

Sample preparation for biological microscopy

Chris Jacobsen

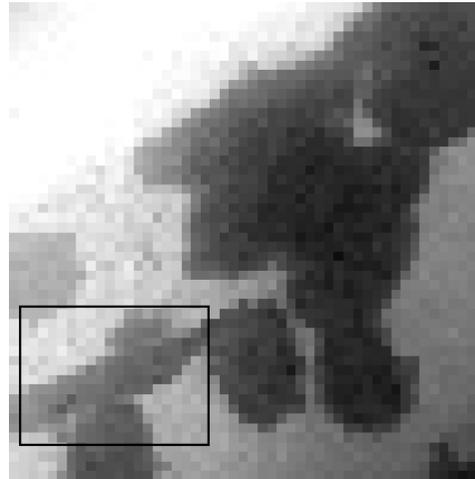
Stony Brook

Growing samples

- Cell culture: need multiple small incubators, and a lab Tsar to avoid culture cross-contamination!
- Need inverted microscope(s) with phase contrast and fluorescence capabilities, plus low-noise digital image capture.
- Optical density measurement.
Microinjection/manipulation?
- Plus other things like autoclaves, glassware washer, laminar flow hoods with UV lamps, centrifuges, DDH₂O...

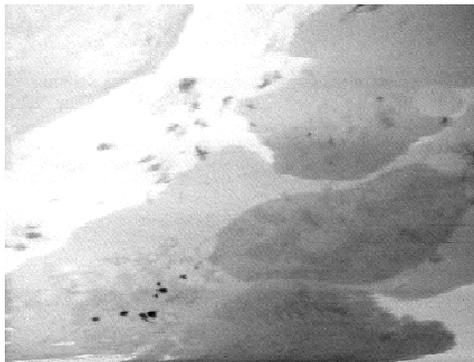
Radiation damage on (initially) living cells

- Chick embryo fibroblasts. Reflux of culture medium every 20 min to keep unexposed cells alive.
- Makes it hard to view living cells!



■ 10 μm
6.0 · 10² Gray, ET=2 min.

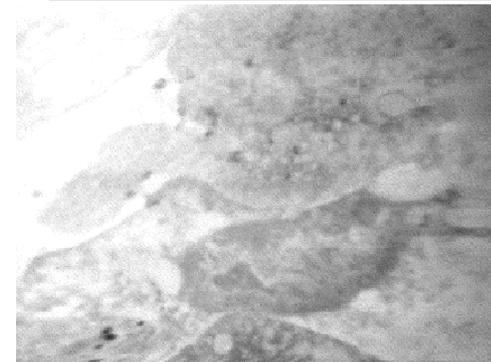
Experiment by V. Oehler, J. Fu, S. Williams, and C. Jacobsen, Stony Brook using specimen holder developed by Jerry Pine and John Gilbert, CalTech. Never properly published, but see Kirz *et al*, *Q. Rev. Biophys.* **28**, 33 (1995)



■ 5 μm
1.2 · 10⁵ Gray, ET=9.5 min.



