

ANISOTROPIC-DIFFUSION FILTER An image processing method for reduction of shot noise without degradation of an image.

AOTF Acousto-optic tunable filters (AOTFs) are birefringent crystals bonded to a piezoelectric transducer. Application of a radio-frequency signal to the transducer generates acoustic waves in the crystal that alter the index of refraction of the crystal and result in diffraction of certain wavelengths of light. A change in the frequency or amplitude of the applied radio signal leads to a change in the wavelength and intensity of the diffracted light. As the radio signal can be altered rapidly, the intensity and wavelength selection are rapidly altered accordingly.

ATOMIC-FORCE MICROSCOPE A type of scanning-probe microscope, in which a fine needle attached to the tip of a soft cantilever scans the surface of a specimen. The shape and physical properties of the surface can be detected from the bending of the cantilever. This type of microscope can be used to manipulate single molecules.

ATTENUATION Blocking or modulation of the excitation light intensity can be accomplished with a series of filters that transmit increasing percentages of the incident light or with an acousto-optic tunable filter (see AOTF).

AUTOFLUORESCENCE The fluorescence from endogenous cell constituents such as NADH, riboflavin and flavin coenzymes, which can contribute to background levels during cell imaging.

CAGED COMPOUNDS Caged fluorophores are fluorophores that have been chemically modified with a caging group that quenches their fluorescence until a brief pulse (usually <100 ms) of ~350-nm light breaks the photolabile bond connecting the fluorophore and the caging group.

CHROMOPHORES Groups with characteristic optical absorptions. They usually contain alternating single and double bonds.

CORRELATIVE MICROSCOPY A combination of methodologies that allows a fluorescent signal that is first seen in living cells to be processed at any given time for immunoelectron microscopy, so that the fine structure and molecular make-up of the carrier that elicited the signal can be revealed.

CRYOEM A technique by which, using a special cryoholder, cryofixed biological samples are directly imaged in the transmission electron microscope under low-dose conditions and at low temperature (at least -170 °C). The sample can be either a frozen layer of suspension that contains many isolated macromolecules for single-particle reconstruction techniques, or a thin (100–500 nm) section.

CRYOFIXATION The rapid freezing of small samples such that cellular components can be immobilized in milliseconds.

FLUORESCENCE CORRELATION SPECTROSCOPY A microspectroscopy technique, in which fluorescence-intensity fluctuations in a small focal volume (~1 μm³) are measured to enable the number, size and movement of fluorescent molecules to be determined at the single-molecule level.

FLUORESCENCE POLARIZATION The absorption of a fluorophore depends on the polarization angle of the excitation light, and fluorescence emission is also polarized. Fluorescence polarization is used to detect the direction and rotation of molecule movement.

FLUORESCENCE QUANTUM YIELD The ratio of photons emitted to photons absorbed. The fluorescence brightness of a species is proportional to the product of its molar extinction coefficient and fluorescence quantum yield. See also the definition for molar extinction coefficient.

FLUORONANOGOLD Fluorescent complexes of small gold clusters that are tagged with an antibody or Fab fragment.

FREEZE SUBSTITUTION A procedure in which, at low temperature, the cellular water of, for example, cryofixed material is replaced by a series of organic solvents, including chemical fixative. As a final step, embedding media are applied (mostly epoxy resins) that, after polymerization, allow further processing of the sample.

GENE GUN Ballistic particle-mediated gene transfer. Complementary DNA molecules are adsorbed to gold particles and shot by a pressure gas jet into tissues or culture cells.

HIGH-PRESSURE FREEZING A cryofixation procedure that uses high pressure to freeze samples efficiently and to minimize the formation of damaging ice crystals.

IMMUNOEM The detection of identified proteins by electron microscopy, which makes use of specific antibodies that are tagged with a marker, usually colloidal gold, for visualization in the electron microscope.

LASER-SCANNING MICROSCOPY A technique for generating an image with a microscope pixel by pixel, which involves sequentially scanning a laser beam through the focal plane of the objective lens and collecting the fluorescence with a single detector.

MAGNETOENCEPHALOGRAPHY An *in vivo* imaging technique that detects tiny magnetic fields generated by electrical current loops, which are typically due to brain activity.

MAGNETOSOMES Magnetic particles that are created within organelles.

MICROSCOPIC MAGNETIC RESONANCE IMAGING A variant of clinical magnetic resonance imaging, which has been adapted for non-invasive studies of small samples that range in size from rats to frog embryos. Typical spatial resolutions are in the range of tens to hundreds of micrometres.

MOLAR EXTINCTION COEFFICIENT The molar extinction coefficient (ϵ) of a species is defined by the equation $A = \epsilon bc$, where A is the absorbance of the solution, b is the path length, and c is the concentration of the species. The fluorescence brightness of a species is proportional to the product of its molar extinction coefficient and fluorescence quantum yield. Highly fluorescent molecules such as rhodamine have high values of both molar extinction coefficient and fluorescence quantum yield. See also the definition for fluorescence quantum yield.

