Molecular Imaging Techniques

Electron Microscopy

TEM – Transmission Electron Microscopy

(Cryo EM)

SEM – Scanning Electron Microscopy

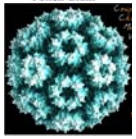
STM / AFM – Atomic Force Microspopy

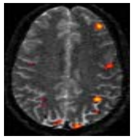
Medical Imaging Methods

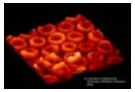
(CAT / PET / MRI / Ultrasound)

X-Ray Crystallography

NMR Spectroscopy



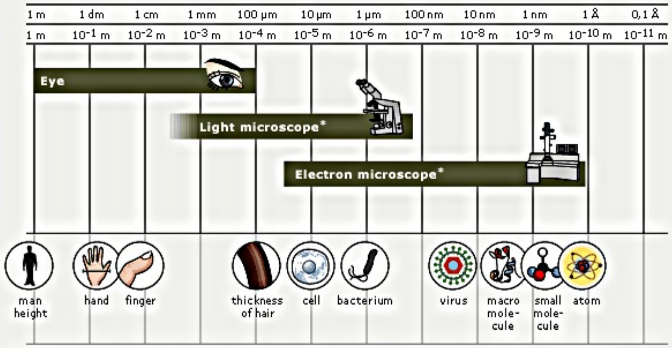




MICROSCOPES BACK

Resolving Power Line

What can you see with the different types of microscopes? The human eye is capable of distinguishing objects down to a fraction of a millimeter. With the use of light and electron microscopes it is possible to see down to an angstrom and study everything from different cells and bacteria to single molecules or even atoms.



* Light microscope includes phase contrast and fluorescence microscopes. Electron microscope includes transmisson electron microscope.

MICROSCOPES BACK

Time Line

14th century – The art of grinding lenses is developed in Italy and spectacles are made to improve eyesight.

1590 – Dutch lens grinders Hans and Zacharias Janssen make the first microscope by placing two lenses in a tube.

1667 – Robert Hooke studies various object with his microscope and publishes his results in Micrographia. Among his work were a description of cork and its ability to float in water.

1675 – Anton van Leeuwenhoek uses a simple microscope with only one lens to look at blood, insects and many other objects. He was first to describe cells and bacteria, seen through his very small microscopes with, for his time, extremely good lenses.

18th century – Several technical innovations make microscopes better and easier to handle, which leads to microscopy becoming more and more popular among scientists. An important discovery is that lenses combining two types of glass could reduce the chromatic effect, with its disturbing halos resulting from differences in refraction of light.

1830 – Joseph Jackson Lister reduces the problem with spherical aberration by showing that several weak lenses used together at certain distances gave good magnification without blurring the image.

* **1878** – Ernst Abbe formulates a mathematical theory correlating resolution to the wavelength of light. Abbes formula make calculations of maximum resolution in microscopes possible.

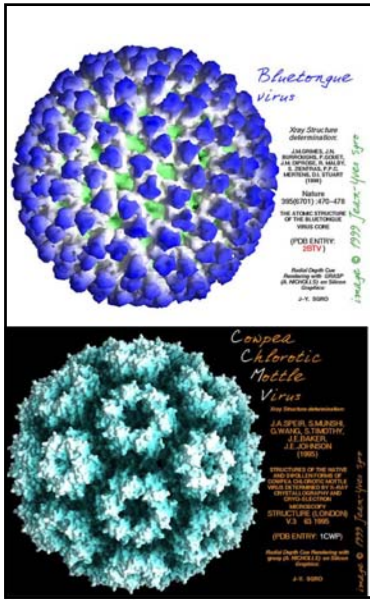
$$d = \frac{0.612\,\lambda}{n \sin \alpha} \sim \frac{\lambda}{2}$$

1903 – Richard Zsigmondy develops the ultramicroscope and is able to study objects below the wavelength of light.
The Nobel Prize in Chemistry 1925 »

1932 – Frits Zernike invents the phase-contrast microscope that allows the study of colorless and transparent biological materials.
The Nobel Prize in Physics 1953 »

