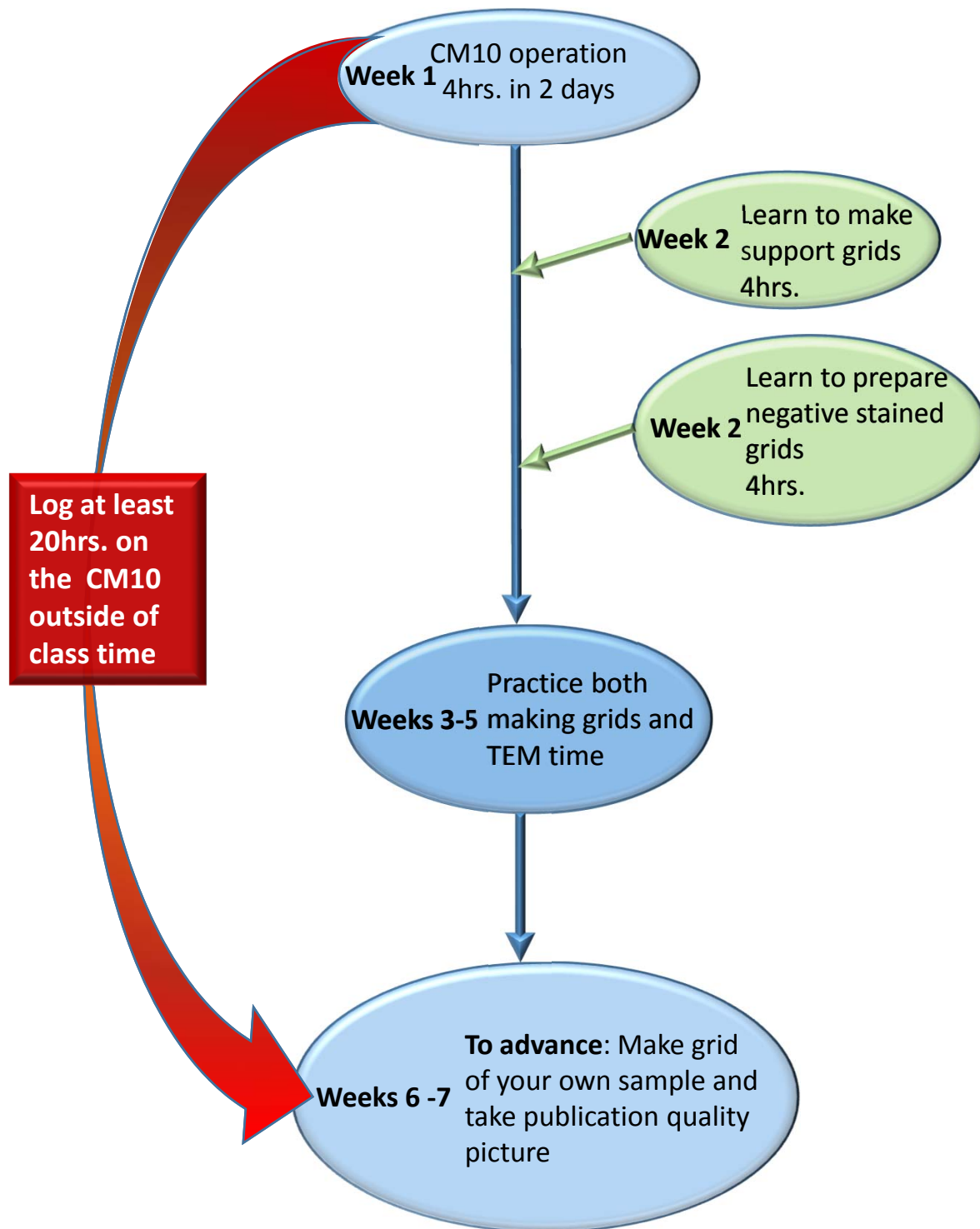


TEM training schedule

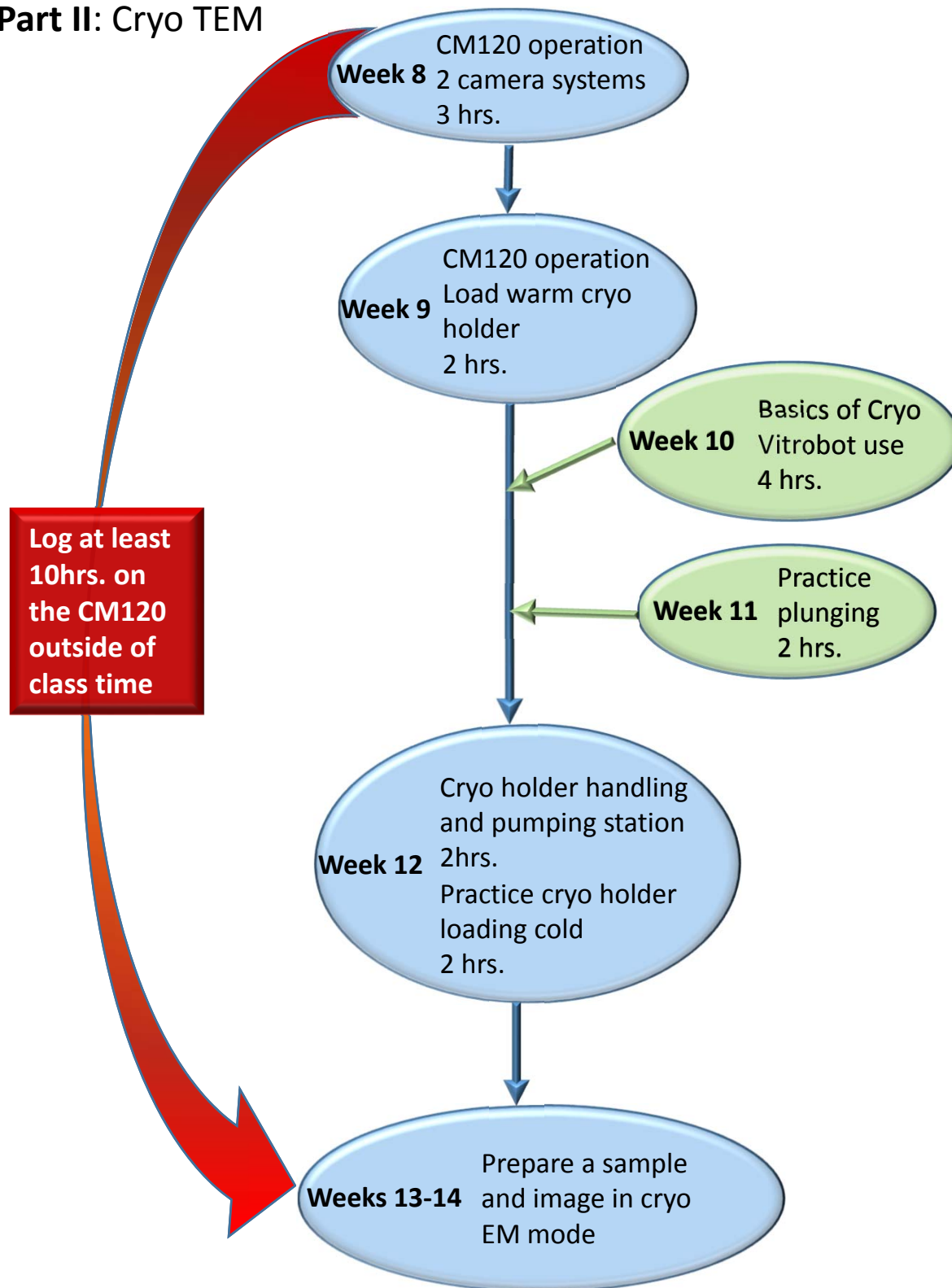
Part I: Room Temperature TEM



Total time commitment Part I: approx. 32 hrs. in the lab

TEM training schedule

Part II: Cryo TEM



Total time commitment Part II: approx.. 25 hrs. in the lab

Materials (you need to bring with you to class)

Part I

- 2 forceps: 2spi.com [Dumont Dumostar Style #5 Tweezer, Biology Tips, 100% Antimagnetic, Matte, 110 mm](#)



- 2 paper clips



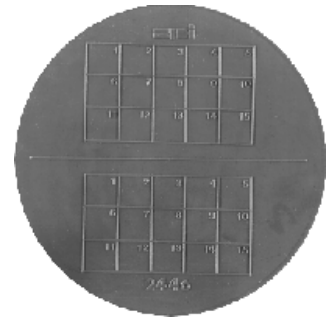
- 3 grid boxes: [SPI Slide-A-Grid Unnumbered TEM Grid Storage Box with Record Keeping Card](#) or

EMS diasum **71150** Grid Storage Box, 50 Capacity



- 1 rubber silicon disc or grid mat

<http://www.2spi.com/item/2446m-ab/grid-handling-grippers/>



Part II

- 5 cryogenic grid storage boxes:

