Introduction - Why electrons?
Concept check questions:

- What are the advantages and disadvantages of electrons compared to photons for microscopy? Neutrons?
- What structural biological technologies give higher resolution information than cryo-EM, and what kinds of samples and questions can they address?
- What structural biological technologies complement cryo-EM at lower resolutions, and what kinds of questions and samples do they address?

Electron guns
Concept check questions:

- Where do the imaging electrons in an electron microscope come from?
- What part of the gun is called the "cathode"? What should be called the "anode"?
- What is the accelerator stack a "stack" of?
- What voltages are typically used in transmission electron microscopes? What kinds of electron wavelengths does this correspond to?
- What does it mean to "condition" the gun?
- What is the difference between spatial and temporal coherence?
- What are the three main types of electron guns? What are the advantages and disadvantages of each?

Electron lenses
Concept check questions:

- What is the defining property of a “lens”?
- Why/how do optical lenses focus light?
- Draw a diagram that shows how a lens can be used to form a magnified image. What parameters determine the magnification?
- How do electron lenses focus electrons?
- Why do electron images rotate in an electron lens?
- What are the four main components of an electron lens system? What does each do?

The column
Concept check questions:

- What are the three main lens systems in an electron microscope called?
- What is meant by a “conjugate plane”?
- What are the special names given to the three independent sets of deflectors?
- What current is controlled by the “filament” knob? “emission”? “spot size”? “intensity”? “focus”? “magnification”?
- What is controlled by the “high tension” knob?
- What is a “crossover”?
- Which knob controls whether the microscope is in “LM,” “M,” or “SA” mode? What currents change?
- What are “pivot points”?
- What does it mean to “align” the microscope?
- What is “hysteresis”?
- What does the “normalize” button do?

The sample chamber
Concept check questions:

- In what directions/ways can the sample be moved while in the microscope?
- What is an “air-lock”, and how it is relevant to the sample chamber?
- Where does the sample rest with respect to the objective lens?
- What is the “pole piece gap”?
- What is a “cryo-box”?
- What is “eucentric height”? Is it different for every grid?

Energy filters
Concept check questions:

- Why are EM energy filters used?
- How are “post-column” filters different from “in-column” filters?
- What is a typical slit width for cryo-EM?
- What is a “zero-loss” peak?
- How could an energy filter allow you to image where a particular element was in the sample?

Electron detectors
Concept check questions:

- Name five different types of electron detectors.
- What is a “CCD”? How do they work?
- What are the advantages and disadvantages of film versus CCDs?
- What is meant by “direct” detector?
- What new capabilities do direct detectors provide?

Vacuum systems
Concept check questions:

- Name four different types of vacuum pumps. How does each work?
- Why are so many different types of pumps needed?
- What is a “backing” pump?

Summary, safety
Concept check questions:

- What is the purpose of the heavy lead shielding covering electron microscopes?
- Why do electron microscopes need chilled water?
- Name three lethal and at least one more non-lethal hazards associated with electron cryo-microscopes.

One-dimensional sine waves and their sums
Concept check questions:

- What four parameters define a sine wave?
- What is the difference between a temporal and a spatial frequency?
- What in essence is a “Fourier transform”?
- How can the amplitude of each Fourier component of a waveform be found?

One-dimensional reciprocal space
Concept check questions:

- What is the difference between an “analog” and a “digital” image?
- What is the “fundamental” frequency? A “harmonic”? “Nyquist” frequency?
- What is “reciprocal” space? What are the axes?
- What does a plot of the Fourier transform of a function in reciprocal space tell you?

Two-dimensional waves and images
Concept check questions:

- What does a two-dimensional sine wave look like?
- What do the “Miller” indices “h” and “k” indicate?

Two-dimensional transforms and filters
Concept check questions:

- In the Fourier transform of a real image, how much of reciprocal space (positive and negative values of "h" and "k") is unique?
- If an image "I" is the sum of several component images, what is the relationship of its Fourier transform to the Fourier transforms of the component images?
- What part of a Fourier transform is not displayed in a power spectrum?
- What does the "resolution" of a particular pixel in reciprocal space refer to?
- What is a "low pass" filter? "High pass"? "Band pass"?

