Two-Dimensional (2-D) Classification	Is a process where individual two-dimensional particle images selected from aligned TEM images, are translationally and rotationally aligned to each other and then computationally classified into classes based on apparent similarities. The grouped particle images in each class are averaged to enhance the overall signal. These 2-D class averages provide valuable information on particle shape, data quality, and heterogeneity present within the data.
Two-Dimensional (2-D) Class Average	An image resulting from the averaging of individual two-dimensional particle images grouped into a single class during two-dimensional classification.
Three-Dimensional (3-D) Classification	Is a process where individual two-dimensional particle images selected from aligned TEM images are translationally and rotationally aligned to each other, then computationally classified into classes based on their apparent similarity to three-dimensional reference volumes.
Three-Dimensional (3-D) Reconstruction	Is a process where individual two-dimensional particle images selected from aligned TEM images are computationally combined using defined Euler angles to yield a three-dimensional object.
Three-Dimensional (3-D) Refinement	Is a process where the Euler angles of individual two-dimensional particles images are optimized based on a three-dimensional reference volume. This iterative process simultaneously maximizes the assignment of Euler angles of the particle images and the contribution of each particle image to the three-dimensional reconstruction. As each iteration progresses and alignment parameters improve, as well as the resulting three-dimensional reconstruction, the sampling rate becomes finer with more high-resolution information being provided until the refinement converges and no more improvements in alignment parameters and/or resolution are attained.
Three-Dimensional (3-D) Variability Analysis	A three-dimensional analysis that attempts to account for continuous conformational variability. There are different implementations of this analysis but the most common is deployed within cryoSPARC.
Ab Initio Model	An unbiased initial model that is generated by aligning individual two-dimensional particle images without prior three-dimensional alignment information. Also referred to as *ab initio* volume.
Ab Initio Volume	See *Ab Initio* Model
Aberrations	Any non-ideal modification to the original object image during the imaging process. These are often a result of imperfections in the geometry of the lenses of the electron microscope with contributions arising from the electron source, apertures, and the camera used to form images. See coma, trefoil, tetrafoil, symmetric aberrations, antisymmetric aberrations, contrast transfer function, spherical aberration, chromatic aberration, …..
Acceleration Voltage	The strength of the electrostatic field (voltage) in the electron gun accelerator stack between the cathode and the anode that determines the velocity at which electrons travel. Commonly, these are expressed in kilovolts (e.g., 80 kV, 100 kV, 200 kV, and 300 kV).

Air-Water Interface (AWI)	The boundary where solution meets air. The chemical properties of this boundary often lead to unwanted effects (e.g., particle behavior) in sample preparation.
Airy Disk	
Amplitude	
Amplitude Contrast	The angular distribution of scattered intensities varies as a function of the atomic composition and density of the object (i.e., more dense object scatter electrons more than less dense objects). Amplitude contrast arises from a loss of amplitude (i.e., electrons) from the beam between neighboring objects. Can be modified by the acceleration voltage and the objective lens aperture. Amplitude contrast does not contribute as much in cryoEM imaging as it does in negative stain imaging.
Anisotropic Magnification	A distortion that results in an image whose magnification varies with direction, and thus leads to a directional scaling of the image. For example, imaging of a circle in a system with anisotrpic magnification will present an image of an ellipse.
Aperture	The ability of a lens to gather and focus radiation arriving from an object that can be focused on an image. Commonly, this refers to the objective lens aperture.
Aperture Contrast	
Astigmatism	An asymmetry in two mutually perpendicular planes that are parallel to the optical axis that result in the focusing of rays at different points. Commonly this value refers to objective lens astigmatism.
Atlas	A low magnification image of the EM grid that can be used for manual and automated data collection. Typically, a series of low magnification images are captured at varying areas of the EM grid and then computationally stitched together.
Automated Data Collection	The process by which a software program automatically collects TEM movies of a specimen based on user-defined parameters. The exact process varies depending on the software utilized (e.g., EPU, Leginon, SerialEM, etc.) but typically involves using images of the specimen collected at various magnifications, including a low magnification atlas and intermediate magnification images. Usually refers to single-particle cryoEM data collection but can also be used for cryoET data collection as well.
B-Factor	
Back Focal Plane	A focal plane located on the opposite side of the object plane with respect to the lens. Commonly, in a TEM this is located below the lens.
Back Projection	The process by which individual two-dimensional particle images are combined with the Euler angles and alignment parameters to generate a three-dimensional reconstruction.
Bandpass Filter	
Beam Deflection Coils	
Beam Size	The overall dimensions of the electron beam illuminating the specimen. Usually defined as the cross-section of the beam, displa ns.
Beam-Induced Motion	Particl M movies that occurs when the specir nergy electrons. There are multiple