* **1938** – Ernst Ruska develops the electron microscope. The ability to use electrons in microscopy greatly improves the resolution and greatly expands the borders of exploration.
The Nobel Prize in Physics 1986 »

* **1981** – Gerd Binnig and Heinrich Rohrer invent the scanning tunneling microscope that gives three-dimensional images of objects down to the atomic level.
The Nobel Prize in Physics 1986 »



Herpes Simplex Virus-1 A-capsid from 400kV Spot-scan Electron Cryomicroscopy

Z. H. Zhou, B.V.V Prasad, J. Jakana, F.R. Rixon, W. Chiu
Baylor College of Medicine, Journal of Molecular Biology

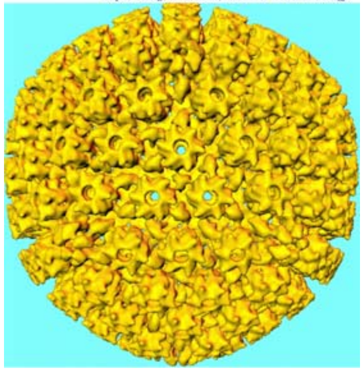
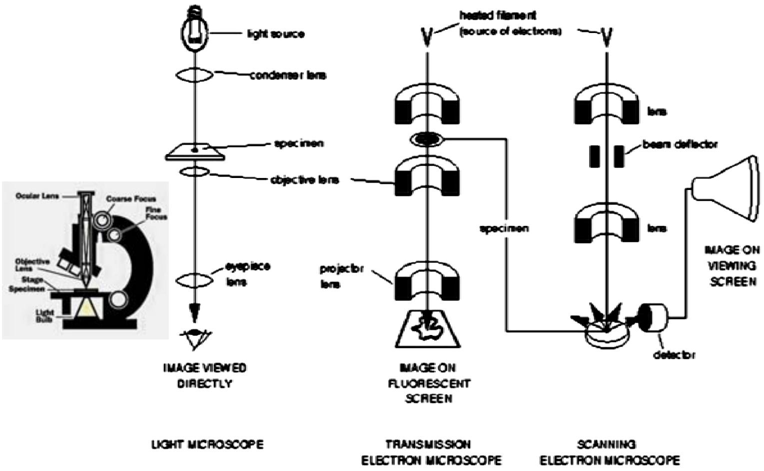
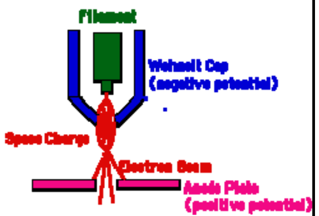


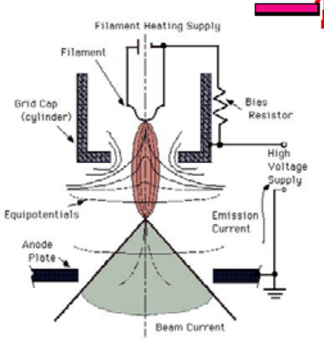
Image Formation



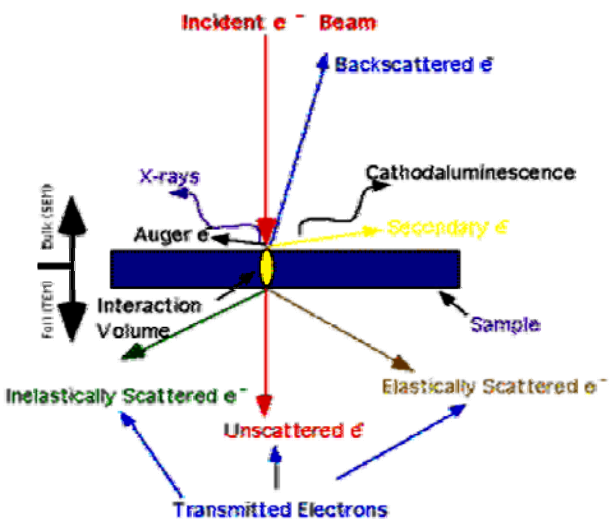


The Electron Source

The electron beam comes from a filament, made of various types of materials. The most common is the Tungsten hairpin gun. This filament is a loop of tungsten which functions as the cathode. A voltage is applied to the loop, causing it to heat up. The anode, which is positive with respect to the filament, forms powerful attractive forces for electrons. This causes electrons to accelerate toward the anode. Some accelerate right by the anode and on down the column, to the sample. Other examples of filaments are Lanthanum Hexaboride filaments and field emission guns.