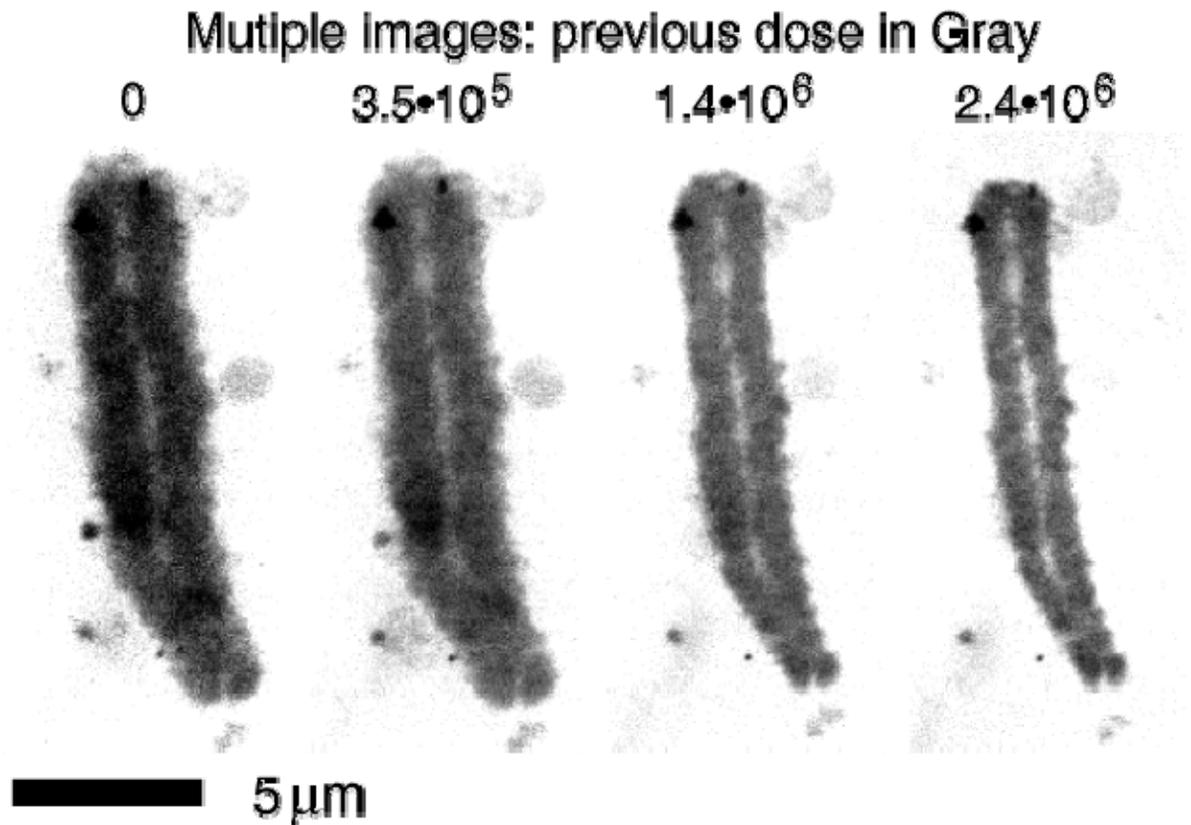
■ 5 μm
2.4 · 10⁵ Gray, ET=17 min.

