What is an image, and why do researchers love to use images in scientific research? Simply put, an image is a visual representation of some measurable property of an object, person, or phenomenon. Imaging systems create and record images for the eye to view and the mind to experience. Some imaging systems create a visual map of what the eye and brain can already see and perceive. Other imaging systems transduce visual information that is imperceptible to the human eye into visible forms. Part of the challenge in the transduction process is to avoid misrepresenting the essential character of the object or phenomenon. Therefore, it is important to choose an imaging system that will do the job and assist in solving a scientific question, rather than a system that is not appropriate for the application or that simply produces beautiful graphics and serves little practical use.

This article serves as a minireview of brain-imaging methods and applications in an introductory format. It highlights some of the popular types of imaging systems, describes briefly how they work, and illustrates their applications and pitfalls in basic neuroscience research.

Brain atlases and the camera lucida

In the past, brain atlases included drawings or photographs of brain sections, with some regions graphically presented and landmarks identified in great detail. A camera lucida (Latin for “light chamber”) was one technique scientists (or artists) could use to create these highly realistic drawings. The camera consisted of a prism, mounted on an adjustable stand at eye level, through which an object’s image was projected onto a sheet of drawing paper; by peering into the camera, the observer could see the object’s projection on the paper and trace it.

Now, as computerized methods and high-resolution scanners supersede older techniques, these conventional brain maps are being replaced by computer-based brain atlases (Roland and Zilles 1994). One of the advantages of more modern techniques is that computerized brain images can compensate for the shrinkage and distortions sometimes seen during sectioning and embedding of postmortem brains. Another is that modern techniques are fast: Scanning brain sections with a high-resolution scanner takes about 10 hours, which is approximately 50 percent faster than the camera lucida, conventional xy mapping, or photographic procedures (Krout et al. 2002). Moreover, computerized atlases can generate databases of three-dimensional information on the brain.

Keywords: brain, visualization, pitfalls, imaging, neuroscience.
With the advent of computerized brain atlases (e.g., the Cerefy Neuroradiology Atlas), researchers can digitally store information on how the brain varies with age, with gender, and across human populations (Toga and Thompson 2001, Nowinski and Belov 2003) and download this information onto local desktop computers. For instance, Mazziotta (2001), in his review of brain mapping, shows several examples in which disease-specific atlases have been created for studying the natural history of neurological disorders.

**Histology and light microscopy**

Histological techniques are still widely used for studying the brain; paraffin, plastic, and frozen sections are popular options that can undergo selective staining. Other histological techniques include enzyme histochemistry, immunocytochemistry, and autoradiography, in conjunction with light and electron microscopy (box 1). In the past, sections were prepared on microscope slides and evaluated with a microscope; now images can be acquired with digital cameras and stored on computer systems. Furthermore, online virtual medical centers provide a variety of online databases with histological images.

The invention of the light microscope brought a previously unseen world to the human eye (see the historical review by Lantos [1983]). Since light microscopy is one of the oldest scientific imaging techniques, it will not be discussed in great detail here. The operation of modern microscopes, although conceptually similar, often requires training to understand not only the physics of the optics but also the associated systems that integrate the hardware with the imaging software. The spatial resolution of traditional microscopy is limited by the wavelength-dependent diffraction of the illuminating light; in theory, visible light resolution is about 0.3 micrometers (μm), but in practice the resolution is somewhat lower. In the optical microscope, image formation occurs at the intermediate image plane, through interference between direct light that has passed through the specimen and light diffracted by structures present in the specimen. The image produced by the objective (i.e., the lens or lens system nearest the object) is such that each image point at the intermediate plane is geometrically related to a corresponding point in the specimen.

**Phase contrast microscopy**

Biological tissues in general, and living cells in particular, are transparent to visible light, thus making traditional bright field and reflection-based techniques (e.g., reflected light microscopy used for opaque specimens) unusable. However, these techniques do slightly alter the phase of the light diffracted by the specimen. This phenomenon was first studied in the 1930s by Frits Zernike, who devised a method that uses destructive or constructive light interference to enhance the contrast between the imaged specimen and the background. Phase contrast microscopy continues to be a widely used and important tool, particularly for the study of cells and tissues in culture. However, it has some disadvantages, such as halo artifacts (halos around the boundaries of images) and image distortion in thick specimens, because of phase shifts occurring in planes other than the one in focus. Also, the system components introduce limitations in the working numerical aperture (a measure of the light-gathering ability of the microscope), causing a reduction in spatial resolution.