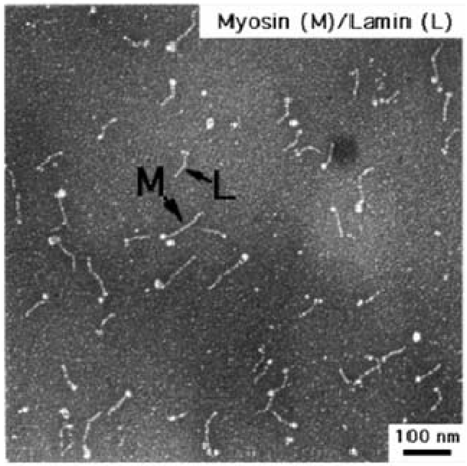


http://mse.iastate.edu/microscopy/source.html



TEM – Transmission Electron Microscope

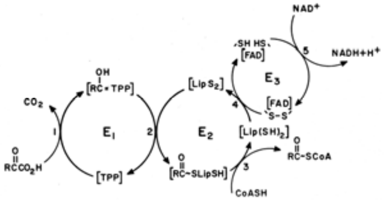
JEOL JEM-2010: 200kV high-resolution TEM with interchangeable polepieces, where one can change from an analytical version (resolution = 0.23nm, +/- 30 degrees tilt) to a high-resolution version (0.19nm, +/- 10 degrees tilt). Double-tilt and heating specimen holders are available on this TEM. http://www.tamu.edu/mic/instruments.html#jem2010

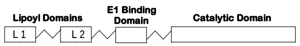


Electron micrograph of a mixture of myosin (M) and nuclear lamin (L) dimers after glycerol spraying/rotary metal shadowing with platinum. Both molecules are composed of two globular heads linked to a common rod-like tail, approximately 100 nm long in the case of myosin and 52 nm in the case of nuclear lamin.

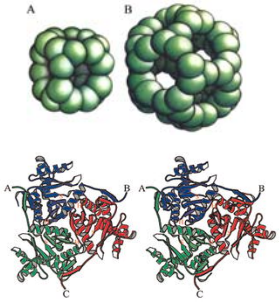
http://www.mih.unibas.ch/Booklet/Lecture/Chapter1/Chapter1.html

Multienzyme Complexes – with Professor Lester Reed

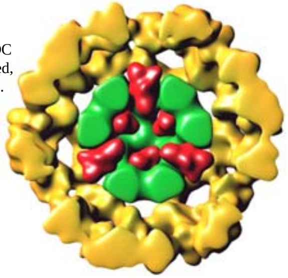




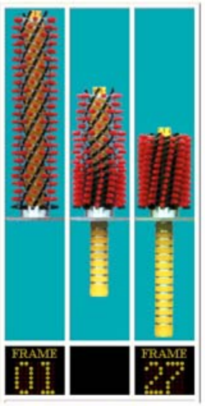
Domain Structure of E2



EM model of PDC (E1 E2 E3) - Reed, Stoops and Zhou.