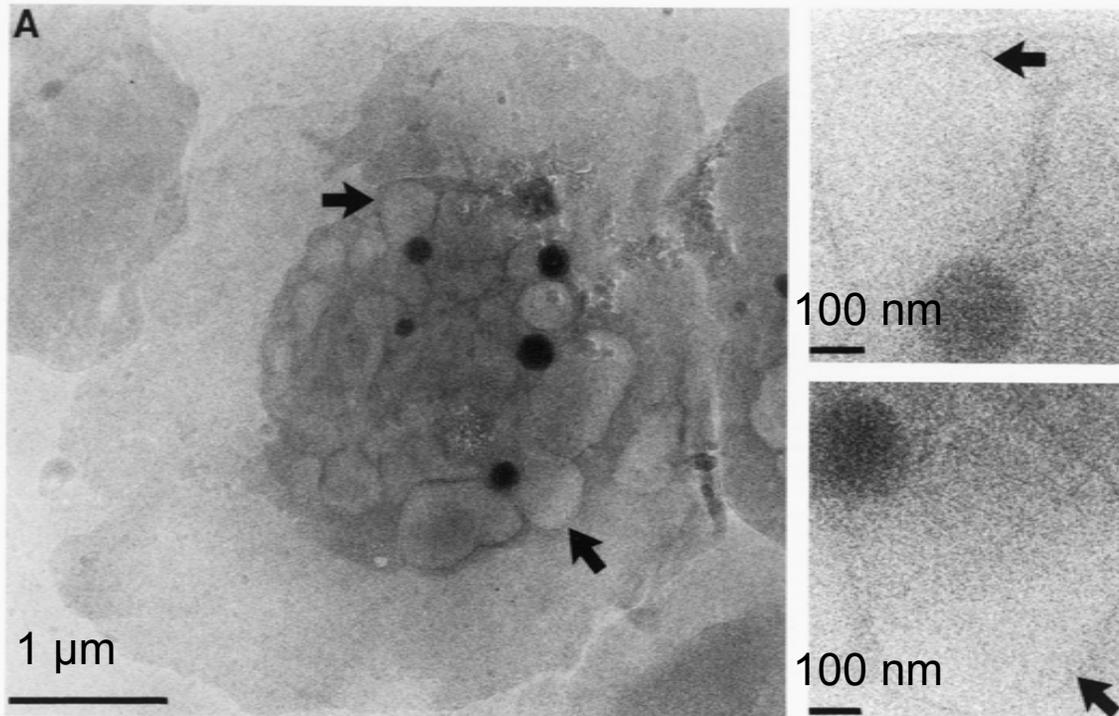


■ 5 μm
3.7 · 10⁵ Gray, ET=24.5 min.

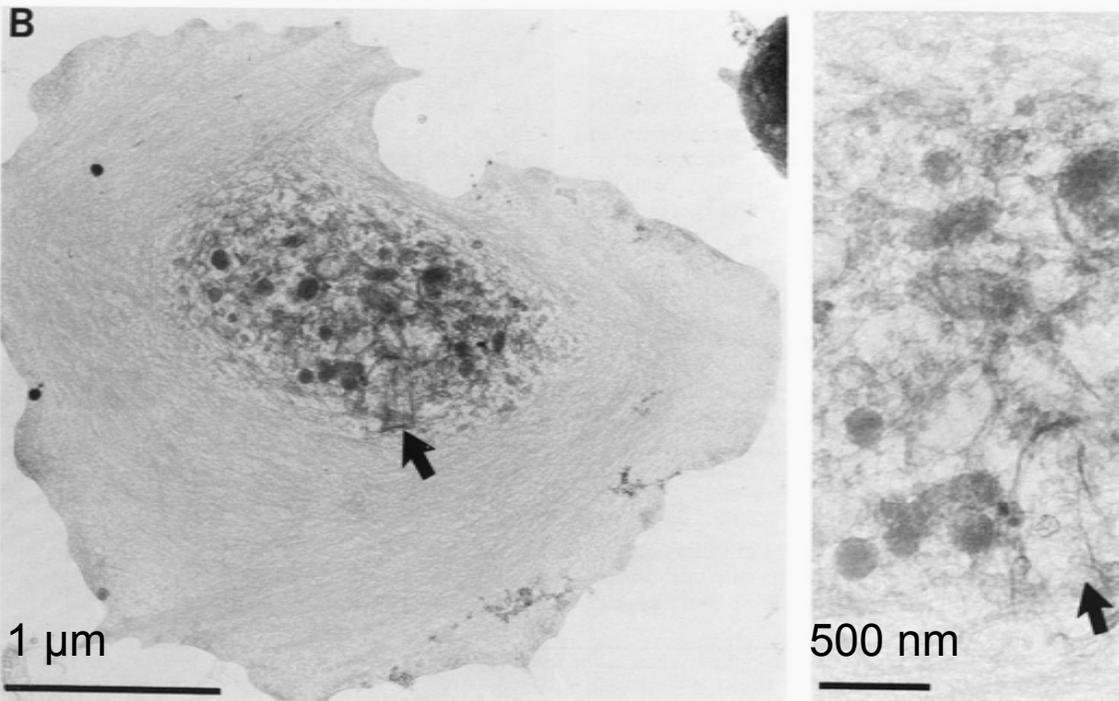
Wet, fixed samples: one image is OK

- Chromosomes are among the most sensitive specimens.
- *V. faba* chromosomes fixed in 2% glutaraldehyde. S. Williams *et al.*, *J. Microscopy* **170**, 155 (1993)
- Repeated imaging of one chromosome shows mass loss, shrinkage