	contributors, including movement of the EM grid, particle translations, and particle rotations, which appear as translations in the EM images. Commonly, this phenomenon is corrected computationally by a process called motion correction.
Box Size	The dimensions (in pixels) of the box that individual two-dimensional particle images are extracted from the aligned EM images and used for downstream processing. See particle extraction.
Bragg's Law	
Central Section	
Charged-Coupled Device (CCD) Camera	
Chromatic Aberration	An aberration that results from electrons of different wavelengths (velocities) leaving a point in object space that cannot be brought to a focus at the same point in image space. Since the focal lengths of electromagnetic lenses are proportional to the velocity of the electrons (e.g., accelerating voltage) electrons of varying velocities will experience different focal points of the same lengths. This contributes to the disc of least confusion in the image plane.
Circle of Least Confusion	In TEM, imaging electrons cannot be focused to a sharp point but rather a circle (or disc) at the image plane. The point at which the envelope of the imaged rays has a minimum diameter is the circle of least confusion. Also referred to as the disc of least confusion.
Circular Mask	A circular area that defines the region of individual two-dimensional particle image (or class average) that is used for image alignment. Information from the image within the mask is retained while information occluded by the mask is dampened or eliminated entirely during alignment. Commonly, the dimensions of the circle are defined by the "inner radius" and "outer radius" which establish the rate of information fall off in the mask. The closer these values are, the "sharper" the fall-off of the mask and, conversely, the greater the difference in these values the "softer" the fall-off of the mask.
Class	An averaged two-dimensional image or three-dimensional volume that comprises a subset of particles within a larger classification run. See two-dimensional classification and three-dimensional classification.
CMOS Camera	
Coherence	
Coincidence Loss	A process in image recording (e.g., electron counting) where multiple electrons hit in a common area of the detector before signal read out, resulting in multiple electron scattering events registering as a single event. This primarily affects low frequency amplitudes.
Collimated Beam/Collimated/Collimation	A beam of parallel electrons that spread minimally as they propagate (i.e., do not diverge as a function of distance).
Coma	An aberration in EM imaging resulting from imperfectly aligned illumination (e.g., collimated beam) that can cause azimuthally varying phase error in the Fourier transforms computed from the EM image[illegible] points that are further from the optica[illegible] error will be observed. Commonly,

	in EM imaging this is associated with a tilt-induced shift of the electron beam frequently used for Image-Beam Shift data collection.
Compositional Heterogeneity	A difference in subunit stoichiometry and/or macromolecular components in an EM sample observed during imaging. Commonly, two-dimensional and three-dimensional classification strategies are employed to sort these complexes for downstream processing.
Compustage	
Condenser Aperture	A solid support film with an empty hole located in the back focal plane of the first (e.g., 1000 mm in diameter) or second (e.g., 70 µm in diameter) condenser lens that is used to limit the amount of electrons continuing down the column. Commonly, smaller condenser two lens apertures are chosen to illuminate the specimen with a more coherent beam at the expense of decreased brightness.
Condenser Lens	An electromagnetic lens that is designed to focus the electron beam emerging from the electron gun onto the specimen to permit optimal illuminating conditions for visualizing and recording the image. Commonly, in EM imaging multiple condenser lenses (e.g., two or three) are “paired” to provide optimal parallel and coherent illumination. These lenses are located between the electron source and the specimen, and are typically named by their location in the column and their proximity to the electron source. For example, condenser lens 1 (condenser 1) is located below the electron gun and above condenser lens 2 while condenser 3 is far from the electron gun, located below condenser lens 2 but above the specimen (objective lens).
Conformational Heterogeneity	A difference in the conformation of a component in an EM sample observed during imaging. Commonly, three-dimensional classification and three-dimensional variability analyses are employed to sort these distinct states.
Contrast Transfer Function	Specifies the relative contrast of features in the image as a function of all spatial frequencies in the image and depends on the spherical aberration, the level of focus of the objective lens (i.e., defocus), the acceleration voltage, and the pixel size.
Contrast Transfer Function (CTF) Estimation	The process of estimating the defocus (both direction) and azimuth of the astigmatism angle of the contrast transfer function using the spherical aberration of the microscope, the acceleration voltage, and the nominal pixel size of the image.
Convergent Rays	The electrons that meet at a point to form the image. Electromagnetic lenses are always convergent since the radial force is directed towards the optical axis.
Convolution	A mathematical operation on two functions that produces a third function. See convolution theorem.
Convolution Theorem	Under suitable conditions, the Fourier Transform of a convolution of two functions is the pointwise product of their Fourier Transforms. In EM, this commonly refers to the convolution between the Fourier Transform of the original particle image with the Contrast Transfer Function to yield the image recorded on the camera.
Counting Mode	A meth[illegible] EM where a direct electron detect[illegible] high frame rate such that each individ[illegible]ntified the moment they reach the