**Differential interference contrast**

The basic differential interference contrast (DIC) system, first devised in 1955, is a modified polarized light microscope. Because the optical components required for DIC microscopy do not obstruct the objective and condenser apertures, the instrument can be employed at full numerical aperture. The results are the capacity to use thick specimens, the elimination of halo artifacts, and a dramatic improvement in resolution. To enhance their quality, images can be manipulated using digital and video imaging techniques that further modify contrast. Typical DIC applications include visualization of living cell cultures, blood cells, subcellular organelles, chromosomes, embryos, and either relatively thick or ultrathin microtome sections (Friedl 2004).

**Comparison of phase contrast and differential interference contrast microscopy**

Both phase contrast microscopy and DIC (table 1) rely on specimen phase differences between sampling and reference beams to produce an image. The two techniques are complementary and are capable of producing high-contrast images of transparent biological specimens that do not affect the amplitude of visible light waves passing through them.

A primary advantage of DIC over phase contrast is that its system architecture makes it possible to utilize the instrument at full numerical aperture, resulting in improved axial resolution. Also, the halo artifact present in phase contrast microscopy is largely absent in DIC images. Another significant advantage of DIC is its capacity for creating images of very small features and of much larger ones at the same time. In phase contrast microscopy, halo shadows center around very fine structures, a problem not presented by DIC. Halo artifacts and image contrast are also affected by the optical thickness of the specimen to a much larger degree in phase contrast than in DIC microscopy.

**Table 1. Comparison of phase contrast and differential interference contrast microscopy.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Phase contrast</th>
<th>Differential interference contrast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Image brightness</td>
<td>1.3</td>
<td>0.4–2.3</td>
</tr>
<tr>
<td>Lateral resolution</td>
<td>Restricted</td>
<td>Superior</td>
</tr>
<tr>
<td>Axial resolution</td>
<td>Poor</td>
<td>Superior</td>
</tr>
<tr>
<td>Illuminating aperture</td>
<td>10% of numerical aperture</td>
<td>Variable</td>
</tr>
<tr>
<td>Halos</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Birefringent specimens</td>
<td>Useful</td>
<td>Not useful</td>
</tr>
<tr>
<td>Birefringent specimen containers</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
Box 1. Imaging techniques used in neuroscience research.

 Autoradiography
In autoradiography, X-ray films are used to visualize molecules or fragments of molecules that have been radioactively labeled. This method can be used to locate tracers within or between cells, or to determine their concentration in tissues. A possible application is the analysis of the length and number of DNA fragments extracted from cells. Autoradiography has a wide range of spatial resolutions, from 0.05 micrometers (µm) to 50 µm.

 Computed tomography
Several generations of computed tomography (CT) scanners have been engineered, and each has its own design for how and when X-ray beams are pulsed (although many systems generate beams continuously). Some designs incorporate a fixed ring of detectors in which the X-ray tube rotates around the patient. In addition, CT uses computers and collimators for image acquisition. Numerous X-ray transmissions are necessary for the construction of a CT image, which is largely dependent on the computer for image processing.

 Electron microscopy
By replacing regular light with electron beams, electron microscopy extends the range of the normal microscope to the near-atomic level. The main advantages are greater magnification and resolution. However, the technique cannot be applied to living cells or tissues.

 Fluoroscopy
Fluoroscopy is performed with a system that uses X-ray equipment, placing the appropriate image receptor into position with the X-ray beam. Using a lowered radiation dose (compared with regular X-ray imaging), it is possible to view continuous movements rather than still images.

 Histochemistry
By combining biochemistry and histology techniques, it is possible to study the chemical constitution of cells and tissues. Applications include enzyme histochemistry (enzyme localization) and immunohistochemistry (localization of antigens through the use of specific antibodies).

 Magnetic resonance imaging
Magnetic resonance imaging (MRI) is a method used for both anatomical and physiological diagnosis or study. It does not interact with biological tissue and is considered noninvasive. MRI equipment includes a large magnet capable of generating a strong magnetic field, a coil (the radio frequency [RF] transceiver) that surrounds the sample, and electronic and computer hardware. Based on magnetic fields, radio waves, and nuclear resonance, MRI detects the local concentrations of hydrogen nuclei in cellular water and lipid molecules. When a patient is placed inside an RF coil, which is then positioned inside a magnet, most of the protons of hydrogen nuclei in the tissue will tend to align parallel along the magnetic field that the magnet creates (like a compass positioned near a magnet). When an electromagnetic RF pulse is applied at the resonance frequency, protons are flipped in space and thus absorb energy. Once the RF pulse is turned off, the absorbed RF energy is emitted at the resonance frequency and is detected as the nuclear magnetic resonance (NMR) signal. The NMR signal, which relays information about spatial coordinates and proton density, is eventually processed into an image. This is a simplistic description of MRI; a more thorough explanation requires a quantum mechanical view, which will not be presented here.