Three-dimensional waves and transforms
Concept check questions:

- What does a three-dimensional sine wave look like?
- What does the third “Miller” index “l” represent?

Convolution and cross-correlation
Concept check questions:

- What is a “convolution”?
- What is the “convolution theorem”?
- What is a “point spread function”?
- What does convolution have to do with the structure of crystals?
- What is “cross-correlation”?
- How might cross-correlations be used in cryo-EM?

Amplitude and phase contrast
Concept check questions:

- Why is it easier to explain amplitude contrast if we envision imaging electrons as particles?
- Why does phase contrast require us to think of imaging electrons as waves?
- What is a “plane wave”? What about a plane wave changes as it travels through a vacuum?
- Explain how/why atoms scatter X-rays.
- Why are there discrete peaks in the scattering from crystals?
- What information is delivered by each peak?

Wave propagation and phase shifts
Concept check questions:

- How is the scattering from an object converted into an image in a microscope?
- What is the relationship between the density of the sample and the wavefunction present on the back focal plane of the objective lens? The image plane? Can you draw a picture showing why?
- How are plane waves represented in an "Argand" diagram? What are the axes?
- Why were Argand diagrams introduced (how do they help us understand wave propagation and interference)?
- How does the phase difference between two waves of identical frequency effect their interference?
- What property of an electron wave gives the probability of its detection at each position?

The contrast transfer function
Concept check questions:

- What two factors make the phase of a scattered component of a wave different from that of an unscattered component?
- The contrast transfer function is typically plotted as a sinusoidally-varying function of what variable (what is the horizontal axis)? What quantity is plotted on the vertical axis?
- What is the CTF's domain and range?
- What does a "contrast transfer" of 1.0 mean? -1? 0?
- Why does the CTF oscillate sinusoidally?
- What four variables appear in the argument of the sine function?

Defocus and its effects
Concept check questions:

- What is a “Thon” ring?
- How can the defocus of a TEM image be determined?
- Why is defocus part of the argument of the CTF sine function?
- Does increasing the current in the objective lens make the image more or less defocussed?
- What is “over-focus”?
- How do heavily defocussed images look different than “closer-to-focus” images?
- What are the advantages of taking pictures far from focus? close to focus?

Envelopes
Concept check questions:

- What effect does partial spatial coherence have on the CTF? Why?
- What effect does partial temporal coherence have on the CTF? Why?
- What is their combined effect?
- How do these effects depend on defocus?

CTF correction
Concept check questions:

- What is a "point spread function"?
- How is the point spread function related to the CTF?
- What is the relationship between the wavefunction that exists on the back focal plane of the microscope and the Fourier transform of the recorded image?
- How (conceptually) can EM images be "CTF-corrected"?
- How can the CTF of a TEM image be determined?
- What special issue arises at CTF-zeros? How can it be handled?
- What would it mean if someone said they "CTF-corrected by phase-flipping only"?
- How can the information loss at CTF-zeros be overcome?

Sample prep - Room temperature methods Concept check questions:

- Why can't cells just be inserted into the microscope and imaged (without any special preparation)?
- What is "chemical fixation", what agents are used to do it, and what are its advantages and disadvantages?
- Once a cell or tissue is chemically fixed, what else is typically done in preparation for traditional "thin section" EM?
- What metal stains are typically used for thin-section EM, and how do they effect the visibility of sample structures?
- How does "metal shadowing" work?
- How is metal shadowing different from "negative staining"?

Sample prep - Methods involving freezing
Concept check questions:

- What problem does high pressure freezing solve?
- What is “low-temperature” embedding?
- What is “cryo-sectioning,” and what artifacts (3) and challenges (several) are associated with it?
- What kinds of samples can be “plunge-frozen”?
- How can focussed ion beams be used to prepare cryo-EM samples?