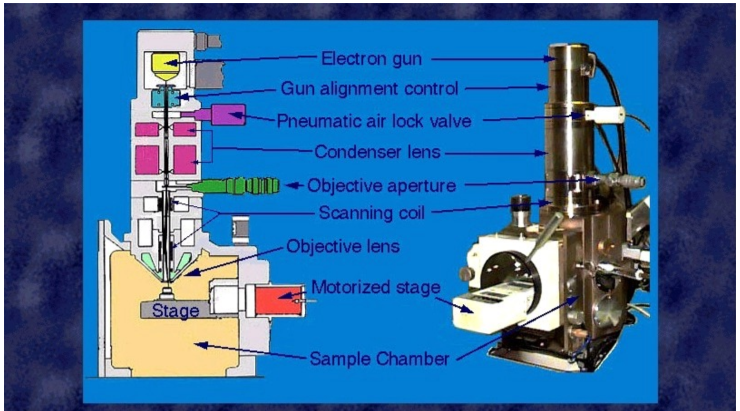
T4 bacteriophage tail sheath motility



http://www.sb.fsu.edu/~caspar/animation/anim1sm.html

SEM - Scanning Electron Microscope

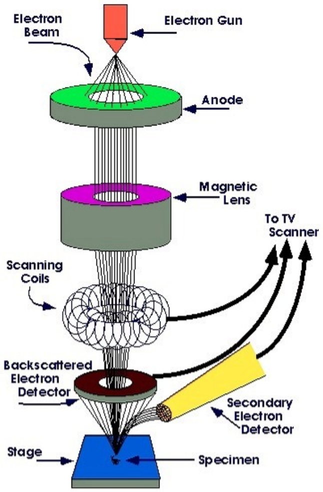
JEOL JSM-6400: This software-oriented, analytical-grade SEM, is capable of acquiring and digitizing images. Acceleration voltages from 0.2 to 40kV, a magnification range of 10 to 300,000x, and a guaranteed resolution of 3.5nm allow an operator to achieve excellent results on a wide variety of samples. http://www.tamu.edu/mic/instruments.html#jsm-6400



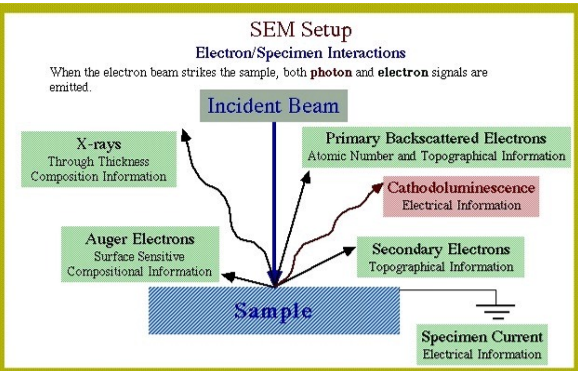
The electron beam hits the sample, producing secondary electrons from the sample. These electrons are collected by a secondary detector or a backscatter detector, converted to a voltage, and amplified. The amplified voltage is applied to the grid of the CRT and causes the intensity of the spot of light to change. The image consists of thousands of spots of varying intensity on the face of a CRT that correspond to the topography of the sample.

http://mse.iastate.edu/microscopy/source.html

The SEM uses electrons instead of light to form an image. A beam of electrons is produced at the top of the microscope by heating of a metallic filament. The electron beam follows a vertical path through the column of the microscope. It makes its way through electromagnetic lenses which focus and direct the beam down towards the sample. Once it hits the sample, other electrons (backscattered or secondary) are ejected from the sample. Detectors collect the secondary or backscattered electrons, and convert them to a signal that is sent to a viewing screen similiar to the one in an ordinary television, producing an image.

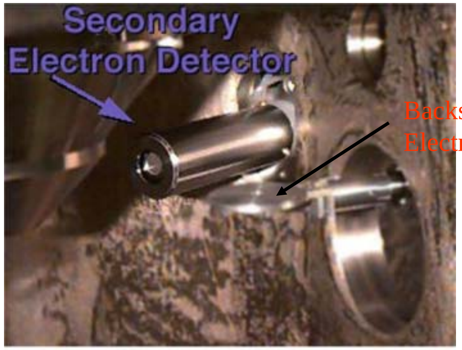


http://mse.iastate.edu/microscopy



While all these signals are present in the SEM, not all of them are detected and used for information. The signals most commonly used are the Secondary Electrons, the Backscattered Electrons and X-rays.

http://mse.iastate.edu/microscopy



http://mse.iastate.edu/microscopy

Secondary electrons are specimen electrons that obtain energy by **inelastic collisions** with beam electrons.