 Positron emission tomography
Positron emission tomography (PET) scanning incorporates a series of radiation detectors arranged in a ring around the subject. These detectors measure high-energy collisions that result in annihilations between positrons and electrons. When these annihilations occur, the masses of both particles are converted into electromagnetic energy (in the form of photons). The photons leave the site of the collision (in the subject) and travel in opposite directions. The detectors then measure and pinpoint the quantity and location of the emissions. As in CT engineering, a number of PET systems have been designed that arrange detectors in different configurations. For PET to function, positron-emitting radionuclides need to be generated with a cyclotron. PET nuclides include carbon (11C), nitrogen (15N), oxygen (15O), and fluorine (18F), to name a few. Fluorodeoxyglucose, or FDG, a glucose analog labeled with 18F, can cross the blood–brain barrier and is useful for mapping glucose uptake.

 Ultrasound imaging
Ultrasound is based on the ability to detect differences in acoustic properties in biological tissue. Ultrasound imaging involves a pulse generator and transducer that produce short pulses of high-frequency (1 to 15 megahertz) electrical oscillations. A transducer (pressed against the subject’s body) transforms the electronic signal into pulses of mechanical vibrations. Pulses of ultrasound energy or vibrations then propagate thorough the tissue and are subject to reflection, refraction, or attenuation, depending on the tissue type or interface encountered. When the ultrasound energy comes in contact with a distinct boundary, the energy is reflected, producing an echo. A transducer then picks up the returning echoes and converts them into images. Strong reflections and echoes give rise to bright spots on the display monitor. Ultrasound is especially effective in the study of soft tissue and is superior to X-ray imaging in this regard.

 X-ray imaging
X-ray images are produced by passing uniform X-ray beams past a sample or body part and capturing the X-ray shadow on film. The sample or tissue interacts with the beam differentially, so that the more beam energy is absorbed by the tissue, the less energy is captured by the film. The uniform beam is created by a high-voltage source and an X-ray tube. Negatively charged electrons “boil off” from a hot cathode in the X-ray tube and are directed toward an anode. As the electrons interact with the anode, two forms of radiation, known respectively as bremsstrahlung (from the German word for braking radiation) and characteristic X rays, are produced from the kinetic energy of the electrons as they collide. The subject’s exposure to radiation can be controlled by amount of current and the duration of the beam through the tube.
An advantage of phase contrast over DIC, on the other hand, is its ability to create images of birefringent specimens. DIC systems rely on polarized light, resulting in image distortion when applied to birefringent specimens or materials, whereas phase contrast uses nonpolarized light and is free of optical disturbances. Tissue cultures are often prepared with polymers that present birefringence, thus making phase contrast the favored imaging system. In general, phase contrast and DIC microscopy should be considered complementary techniques. Only by taking full advantage of both techniques is it possible to completely investigate specimen properties, dynamics, and morphology.

**Fluorescence microscopy**

The basis for fluorescence microscopy is the property of some atoms and molecules to absorb light at a particular wavelength and subsequently to emit light at longer wavelength. In practice, this is achieved by illuminating the object with monochromatic light at the wavelength that excites fluorescence emission in the fluorophores (fluorescent molecules) of the sample (figure 1). The object is viewed using filters that exclude the illuminating wavelength and select the wavelength of the light emitted by the fluorophores. Each fluorophore behaves as a separate light source, thus avoiding imaging limitations caused by diffraction. Using several different fluorophores with different optical properties, it is possible to create images of living cells in which different organelles appear in different colors. Characteristic problems of fluorescence microscopy include photobleaching (permanent loss of fluorescence due to photon-induced chemical damage and covalent modification) and photodamage (tissue damage induced by excessive absorbed energy), which are critical factors when imaging living cells.