MULTI-PHOTON MICROSCOPY A microscopy technique that uses simultaneous absorption of two or more photons of a long wavelength to excite fluorophores that are normally excited by a single photon of shorter wavelength. The use of the longer excitation light reduces photodamage and allows excitation of fluorophores located deep within thick samples.

NANOPARTICLES Particles with controlled dimensions on the order of nanometres. Examples include colloidal gold, magnetite particles, and luminescent semiconductor aggregates that are also known as 'quantum dots'.

NANOPROBE SCANNING A family of imaging techniques in which the interaction of a sharp, nanometre-sized tip with a specimen is measured as the tip is mechanically scanned over the specimen surface.

OPTICAL COHERENCE TOMOGRAPHY An *in vivo* imaging technique that sends out femtosecond infrared pulses and uses optical interference to sense reflections from tissue inhomogeneities.

OPTICAL TWEEZERS A laser light that is focused by the objective lens of a microscope can be used to trap a plastic bead with a diameter of 0.1–10 μm. The trapped bead can be used as a 'handle' to allow the manipulation of molecules.

PATTERN PHOTBLEACHING Marking geometrical patterns on biological structures that have amorphous shapes, in order to measure structural dynamics.

PHOTOTOXICITY Damage of the living specimen following excessive illumination on fluorescence microscopes. Phototoxicity increases with shorter wavelengths.

POSITRON EMISSION TOMOGRAPHY An *in vivo* imaging technique that detects the location of positron-emitting isotopes by virtue of the pair of γ rays that are emitted when the positrons encounter electrons.

PROJECTION Reduction of dimensionality. For example, a 3D image ($x/y/z$) can be projected into the x/y plane by assigning the maximum intensity that can be found along the z axis at each x/y position to a single projection image. This generates a 2D maximum intensity projection.

SCANNING NEAR-FIELD OPTICAL MICROSCOPY An evanescent field from a small aperture at the tip of an optical fibre is used to excite fluorophores in this type of microscope. The fibre is scanned over the specimen to obtain 2D images. The optical resolution, which is limited to the size of the aperture, is very high (~30 nm).

SEGMENTATION The identification of objects above background noise using image-processing methods. Can be achieved by detecting either object boundaries (contour-orientated segmentation) or whole objects (region-based segmentation).

SINGLE-PAIR FLUORESCENCE RESONANCE ENERGY TRANSFER Fluorescence resonance energy transfer (FRET) that occurs from a single donor fluorophore to a single acceptor molecule is called single-pair FRET.

SINGLE-PHOTON EMISSION COMPUTED TOMOGRAPHY A nuclear-medicine imaging technique that reconstructs the distribution of a γ -emitting radionuclide tracer in a subject in 2D or 3D.

SPECTROSCOPIC OR CHEMICAL-SHIFT IMAGING A variant of magnetic resonance imaging that generates individual nuclear magnetic resonance spectra from a grid of subvolumes in an object. In addition to the more conventional water and lipid magnetic resonance images, analysis of the resulting spectra allows maps of specific metabolites to be reconstructed.

SUPERPARAMAGNETIC MAGNETITE NANOPARTICLES Nanometre-sized particles of magnetite (Fe₃O₄), which locally amplify an external magnetic field, but are too small to maintain their own field in the absence of an external field.

SURFACE RECONSTRUCTION Visualization of surface polygons from arbitrary angles in a graphical viewer. Requires previous object identification using segmentation techniques.

TAPPING MODE A form of atomic-force microscopy in which the tip is vibrated perpendicular to the specimen plane to avoid gouging the specimen as the tip is scanned laterally.

TOKUYASU CRYOSECTIONING TECHNIQUE An immunoelectron-microscopy procedure in which cells are chemically fixed and infiltrated with cryoprotectant before they are frozen by being plunged into liquid nitrogen. Sectioning is carried out at a low temperature, after which the frozen sections are transferred to room temperature, thawed, subjected to immunolabelling and, only then, embedded in a thin layer of support and contrasting film.

TOMOGRAM A 3D image, which is computed from a series of images that are acquired by tilting a 200–500-nm specimen from, for example, -70° to +70° using 1° increments.

TOTAL INTERNAL REFLECTION When a light beam strikes the interface of two media of different refractive indices, at an angle beyond the critical angle, all of the light energy is reflected back into the incident medium. In this situation, an 'evanescent field' develops at the interface with an energy that decreases exponentially into the medium with the lower refractive index. This allows selective excitation of fluorophores located within ~100 nm of the interface.

ULTRASMALL GOLD Gold clusters of 0.8–1.4 nm that are often attached to an antibody or Fab fragment. The clusters are visualized in the electron microscope by using a silver- or gold-based size-enhancement procedure.

VOLUME RENDERING Computer visualization of 3D images from arbitrary angles without explicit definition of surface geometry.

ZOOM The optical zoom lens system assists the objective in image magnification. It determines the size of the scan region and the apparent magnification of the image. Pixels in a zoom-1 image have areas twice the width and length of those in a zoom-2 image. Increasing the zoom magnification leads to increased irradiation time per unit area and therefore to increased photobleaching.