Elastic scattering results in little (<1eV) or no change in energy of the scattered electron, although there is a change in momentum. Since momentum, p=mv, and m doesn't change, the direction of the velocity vector must change. The angle of scattering can range from 0-180 degrees, with a typical value being about 5 degrees. **Elastic scattering** occurs between the negative electron and the positive nucleus. This is essentially Rutherford scattering. Sometimes the angle is such that the electron comes back out of the sample. These are **backscattered electrons**.

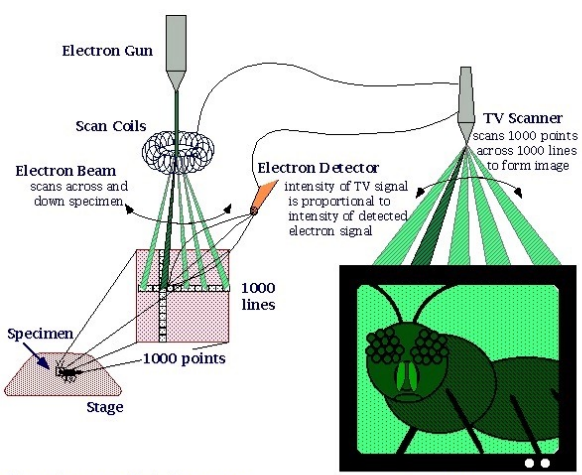
Sputter Coater

http://mse.iastate.edu/microscopy

A sputter coater coats the sample with gold atoms. The purpose is to make non-metallic samples electrically conductive.

The sputter coater uses argon gas and a small electric field. The sample is placed in a small chamber which is at vacuum. Argon gas is then introduced and an electric field is used to cause an electron to be removed from the argon atoms to make the atoms ions with a positive charge. The Ar ions are then attracted to a negatively charged piece of gold foil. The Ar ions act like sand in a sandblaster, knocking gold atoms from the surface of the foil. These gold atoms now settle onto the surface of the sample, producing a gold coating.

How an Image is Produced



http://mse.iastate.edu/microscopy

TV Screen

SEM Images

http://mse.iastate.edu/microscopy

Chigger Mite

Deer Tick

Pollen Grain

Pollen Mix

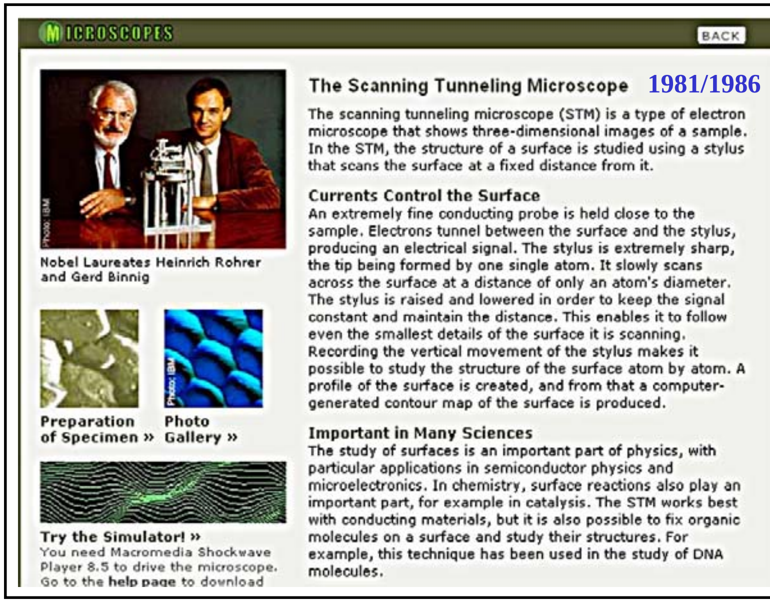
"Seeing" as the Blind Person "Sees"

The microscope can be regarded as an extension of the human eye. But sight is not the only sense we use to orientate us in our surroundings, another is touching and feeling.