**Confocal microscopy**

A conventional fluorescence microscope bathes the entire specimen in high-intensity light from a mercury or xenon source (figure 2). This results in a significant amount of signal caused by autofluorescence and by emitted background light originating from areas above and below the focal plane. Secondary fluorescence emitted by the specimen often occurs through the excited volume of tissue and obscures the resolution of features that lie in the objective focal plane. The problem is relevant in thicker specimens (> 2 μm), in which most of the fine details are lost because of the high degree of noise.
Confocal microscopy, by contrast, exploits a different method of illumination to form an image, scanning one or more focused beams of light across the specimen. The point of illumination in the specimen is brought into focus by the objective lens and laterally scanned with a device under computer control. The light from the specimen is detected by a photomultiplier tube through a pinhole, which eliminates out-of-focus light by acting as a spatial filter, and the output is displayed by the computer. Confocal spot size ranges from approximately 0.25 to 0.80 µm in diameter and 0.5 to 1.5 µm deep, depending on the microscope design, laser wavelength, objective characteristics, and specimen.

A major feature of confocal microscopy is its ability to optically section a specimen, allowing the collection of thin (0.5 to 1.5 µm) optical sections from thick specimens (up to 50 µm). Thanks to spatial filtering, background noise is significantly reduced and the resolution along the z-axis greatly increased in comparison with wide-field fluorescence. Data can be collected from stained specimens using single-, double-, triple-, or multiple-wavelength modes of illumination, and the images collected with the various illumination and labeling strategies will be in register with each other. Live cell imaging and time-lapse sequences are possible, and digital image processing methods applied to sequences of images allow not only three-dimensional representation of specimens but four-dimensional imaging (through the time-sequence presentation of three-dimensional data; Emptage 2001). The effects of photobleaching and photodamage can be further reduced by combining this technique with non-destructive ones, such as DIC and phase contrast, which can be used to locate the specific area of interest in a specimen before proceeding with the fluorescence analysis.

**Multiphoton fluorescence microscopy**

Multiphoton fluorescence microscopy (MFM) combines laser scanning microscopy with long-wavelength multiphoton fluorescence excitation to capture high-resolution, three-dimensional images of stained specimens. The technique is based on the capability of fluorophores to be excited not only by photons at a wavelength corresponding to the absorption band for fluorescence but also by those at twice the wavelength. The latter absorption only produces fluorescence if two photons are absorbed within a few femtoseconds of each other (1 femtosecond = one quadrillionth [10^{-15}] second); this is accomplished with high-power pulsed lasers, which generate a significant amount of power during pulse peaks but have an average power that is low enough not to damage the specimen. Fluorescence excitation will occur only following two-photon excitation in a small volume at the focal point of the microscope, resulting in reduced photobleaching and photodamage (Piston 1999, Kawano et al. 2003).
The lack of absorption from out-of-focus fluorophores allows light to penetrate deeper into the specimen, permitting the imaging of samples up to three times thicker than those used in confocal microscopy. MFM permits scanning in the xy plane and through the depth of the specimen, allowing the three-dimensional imaging of living cells and tissues (Tauer 2002). However, confocal microscopy has a slightly higher spatial resolution, because it uses shorter wavelengths, resulting in lower diffraction. In MFM, the low background noise and the absence of pinholes make the efficiency of the process much higher in comparison with both wide-field and confocal microscopy.

**Time-lapse microscopy**

Most of the current microscopy techniques can be used in real time, creating high-resolution, time-lapse images of living cells. Through the use of these techniques, a variety of molecular mechanisms are now being uncovered. For example, with regard to time-lapse imaging of dendritic spines, mechanisms involving changes in intracellular calcium concentration are being unraveled that may control both the formation or elongation and the pruning or retraction of spines (Segal 2002). In another example, time-lapse imaging has helped researchers view the nuclear envelope, a highly dynamic structure that reversibly disassembles and re-forms at mitosis and breaks down during apoptosis (Buendia et al. 2001). Time-lapse imaging has also been used in conjunction with green fluorescence protein, which can be introduced into intracellular structures and proteins, making them fluorescent and permitting visualization of them in living cells.