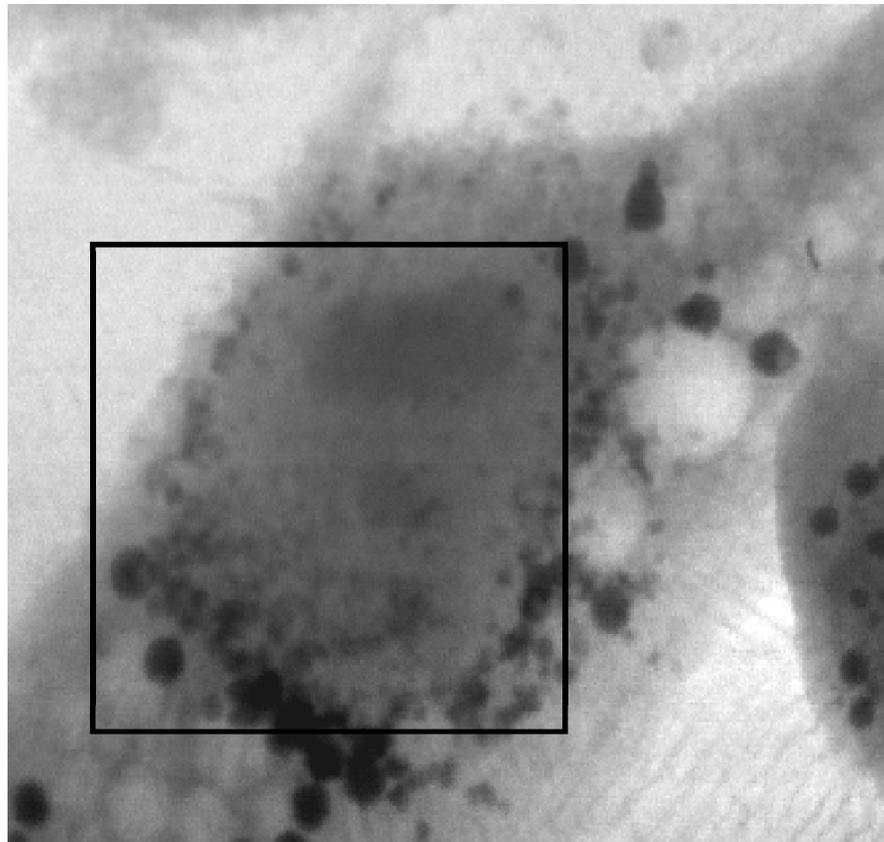
Frozen hydrated



2% glutaraldehyde fix
1% OsO₄ postfix
critical-point dry

- Human blood platelets
- 1 MeV transmission electron microscope (JEOL-1000)
- O'Toole, Wray, Kremer, and McIntosh, *J. Struct. Bio.* **110**, 55 (1993)

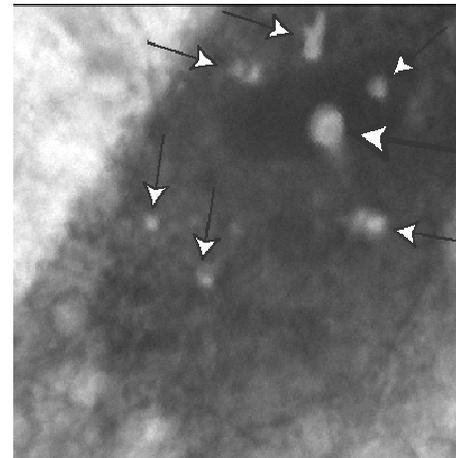
Radiation damage resistance of wet specimens at liquid nitrogen temperature



Frozen hydrated image **after** exposing several regions to $\sim 10^{10}$ Gray

Maser et al., *J. Micros.* **197**, 68 (2000)

After warmup in microscope (eventually freeze-dried): holes indicate irradiated regions!



— 7 μm

Cryo specimen preparation

- Cryo prep lab should include cryo plunger, high pressure freezer, cryo ultramicrotome, and LN₂ storage vessels.
- One approach: mount delicate sample in a cartridge/crystal pin mount once, and move cartridge from technique to technique.
- Evaluation of specimen quality: cryo light microscopy (gives new science opportunities!), lab x-ray source for checking for ice crystallization diffraction rings.
- Specimen preselection: indexing between cryo light microscope and x-ray/IR microscopes and nanoprobes.