The **"finger" in this case is a very fine needle** which is moved across the surface of the structure to be investigated. By registering the needle's movements in the vertical direction as it traverses the surface, a sort of topographical map is obtained.

Two breakthroughs –

1. The so-called **tunnelling effect** - a method for keeping the tip of the needle at a very small and exact constant distance from the surface was developed, thus eliminating the mechanical contact between the needle and the surface. This involves **applying a potential between the needle tip and the surface so that an electric current flows between the needle and the surface without actually touching them**, provided that the tip of the needle and the surface are close enough together.
2. To produce extremely fine needles so that **the tip consists of only a few atoms**.


MICROSCOPES BACK

The Scanning Tunneling Microscope 1981/1986

The scanning tunneling microscope (STM) is a type of electron microscope that shows three-dimensional images of a sample. In the STM, the structure of a surface is studied using a stylus that scans the surface at a fixed distance from it.

Currents Control the Surface

An extremely fine conducting probe is held close to the sample. Electrons tunnel between the surface and the stylus, producing an electrical signal. The stylus is extremely sharp, the tip being formed by one single atom. It slowly scans across the surface at a distance of only an atom's diameter. The stylus is raised and lowered in order to keep the signal constant and maintain the distance. This enables it to follow even the smallest details of the surface it is scanning. Recording the vertical movement of the stylus makes it possible to study the structure of the surface atom by atom. A profile of the surface is created, and from that a computer-generated contour map of the surface is produced.

Important in Many Sciences

The study of surfaces is an important part of physics, with particular applications in semiconductor physics and microelectronics. In chemistry, surface reactions also play an important part, for example in catalysis. The STM works best with conducting materials, but it is also possible to fix organic molecules on a surface and study their structures. For example, this technique has been used in the study of DNA molecules.

Nobel Laureates Heinrich Rohrer and Gerd Binnig

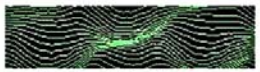
Preparation of Specimen »

Photo Gallery »

Try the Simulator! »

You need Macromedia Shockwave Player 8.5 to drive the microscope. Go to the help page to download

Preparation of Specimen » Photo Gallery »



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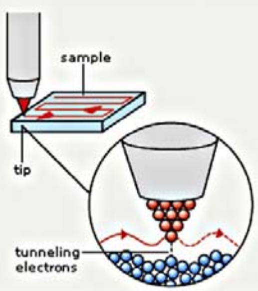
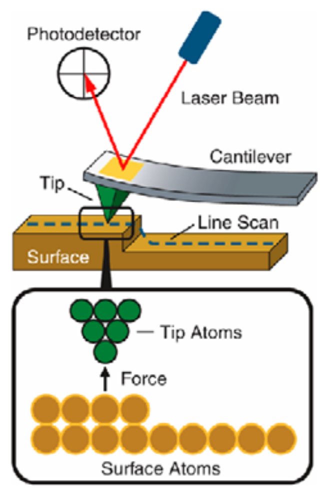
STM, *S*canning *T*unneling *M*icroscopy or the *S*canning *T*unneling *M*icroscope, is an excellent technique, but STM is ***limited to imaging conducting surfaces***

→ **AFM** **(~1986)**



Related Laureates:
The Nobel Prize in Physics, 1986
- Gerd Binnig and Heinrich Rohrer »

AFM – surface topography

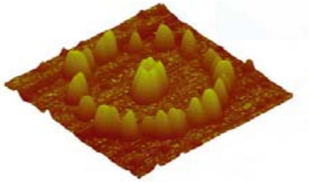


*A*tomic *F*orce *M*icroscopy (AFM) or or *S*canning *P*robe *M*icroscopy (SPM) is often called the "Eye of Nanotechnology" - a high-resolution imaging technique that can resolve features as small as an atomic lattice in the real space. It allows researchers to observe and manipulate molecular and atomic level features.