**X-ray imaging**

X-ray imaging, which was initially developed by Röntgen in 1895, is the oldest in vivo imaging technique. X rays are short-wavelength electromagnetic waves with high energy (5 to 150 kiloelectron volts) that differentially penetrate solid objects and tissues according to their absorbance, which is proportional to their density. To produce an X-ray image, radiation passes through the subject, resulting in an image on photographic film that displays the variation in the subject’s density. Radiation intensity is defined as dose rate (intensity per unit of time) and the X-ray quanta per exposure as dose. High-quality images depend on an adequate dose rate. If the intensity of the X rays is too low, the target will not be penetrated adequately, and the image will be underexposed; if the dose is too high, the image will be overexposed. Contrast is very good between hard and soft tissue, and it may be further increased by introducing strong X-ray absorbers, making it possible to view otherwise invisible structures. Some X-ray systems are more sophisticated than others at measuring the dose, which ultimately has an effect on the sensitivity of dose rate adjustments. Spatial resolution is high in the X-ray system, on the order of less than 0.1 millimeter (mm), but is limited by the detector resolution and the focal spot size of the X-ray beam. However, one major pitfall is the obvious loss of depth information.

**Computed tomography**

Computed tomography (CT) also uses X rays to visualize body structures (box 1). Computed tomographic images are reconstructed from a large number of X-ray transmission measurements throughout the target. Beams are passed through the region of interest from several different angles to create cross-sectional absorbance maps, which can be assembled by a computer into a three-dimensional picture. Different designs exist for the CT machinery, with progressively shorter acquisition times and better resolution. The latest generations of CT scanners can reconstruct acquired images in less than 1 second, and some designs can acquire tomographic sections in less than 50 milliseconds, making them usable in real-time imaging. Spatial resolution is also very high, with scanned volume elements (voxels) in the range of 0.4 x 1.0 x 2.0 mm or better.

**Magnetic resonance imaging and magnetic resonance spectroscopy**

Magnetic resonance imaging (MRI) exploits the magnetic properties of nuclear magnetic resonance (NMR) of nuclei with an odd number of protons or neutrons, such as hydrogen (1H), carbon (13C), phosphorus (31P), sodium (23Na), and fluorine (19F). Each of these atoms absorbs radio waves at different frequencies and emits a unique, identifiable radio frequency (RF) signal that can be recorded. The MRI signal has been well studied and is regulated by parameters dependent on the biophysical properties of the sample under examination.

MRI techniques are experiencing a powerful surge of activity throughout the scientific community, where they are developing at an extremely fast rate. MRI has the significant advantage of being noninvasive, and can provide information about anatomical abnormalities in living beings (figure 3), whereas histological and microscopy techniques are limited to tissue sections. However, there are limitations that should be considered when using this technology, such as motion artifacts and samples with low contrast caused by pathological states (Moritani et al. 2000). There are also huge gaps in the basic understanding of the method (box 1) and its use with some of the newer fast imaging techniques. The spatial resolution of MRI is comparable to that of CT, but the latter usually allows faster acquisitions. MRI scans can be repeated as many times as needed, whereas the radiation associated with X rays limits the number of CT scans that can be performed safely on a human subject.

The rapidly evolving functional magnetic resonance imaging (fMRI) technology, which relies on the different magnetic properties of oxygenated and deoxygenated blood, permits the visualization of physiological processes. Methods of fMRI, such as BOLD (blood oxygen level–dependent) and perfusion MRI, are used for determining neuronal activity, tissue oxygenation levels in various brain regions (an indirect detection), and relative changes in cerebral blood flow (Matthews and Jezzard 2004). In the diffusion-weighted imaging (DWI) modality, in which the change in the measured RF signal...
makes it possible to visualize the diffusion of protons, MRI is quite a sensitive technique for detecting early changes in edema formation and for identifying areas of diffuse injury (Albensi et al. 2000). A further improvement, which does not suffer from DWI’s sensitivity to motion artifacts, is diffusion tensor imaging (DTI). Both techniques rapidly dephase and rephase protons with a transverse magnetic field and measure the variations in the RF signal, which are caused by moving nuclei. The main advantage of DTI over DWI is that it can give information on the direction as well as the magnitude of flow (Gillard et al. 2001, Urresta et al. 2003, Guadagno et al. 2003, Rohde et al. 2004).

The principles of NMR have also been applied to study the properties of individual chemical species in humans and other animals. Instead of focusing on single nuclei, magnetic resonance spectroscopy (MRS) modulates the spatial encoding of the RF signal to study the entire emitted spectrum of a selected finite volume. The local field influencing the protons is determined by their chemical environment, and the spectra provide specific information on the chemical species bonding the protons, along with their changes in concentration on distribution. The resulting data can be used to study hydrogen, carbon, phosphorus, and fluorine nuclei, and their correlation with metabolic processes (Jones and Waldman 2004, Sinha et al. 2004).