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Cryo-fluorescence microscopy facilitates correlations between light and cryo-electron microscopy and reduces the rate of photobleaching

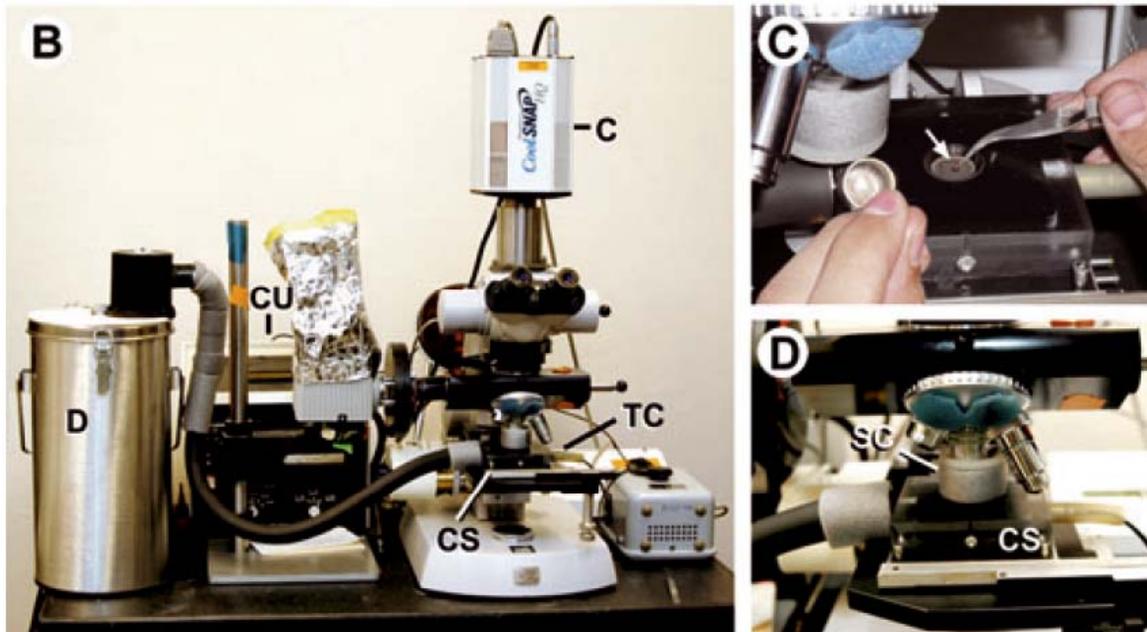
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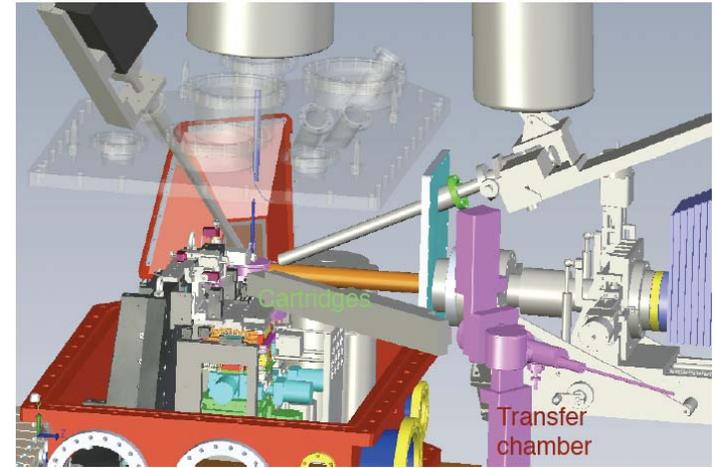
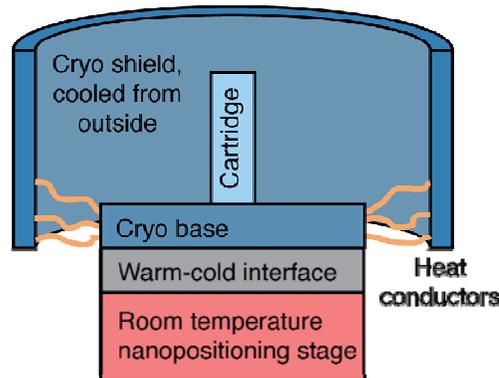
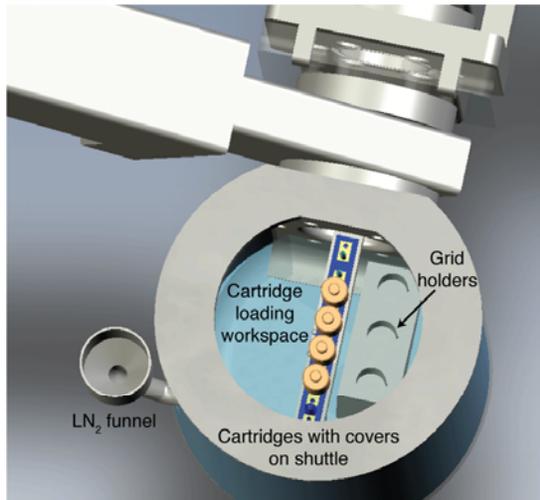
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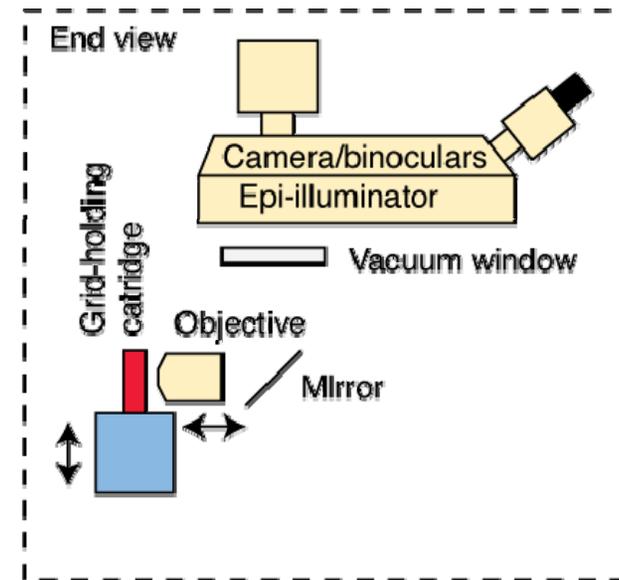
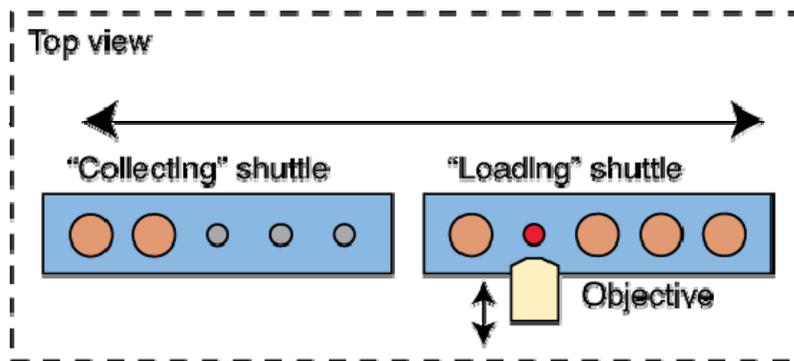
See also Sartori *et al.*, *J. Struct. Bio.* **160**, 135 (2007).



Cryo system: Xradia example



- Mount fragile grid in cartridge once.
- Transfer cartridge between visible light and various X-ray microscopes (including scanning, tomography).
- Robotic sample insertion in microscope.



Conclusion

- Sample prep is both important, and challenging, in biological microscopy.
- In fact, sample prep is hard enough that you really want a dedicated microscope - so when the prep finally works, you don't have to reconfigure the microscope!
- As spatial resolution is improved, radiation dose goes up (at about the fourth power!) so cryo microscopy becomes increasingly important.
- The success of NSLS II in biological microscopy will require beamlines, microscopes, **and** cryo sample preparation facilities!