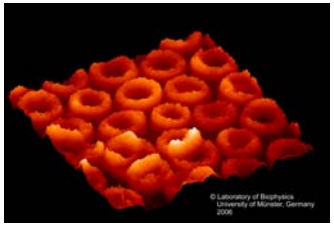
AFM works by bringing a cantilever tip in contact with the surface to be imaged. An ionic repulsive force from the surface applied to the tip bends the cantilever upwards. The amount of bending, measured by a laser spot reflected on to a split photo detector, can be used to calculate the force. By keeping the force constant while scanning the tip across the surface, the vertical movement of the tip follows the surface profile and is recorded as the **surface topography by the AFM**.

AFM has much broader potential and application because it can be used for imaging any conducting or non-conducting surface.

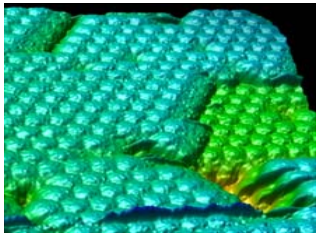
Examples of AFM Images



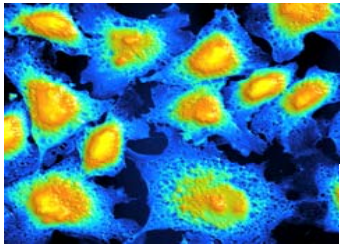
Self-assembled nanoparticles on a pre-patterned substrate – “chemical lithography”. Photo credit: Prabhakaran, et al.



Red blood cells



Virus Crystal



Chinese Hampster Ovary Cells

Medical Imaging - Radiology

MRI (or NMRI) - Magnetic resonance imaging (MRI) is an imaging technique used primarily in medical settings to produce high quality images of the inside of the human body. MRI is based on the principles of nuclear magnetic resonance (NMR). MRI is a noninvasive imaging technique that does not use x-rays. The fluid contrast between structures in the brain can then be visualized.

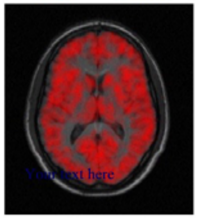
CAT (or CT) - Computerized Axial Tomography or computerized tomography. A CT scan is essentially a computerized assembly of several x-ray images taken from a series of different angles. With a CT, the resolution is much better than conventional x-rays, and the detail that can be seen is much greater. As with all other typical x-rays, the procedure is radiographic and the patient's body is exposed to a small amount of radiation during the scan.

PET - positron emission tomography (PET); PET produces images of metabolic activity as opposed to images of the body's physical structures that are derived from other imaging techniques (MRI / CT). For a PET scan, a small amount of radioactivity is attached to biological substances that are similar to those already found in the body. These radioactive agents, once introduced into the body, are processed by organs and tissues as part of their normal function. The PET scanner is able to detect the location of the radiation in the body. A computer then creates a picture of the activity using colors to highlight the different levels of function.