**Positron emission tomography**

Positron emission tomography (PET) is a method for measuring concentrations of positron-emitting radioisotopes (tracer compounds) using an elaborate array of detectors (box 1). Tracer compounds typically contain carbon (¹¹C), nitrogen (¹⁵N), oxygen (¹⁵O), or fluorine (¹⁸F). After administering these compounds, it is possible to visualize the location of positron activity within an organism. Historically, positron emission was located using a large ring of detectors; however, new designs have been developed over the last 10 years to create smaller, less expensive, and higher-resolution systems for animal studies, such as microPET. The microPET designs rely on miniaturized components and allow spatial resolution of approximately 1 mm, compared with approximately 3 mm for larger, human-oriented systems (Roselt et al. 2004, Yang et al. 2004).

Applications of PET include radio labeling of drugs to trace their uptake in specific body regions. PET also has the potential to locate molecules of drugs or other chemicals directly in the brain (Mathis et al. 2004, Sadowski et al. 2004, Shimoji et al. 2004). A major limit to the diffusion of PET as a research tool is the short half-life of positron-emitting compounds, which must be produced close to the facility where they will be used. To overcome the limited spatial resolution, newer designs often integrate PET with MRI or CT.
to provide simultaneous imaging of fine anatomical details and functional data (Bockisch et al. 2004, Kapoor et al. 2004).

**Ultrasound and transcranial doppler**

Ultrasound imaging is a common technique (box 1) that uses high-frequency sound waves to visualize objects. When an ultrasound wave interacts with a surface, the wave rebounds and an “echo” is recorded. A modification of ultrasound imaging is transcranial doppler, a technique for measuring relative changes in cerebral blood flow. The technique is now well established with different disease processes, including ischemic cerebrovascular disease, subarachnoid hemorrhage, and cerebral circulatory arrest (Alexandrov and Joseph 2000). Ultrasound pulses, at approximately 2 megahertz (MHz), are aimed toward the sample using a handheld transducer. The frequency shift in the reflected sound indicates the velocity of sound in the sample or subject. Velocities from the cerebral, internal carotid, basilar, and vertebral arteries can be sampled by altering the transducer location, angle, and depth setting. The spatial resolution of ultrasound depends primarily on the frequency used; at 5 MHz, for example, axial resolution is 0.47 mm, while lateral resolution is about 1 to 5 mm, depending on the depth of the sample. By changing ultrasound frequency and using focused acoustic lenses, it is possible to increase both axial and lateral resolution, but with a loss of penetration (usually 10 to 20 centimeters, depending on frequency and system design).

**Fluoroscopy**

A fluoroscopy system is fundamentally a video X-ray machine, capable of taking continuous pictures of an object using only a small X-ray dose (box 1). Instead of acquiring the image on a film, fluoroscopy relies on X-ray detector systems capable of producing real-time images, which are then displayed on a monitor. However, because these systems require lower X-ray doses (for safety reasons) and faster detectors, the spatial resolution of fluoroscopic images (0.13 mm) is lower than that of static X-ray images (0.08 mm). Fluoroscopy is commonly used in angiography for studying blood vessels in the brain or guiding catheters (Lipsitz et al. 2003).

**Optical imaging of intrinsic signal**

It has been known for several decades that intrinsic changes in the optical properties of tissue are dependent on electrical and metabolic activity (Hill and Keynes 1949, Grinvald et al. 1982). The first optical recording of neuronal activity was by Hill and Keynes (1949), who detected light-scattering changes in nerves that were firing. However, most intrinsic optical signals are barely detectable, and it has only recently become possible to use optical detection of intrinsic signals for imaging the brain after adding a voltage-sensitive dye or fluorescent marker to the tissue. The use of voltage-sensitive dyes permits the visualization of brain activity with a spatial resolution of 50 to 100 microns. The intrinsic signal can then be detected by a normal microscope and, when appropriately evaluated with imaging software, can give a bidimensional image of brain structures in which color intensity is associated with and proportional to the intensity of firing of nerve cells (Hochman et al. 1995, 1999). The functional correlates of the intrinsic signal seem to implicate ionic shifts across membranes: in particular, changes in potassium (K⁺), chloride (Cl⁻), and sodium (Na⁺) concentrations (Schwartzkroin et al. 1998, Hochman et al. 1999). Given that large changes in extracellular K⁺, Cl⁻, and Na⁺ occur during intense neuronal activity (Janigro et al. 1997, D’Ambrosio et al. 1998), it seems that changes underlying the intrinsic signal may, to some extent, also be implicated in the generation of fMRI signals. In fact, the accumulation of Na⁺ in cells and the leakage of K⁺ into extracellular space activate energy-depleting mechanisms that cause hydrolysis of ATP (adenosine triphosphate) to adenosine and may activate cerebral blood flow. Ionic changes in the extracellular space may also cause large field potentials that affect cerebral blood flow through the K⁺-mediated mechanisms described above (Dombrowski et al. 2001). What seems to be most important in the generation of these intrinsic signals is the size of the extracellular space, which in turn is proportional to the osmotic content of intracellular versus extracellular compartments (Schwartzkroin et al. 1998, Hochman et al. 1999). A change in osmolarity in each compartment occurs at each action potential, and the cumulative effect of these changes may be large enough to cause visible changes in reflected light or in fMRI signals.