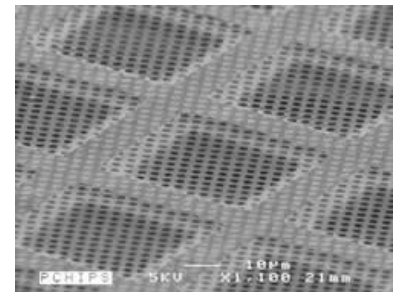
71166-10-W Cryo Grid Box, Round, w/Lid, White or blue

<http://www.emsdiasum.com/microscopy/products/grids/accessories.aspx#71166>



- 1 box (50 grids) of C-flat™ Holey Carbon grids

www.protochips.com CF-1.2/1.3-4C-50 #73137



Topics to be covered

Part I: Room temperature TEM

- **Week 1: CM10 operation**, 2 hrs. group session, and 2hrs. one on one session: Through a series of lectures (plus reading material) and laboratory sessions the students will learn:

- How to safely and effectively operate the CM 10 TEM.
- Start the high tension and bring up the filament.
- Learn to stigmatize the condenser lens.
- Align the pivot points.
- Load specimen (provided by the staff).
- Eucentric stage height.
- Learn to stigmatize the objective lens.
- Learn basic camera functions.

After this initial week the students are required to log at least 20 hrs. on the CM10 TEM (preferably 2 hrs. twice a week) on their own until the end of week 7.

- **Week 2: Support film on grids and negative staining**, 4 hrs. group session twice a week.

This week the students will learn:

- To cast formvar films and produce support film on grids.
- To operate the carbon evaporator to carbon coat the formvar support films.
- To produce carbon films on mica sheets for high resolution support film grids.
- To operate the Denton glow discharge device.
- To negative stain viral particles (provided by the staff).

Topics to be covered

Part I (cont.)

- **Weeks 3-5:**

- Practice both making support film grids, negative stain and imaging with viral samples provided by the staff.

If the student can successfully accomplish the task they can start using their own sample material.

- **Week 6-7: Final assessment: Lab Practical**

- In order to move to part II of the course the student is required to demonstrate proficient operation of the CM10 TEM to the Electron Microscopy Facility personnel and to produce publication quality images of their own sample (if they have one).

Topics to be covered

Part II: Cryo TEM

- **Week 8: Operation of CM120 TEM**, 3hrs group session.

The student will transition from the CM10 microscope to the CM120 and learn:

- Alignment procedures for 120 Kv.
- Tietz camera system.
- Orius camera system.
- Low dose imaging on NS stained sample provided by the staff.

After this initial week the students are required to log at least 10 hrs. on the CM 120 TEM on their own until the end of week 14.

- **Week 9: CM120 TEM use**, 2hrs group session.

-Learn to handle and load the cryo holder at room temperature.

The student should practice on their own at least one more time this week.

- **Week 10: Basics of Cryo Electron Microscopy and preparation of cryo samples**, 4hrs group session.

- Observing biological material (protein complexes, virus, liposomes, exosomes-particulate material) in their native configuration by freezing these materials instantaneously in a layer of vitreous ice.
- Vitrobot operation.
- Safety issues while handling ethane gas.

Topics to be covered

Part II (cont.)

- **Week 11: Practice plunging grids on your own**, at least 2 hrs. independent work.
- **Week 12: (Cold) Cryo holder handling and pumping station**, 2 consecutive days.
2 hrs group session-**day 1**

Note: The Cryo holder is a very delicate and expensive piece of equipment (\$50,000.00). Extreme care should be taken when handling this device, if it is damaged it will take weeks to be repaired and this will mean down time for everyone.

- Learn about vacuum systems and the proper use of the conditioning and pumping station to get the cryo holder ready for the cryo session.

2hrs group session-day 2****

- Cooling cryo holder in portable cryo station and loading cryo grids.
- Load the cryo holder into the microscope.
- Imaging cryo grids in low dose (cryo grid screening).

Topics to be covered

Part II (cont.)

- **Weeks 13-14:**

- Practice both making cryo grids and imaging them with viral samples provided by the staff under Electron Microscopy Core personnel supervision.

If the student can successfully accomplish the task they can start using their own sample material.

Final assessment: Lab Practical

-In order to become an independent user and be able to submit material to the High Resolution Cryo EM Facility the student is required to demonstrate proficient use of the CM120 TEM to the Electron Microscopy Core personnel and to produce publication quality images of their own sample (if they have one).

Work flow for a particulate sample from tube to high resolution cryo EM