	detector. This has the benefit of eliminating Landau noise that results from individual electrons depositing different amounts of energy into the detector, improving the detective quantum efficiency of the camera.
Cross-linking	
Cryogen	A liquid that has a fast cooling rate (e.g., 10^4 K/s or greater) maintained at a cold temperature (e.g., <160 K) used for vitrifying specimens for cryoEM imaging. Common cryogens of choice are ethane, propane, and mixture of ethane:propane.
Cryogenic Electron Microscopy	A cryomicroscopy technique applied to samples cooled to cryogenic temperatures. For biological samples, structure is preserved by embedding the specimen in an environment of vitreous ice maintained within an EM grid.
Crystalline Ice	Water that has been cooled sufficiently slow to allow for the formation of ordered water molecules (i.e., ice) such that the ordered ice structure can be observed in the Fourier Transform of the aligned EM images.
Cubic Ice	Also referred to as ice Ic, is a metastable variant of ice that forms between 130 and 220 kelvins. Upon warming, it transforms into the thermodynamically more stable version ice Ih.
De novo Modeling	
Deconvolution	
Defective Pixel	A pixel in a digital camera that may appear in the final image as a bright white or black spot, sometimes referred to as “hot” or “dead” pixels, respectively. These pixels are unable to record information from the specimen.
Defocus	A form of phase-contrast imaging where the microscope’s objective lens is intentionally focused beyond the specimen by a distance of a few microns, typically a range between 0.5-2.5 µm, to generate contrast in the recorded image. During CTF estimation this value is calculated from the data. Commonly, due to variations in beam tilt pivot points and imperfections in the specimen, the value set at the transmission electron microscope is not what is recorded in the final images.
Defocus Range	The difference between the largest and smallest defocus values allowed for imaging the specimen. Typically, images are recorded with a defocus value randomly chosen between the user-defined upper and lower limit of the defocus range.
Denoising	
Depth of Field	A property of the objective lens that allows for a specimen to move along the optical axis (i.e., in Z) and can still give a maximally sharp image at the position of the image plane. This distance, referred to as Do, is related to the depth of focus. Typically, for modern electron microscopes, this distance is greater than the thickness of the specimen. Importantly, this distance decreases with increasing electron wavelength.
Density Subtraction	
Detective Quantum Efficiency	

Diffracted Beam	An electron beam that has been scattered (e.g., bending around atoms) by the specimen.
Diffraction	See electron diffraction.
Diffraction Lens	
Diffraction Plane	
Direct Electron Detector	
Disc of Least Confusion	See Circle of Least Confusion.
Divergent	
Divergent Rays	
Dose	The measure of radiation energy absorbed by the specimen. Specimen damage is proportional to the total dose which is th product of the dose rate and the exposure time. Note, this is different that total exposure.
Dose-Weighting	The process of applying different weights for each movie frame and the information content of each movie frame that is combined during motion correction and image summation to account for the loss of high-resolution information during dose accumulation in the specimen.
Double-Condenser Lens System	The pairing of two (or more) condenser lenses in a TEM that affords considerable flexibility in the illuminating system by allowing for a wider range of intensities with a given gun adjustment. The excitation (or strength) of each lens is simultaneously adjusted to keep the image in focus.
Downsampling	
Drift	
Electron Diffraction	The phenomenon resulting from interaction between electrons and crystalline materials, producing a pattern of rings or spots that characterize the sample.
Electron Gun	The source of electrons in a transmission electron microscope. Traditional TEMs used a filament whereas modern TEMs use a field emission gun.
Elastic Scattering	A form of non-radiative interaction between a projectile particle (i.e., electron) and the target specimen in which the kinetic energy of a particle is conserved and no internal energy is deposited into the specimen being imaged. In TEM imaging, these are productive scattering events that lead to usable signal in the recorded images.
Electromagnetic Lens	
Electron Beam	
Electron Microscopy	
Electron Microscopy Data Bank (EMDB)	
Electron Optics	
Electron Speed/Velocity	
Electron Tomography	
Electron Scattering	
EM Grid	
Energy Filter	
Envelope Function	
Eucentric Height	