Another observation that may contribute to a unifying theory of brain imaging is that most of the excitation in the brain is achieved through the release of glutamate by presynaptic cells and the binding of glutamate to postsynaptic receptors and glial cells. Glutamate, unlike other neurotransmitters, is not readily metabolized in the extracellular space, and the cessation of glutamatergic signals occurs through the uptake of glutamate into glia (Bergles and Jahr 1998). This uptake depends on Na⁺ gradients and therefore affects the ratio between osmotic content in the cell and in the extracellular space. Maintenance of Na⁺ gradients is difficult, and ATP consumption accompanies each cycle of the Na⁺–K⁺–ATPase pump. Recently, it has been suggested that blood oxygen level–dependent fMRI responses in the individual cortexes of monkeys depend primarily on the strength of synaptic transmission rather than on the cellular output, further supporting the hypothesis that changes in glutamate uptake, which are proportional to Na⁺ gradients, energy status, and K⁺ concentrations, may ultimately be responsible for imaging signals.

**Imaging invertebrates**

In general, there is nothing unique about imaging invertebrate as opposed to vertebrate tissue, apart from the fact that most invertebrate organisms are smaller than vertebrates. Therefore, many imaging techniques can be directly transferred to invertebrate systems. For example, in the leech, investigators have used voltage-sensitive dyes to visually identify neurons that might be involved in the neural network that generates swimming behavior (Taylor et al. 2003). More sophisticated...
systems, such as MRI, have also been used for imaging invertebrate systems. For instance, contrast-enhanced magnetic resonance histology was used in the squid to identify individual cell bodies, and the results were compared with those of conventional histology. The receptor cells of the squid’s proprioceptive neck receptor organ were labeled with paramagnetic cobalt (II) ions by conventional cobalt iontophoresis. Stimulated echo images were then obtained using a 9.4-Tesla magnet and followed by conventional histological treatment and light microscopy (Gozansky et al. 2003). In a more unusual example, MRI was recently used on a moldic fossil dicynodont (a small therapsid reptile) from the Permian sandstone formation of Elgin, Scotland (Clark et al. 2004). The use of MRI is highly advantageous for paleontologists, since it provides a nondestructive technique for examining fossils preserved over geologic time.

**Three-dimensional imaging techniques: Advantages and disadvantages**

Although all of these imaging techniques can be useful in both clinical and basic research, it is important to stress that the two applications differ greatly in their requirements. For example, in the clinical context, a common question is whether CT is better than MRI for imaging the brains of stroke or trauma patients. The answer may depend on the institution and on other factors, such as the practicality or availability of a particular machine. CT is quicker and easier to operate, but it presents radiation exposure issues; MRI, although more complicated, usually gives better image quality and more interesting data, especially with fMRI techniques. When dealing with animal subjects, the governing factors are different: Cost-effectiveness, accessible instrumentation, and the amount of potential obtainable information are relevant, while subject comfort and safety are less so. Another limiting factor in animal research is the size of the system, which is usually designed to work on humans; animals that are either too big or too small require different designs, such as the micro-CT. Micro-CT systems allow spatial resolutions as high as 5 µm, with a maximum scanning area equivalent to an A4 paper sheet, and can be used easily to visualize fine anatomical features in small animals (Ritman 2002, Batiste et al. 2004, De Clerck et al. 2004).