Sample prep - Grids
Concept check questions:

- What are the most common materials used to make grids?
- If you wanted to culture cells on grids, which grids would be better - copper or gold?
- What does “250 mesh” mean?
- What is a “slot” grid? A “finder” grid?
- What is formvar?
- What is the difference between “holey” carbon and “Quantifoil” coatings?
- What is a carbon evaporator, and how does it work?
- What is “glow discharging,” and why is it done?
- What is “cryo-crinkling”, and what are some ways to reduce it?

3D reconstruction
Concept check questions:

- How can 3-D reconstructions be calculated from 2-D projections in real space?
- What is the "projection theorem"? Draw it.
- How are 3-D reconstructions calculated from 2-D projections in reciprocal space?

Dose limitations
Concept check questions:

- How do imaging electrons damage biological samples?
- How can radiation damage be recognized in images?
- How can the rate of this damage be assessed quantitatively?
- What is the effect of temperature on the rate of radiation damage?
- What disadvantage is there to imaging at temperatures less than 40K?
- For what kinds of samples can radiation damage be overcome? How?
- What are the three basic modalities of cryo-EM? How are they different? What kinds of resolutions can be expected from each? Why?

Tomography - Intro
Concept check questions:

- What is a “tilt-series”?
- What range of angles is typically imaged?
- What is the “missing wedge”?
- How are interpolations involved in the calculation of a tomogram?
- What does each word in “serial section montage tomography” signify?
- How large or small a sample can be imaged by electron tomography?
- How is “volume rendering” different than showing an “isosurface” or single slice?

Tomography - Data collection and reconstruction Concept check questions:

- What is "eucentric height"? Is it different for every sample?
- What is the "tilt axis offset"? Is it different for every sample?
- When speaking about automatic sequential tilt-series acquisition, what is "tracking"? What is "targeting"?
- How is the "predictive" tracking method different from the "focus position" method? Which is faster? Why would the slower method ever be used?
- What is a "low mag atlas" and why would one be recorded?
- What microscope operations are used to set specimen height automatically? Why?
- What microscope operations are used to focus objects automatically? Why?
- What about each image has to be determined to "align" a tilt-series? How are these parameters found?
- What steps of data collection and 3-D reconstruction have to be done by the investigator, and which are typically automated?

Tomography - Identifying objects in tomograms
Concept check questions:

- What does "CLEM" stand for?
- What are the advantages and disadvantages of doing the light microscopy at room temperature and cryo-temperatures?
- What is "cryo-PALM"? Bonus exercise and question: Estimate the localization precision of cryo-PALM from the example shown. Do you know what factors limit the localization precision in this experiment?
- How can small molecule inhibitors or genetic manipulations be used to identify objects in tomograms?
- What is "template matching"? In the slide on template matching, four variables were listed as arguments in the cross-correlation function - what were they and why was each present?
- What kinds of macromolecular complexes are likely to identifiable within a cell by template matching?
- What is "visual proteomics"?
- What ideas have been tried so far to label objects of interest inside cells with electron dense tags?

Tomography - Limitations
Concept check questions:

- What is the fundamental resolution limitation in tomography for native samples?
- What is the fundamental resolution limitation for stained samples?
- Name and explain four other resolution limitations in tomography.
- What is the "missing wedge"? Why is it missing? What effect does it have on reconstructions?

Tomography - Parameters and handedness
Concept check questions:

- There are many choices involved in the design of a tomography experiment. Each is a balance between opposing considerations (more/higher/smaller is good, but only up to some point). Explain the compromises involved in the choice of magnification, total dose, tilt-increment, exposure time per image, and defocus.

- Which steps of a tomography project influence the handedness of the final reconstruction?

- What can be done to ensure that the handedness of chiral features in tomograms is interpreted correctly?

Intro to single particle analysis
Concept check questions:

- Any two projection images of the same object share at least one feature - what is it?
- How can this fact be used to align particle images?
- What kinds of samples are amenable to single particle analysis, and what kinds of resolutions have been obtained in the best cases?
- Name 6 advantages of single particle analysis (as compared to other popular structural techniques like X-ray crystallography and NMR spectroscopy).
- Describe 5 limitations.