Euler Angle	
Ewald Sphere Correction	
Exposure Time	
Field Emission Gun	A type of electron gun that uses a sharp pointed emitter that is held at several kilovolts negative potential relative to a nearby cathode so that there is a sufficiently large potential gradient to cause field electron emission. FEGs produce an electron beam that is typically greater than 1000X brighter and more coherent than filament-based electron guns. Commonly, modern TEMs use a Schottky type emitter in which thermionic emission is enhanced by barrier lowering in the presence of a high electric field.
Field of View	
Filament	A type of electron gun commonly found in entry level TEMs consisting of a thermionic cathode wire made of tungsten or lanthanum bromide (LaB6) in a “V” shape surrounded by a shield (e.g., Wehnelt) with a circular aperture. Electrons emitted by the filament are accelerated across a potential difference between the cathode and the anode before traveling to the condenser lenses.
Filter Paper	A semi-permeable soft paper barrier that has high wet strength and high wicking capabilities for the removal of liquid from a grid surface. Commonly, Whatman #1 qualitfilter paper is used in the preparation of cryoEM samples.
Focal Length	
Focal Point	
Foil	
Forward Projection	
Fourier Crop	
Fourier Ring Correlation	
Fourier Shell Correlation	
Fourier Sum	
Fourier Transform	
Frame Alignment	
Frame Grouping	
Frequency	
Fresnel Fringes	
FSC Resolution	
Gain Correction	
Gain File	
Glow Discharging	
Gold-Standard Three-Dimensional Refinement	
Gun Tip	
Helical Parameters	
Hexagonal Ice	Also referred to as ice Ih, is the only form of ice that is known to naturally occur on earth.
High-Pass Filter	
Homology Modeling	
Hot Pixel	

Ice Thickness	
Illumination	
Image Plane	
Inelastic Scattering	A form of radiative interaction between a projectile particle (i.e., electron) and the target specimen in which projectile particle loses kinetic energy that is absorbed by the specimen being imaged. In TEM imaging, these are non-productive scattering events that lead to increased noise in the recorded images.
Interference	
Interference Contrast	
Intermediate Lens	
Inverse Fourier Transform	
Ionizing Radiation	
Laplacian of Gaussian (LoG) Particle Picking	
Layer Line	
Liquid Ethane	
Liquid Nitrogen	
Liquid Propane	
Lorentz Force	
Low-Pass Filter	
Magnification	
Magnification Distortion	
Manual Picking	
Map Sharpening	
Marginal Rays	
Masking	
Mass Thickness	
Mean Free Path	
Mechanical Drift	
Mesh	
Microcrystal Electron Diffraction	
Minicondenser Lens	
Monochromatic	Radiation of a single wavelength or frequency.
Motion Correction	The computational process of pixel-by-pixel alignment of frames within a movie. There are several algorithms currently employed in EM processing that utilize either whole-frame alignment or patch-based alignment.
Movie	A series of recorded two-dimensional images assembled in a time-dependent manner as an EM specimen is illuminated with an electron beam. Modern day cameras (e.g., direct-electron detectors) used for EM imaging save exposures in this format.
Movie Frame	A two-dimensional image averaged across a finite time window from a movie collected during exposure of the specimen with the electron beam. Commonly, these represent 25-250ms of a longer exposure typical
Movie Summation	The pi gnal across frames within a movie. This c ed or aligned movies.

Multi-body Refinement	
Negative Stain	
Noncoherent	
Nyquist Frequency	Named after Harry Nyquist, this is a characteristic of the sampling of the EM information that results in conversion of the continuous signal or function into a discrete sequence. This is a result of the physical pixel boundaries in direct electron detectors.
Nyquist Limit	The resolution limit of information present in EM images. Commonly, this is 2X the pixel size of the individual particle images used for processing. For example, particle images at a pixel size of 1.00 Å will have their information limited to ~2.00 Å resolution due to the Nyquist frequency.
Objective Lens	The most critical lens of the electron microscope since it determines the resolving power of the instrument. It performs the first stage of imaging and aberrations present in the image formed will be subjected to further magnification by the projector lens systems. Modern transmission electron microscopes utilize a split objective lens with the specimen is physically being placed between the upper and lower objective lens pole pieces. Physically, the objective lens is located after the condenser lenses and before the projector lenses.
Objective lens aperture	A solid support film with an empty hole (e.g., 100 µm in diameter) located in the back focal plane of the objective lens that is used to limit the amount of high-resolution radiation to improve image contrast. The size of the aperture will determine the cut-off resolution with smaller diameter apertures removing more high-resolution information. For example, on a Titan Krios at 300 kV a 100 µm objective lens aperture will eliminate scattering information at ~1.8 Å resolution and beyond.
Optical Axis	
Pole Pieces	
Spherical Aberration	This constitutes one of the principal factors limiting the resolution of the electron microscope. The power of the lens is greater for electrons further from the optical axis at which they pass through the lens, resulting in electrons entering the lens further from the optical axis b gly. Conversely, electrons entering the ler are converged less strongly. This

	causes the rays from one object point to not cross the corresponding image point but rather distributed across a surface with a diameter referred to the circle of least confusion. Commonly, spherical aberration values are 2.7 mm (e.g., Titan Krios, Talos Arctica) or 2.0 mm (e.g., Tecnai T12)