Another common question is whether fMRI is more or less efficient than PET in obtaining functional data. Nuclear medicine techniques such as PET and single-photon emission computer tomography, or SPECT, are sensitive enough to detect nanomolar concentrations, whereas MRI methods typically detect concentrations in the millimolar range. However, MRI is still more cost-effective, and it does not require the expensive tracers used in PET imaging, nor does it raise safety issues related to the use of radioactive compounds (MRI does have its own safety issues, related to the strong magnetism generated and the proximity of magnetically coded materials such as credit cards.) When dealing with tiny animals, spatial resolution becomes very important, since the region of interest becomes critically small; therefore, dedicated machinery, such as micro-PET, becomes extremely desirable to obtain significant data. A comparison of the in vivo imaging techniques, including data on their spatial resolution, physical principles, and general applications, is presented in table 2.

**Conclusions and future trends**

It is often difficult to decide when one technique is better than another for solving a scientific question, and none of the methods presented here is intrinsically weaker than the others. An fMRI image can sometimes have the same informative content as an expensive PET image, but the increasing availability of PET can lead scientists to use it despite its disadvantages (lower spatial resolution, exposure to

<table>
<thead>
<tr>
<th>Imaging technique</th>
<th>Imaged signal</th>
<th>Imaged characteristic</th>
<th>Spatial resolution (millimeters)</th>
<th>Acquisition time</th>
<th>Real-time data</th>
<th>Three-dimensional imaging</th>
<th>Functional data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Computed tomography</td>
<td>X-ray transmission</td>
<td>Tissue density</td>
<td>0.4 (x, y), 1.0–2.0 (z)</td>
<td>20 seconds (whole organ), 50 milliseconds (section)</td>
<td>Yes (some designs)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Fluoroscopy</td>
<td>X-ray transmission</td>
<td>Tissue density</td>
<td>0.1 (planar)</td>
<td>33 microseconds per frame</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>MRI</td>
<td>Emitted radio frequency</td>
<td>Proton density, relaxation time, flow rates</td>
<td>0.1–1.0 (x, y), 1.0–5.0 (z)</td>
<td>Minutes (whole organ), 50 milliseconds (section)</td>
<td>Yes (some modalities)</td>
<td>Yes</td>
<td>Yes (with fMRI)</td>
</tr>
<tr>
<td>PET, SPECT</td>
<td>Emitted gamma rays</td>
<td>Tracer uptake</td>
<td>3.0–7.0</td>
<td>500 milliseconds</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>Reflected ultrasound</td>
<td>Tissue elasticity mismatch</td>
<td>0.3–0.5 (depth), 1.0–5.0 (lateral)</td>
<td>Real-time (instantaneous)</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>X-ray imaging</td>
<td>X-ray transmission</td>
<td>Tissue density</td>
<td>&lt; 0.1 (planar)</td>
<td>Not applicable</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

fMRI, functional magnetic resonance imaging; MRI, magnetic resonance imaging; PET, positron emission tomography; SPECT, single-photon emission computer tomography.
radioactive tracers). Similarly, when dealing with soft tissues, CT scans can produce more attractive (but less informative) images than ultrasound, encouraging scientists to use CT for the wrong applications. It is important to use the right technique for the right application. We recommend these guiding principles for choosing an imaging method:

- Identify the object of the analysis (e.g., anatomical versus functional studies).
- Select a subset of imaging techniques capable of producing the desired type of image.
- Within the chosen group of techniques, evaluate the risk–benefit ratio and cost-effectiveness of each technique.
- Design an experiment that takes full advantage of the selected imaging modality (or modalities), avoiding pitfalls whenever possible.

With the rapid evolution of technology, scientific investigators will be constantly challenged with maintaining and broadening their understanding of instrumentation, computer software, and practical scientific applications for visualizing brain tissue. For example, the use of tracers associated with protein transcription allows the visualization of gene expression by whole-body imaging with fMRI or PET, promising a transformation in basic research on apoptosis, toxicology, and gene therapy (Groot-Wassink et al. 2004, Poгре and Slikker 2004, Wu et al. 2004). The race to develop different imaging techniques will lead to faster, smaller, less expensive, and more efficient instrumentation, and new applications will undoubtedly emerge. Although microscopy will remain fundamental for imaging, the major advances will be in imaging techniques, as MRI modalities such as DTI and fMRI compete with CT and PET to become less invasive and to provide higher resolution and greater amounts of functional data.

A picture is worth a thousand words. By acquiring images that provide information on molecular events, gene expression, biochemical subsystems, anatomical modifications, and physiological perturbations, researchers are altering our perceptions of anatomy, disease mechanisms, and drug interventions far beyond what Wilhelm Röntgen, the creator of the first X ray, may have ever imagined.

References cited


excitation pulses. Biochemical and Biophysical Research Communications 311: 592–596.