Single particle analysis: Special sample prep issues
Concept check questions:

- Why do most projects begin with “negative staining”? What is “negative” about it?
- What is “cryo-negative staining,” and why might one do it?
- Explain the “GraFix” method.
- Name three ways to stabilize membrane proteins for cryo-EM imaging.
- What is an “affinity grid”?

Single particle analysis - Data collection
Concept check questions:

- How do programs such as Leginon record good single particle images automatically (what is the sequence and logic of their operations)?

- In addition to single projection images, sometimes pairs of images are recorded. What kinds of pairs are recorded, and why?

- What kinds of beam-induced specimen movement are common and what can be done to reduce or mitigate the problems this causes?

Single particle analysis - Reconstruction basic workflow Concept check questions:

- What methods are used to identify individual particles in cryo-EM images?
- After particles are picked, the next step in single particle image processing is typically to classify the images. What factors make different particle images look different?
- What characteristics are the same and which are different about images in the same "class"?
- Describe two different methods to classify single particle images.
- What is an "eigenimage"?
- How can the relative orientations of different class averages be found?
- Single particle reconstruction is an iterative process. What are the basic steps being iterated? How does one know when to stop iterating?
- Describe one way "maximum likelihood" methods can be used in single particle reconstruction.

Single particle reconstruction - additional topics
Concept check questions:

- Name 5 ways to generate an initial model.
- What are the differences between a "random conical tilt" and "orthogonal tilt" reconstruction, and why are these produced?
- Why are focal pairs of images sometimes recorded?
- Name three resolution-enhancing steps made possible by recording images on a direct detector in "movie-mode".
- Name two kinds of heterogeneity that can be detected and overcome with single particle methods.
- Given what you learned about how maximum likelihood methods could be used to produce a single particle reconstruction, how might they be used to sort out heterogeneity and produce multiple 3-D reconstructions from a single data set?
- What process parameters are typically changed from iteration to iteration in a single particle reconstruction?
- Where can a good list of single particle software packages be found?
- The "Einstein from noise" demonstration is famous in cryo-EM for illustrating what?
- Name three ways reference bias can be introduced into a single particle reconstruction, and how one can know if his/her structure is biased?

Single particle analysis - Interpretation and limitations
Concept check questions:

- How can EM be used to characterize particle flexibility?
- When should one try to classify particles into distinct conformational states and solve structures of each one, and when should one conclude their particle is simply "flexible" (i.e. it exhibits a continuous range of motion)?
- Why do some particles exhibit preferred orientations? How can that problem be handled?
- Why can the effects of partial spatial coherence and translational alignment errors both be understood as envelopes?
- What is similar about partial temporal coherence and errors in defocus determination?
- How many images are typically needed for a near-atomic-resolution single particle reconstruction? What factors influence this number?
- What is "MDFF", and when is it used?

2-D crystallography - Intro and sample prep
Concept check questions:

- What is a “2-D crystal”?
- When is 2-D crystallography the cryo-EM approach of choice?
- Describe a method for inducing a protein of interest to form a 2-D crystal.
- In addition to plunge-freezing, what other way have 2-D crystals been stabilized for EM imaging?

Fourier transform of a 2-D crystal
Concept check questions:

- Why does the Fourier transform of a crystalline object have discrete spots separated by pixels with near-zero amplitudes?
- What is the convolution theorem, and what does it have to do with crystallography?
- What does the Fourier transform of a 2-D crystal look like?
- What is the "missing cone," why is it "missing," and what effect does it have on 2-D crystallographic reconstructions?

2-D crystallography - Data collection and reconstruction Concept check questions:

- What is the difference between “imaging” and “diffraction” modes on an EM?
- Why are both images and diffraction patterns of 2-D crystals recorded in a 2-D crystallography project?
- Why are images of both untilted and tilted samples recorded?
- How is all the data from all these images and diffraction patterns merged to produce the reconstruction?
- What is crystal “unbending”? How and why is it done?
- Describe four common challenges in 2-D crystallography projects.

Helical tubes
Concept check questions:

- How are helical tubes related to 2-D crystals?
- Why are helical tubes particularly good samples for cryo-EM reconstruction?
- What does the diffraction pattern of a helical tube look like?