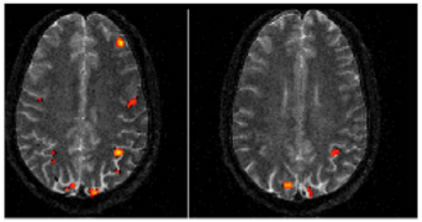
Medical Imaging

CAT scan - ear canal

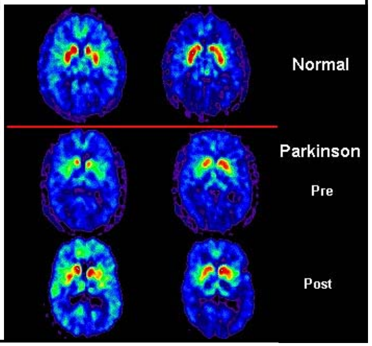
PET - brain



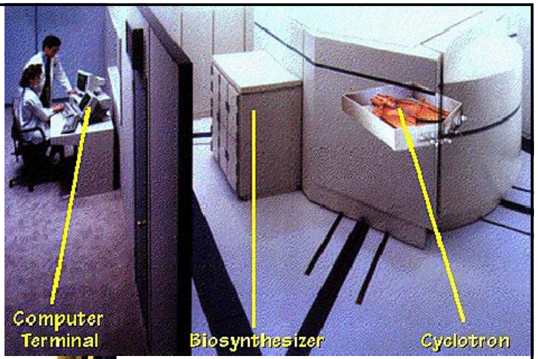
MRI - brain

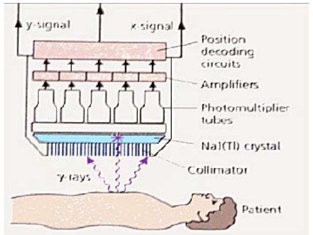


http://www.macalester.edu/~psych/whathap/UBNRP/Imaging/mri.html



PET Scans





Ultrasonography:

Introduction to Ultrasound Imaging

Ultrasound scanners - a form of 'medical' Sonar

SONAR = **Sound Navigation and Ranging**

RADAR = **Radio Detection and Ranging**

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1877 - Lord Rayleigh – "The Theory of Sound" – sound waves

1912 - Underwater navigation - submarines WWI, Titanic sank

1935 - First practical RADAR using electromagnetic waves

1940s – Ultrasound therapy: **arthritis**, **craniotomies**

1952 – John Wild – "Application of Echo-Ranging Techniques to the Determination of Structure of Biological Tissues"

1958 – "Investigation of Abdominal Masses by Pulsed Ultrasound" (an important early paper on medical diagnostic uses of ultrasound)

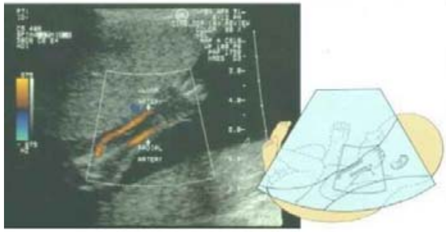
What are Obstetric Ultrasound Scans?

Obstetric Ultrasound is the use of ultrasound scans in pregnancy. Since the late 1950's ultrasonography has become a very useful diagnostic tool in Obstetrics. Currently used real-time scanners using very high frequency sound waves of between 3.5 to 7.0 megahertz (i.e. 3.5 to 7 million cycles per second) can provide a continuous picture of the moving fetus can be depicted on a monitor screen. and growth in the fetus. The conducting gel is non-staining but may feel slightly cold and wet. There is no sensation at all from the ultrasound waves.

Transcucer (probe) on the abdomen

3-D scan of fetal face

Ultrasound
and
3D Ultrasound



The fetal arm with the major arteries (radial and ulnar) clearly delineated.

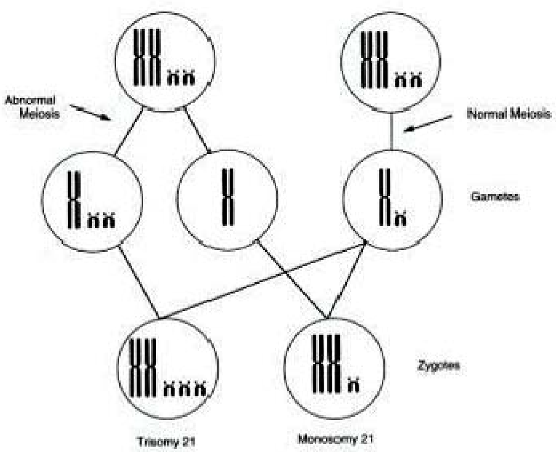
Inherited Abnormalities

	$/10^3$ live births
Down's Syndrome	1.3
Cystic Fibrosis	0.4
Familial Hypercholesterolaemia	2.0
PKU	0.1
Hypothyroidism	0.25

Downs Syndrome - Trisomy 21

- First described **1866 - JLH Down**
- Clinical Features
  - Average life expectancy 30 years
  - Characteristic phenotype
  - Learning disability (IQ 20-60)
  - Developmental delay / Hypotonia
  - Delayed puberty / Early menopause

The Downs Report – Clinical Background – Non Disjunction Diagram



Are any prenatal tests available to detect Down syndrome?

Yes. There are several types of testing available.

Screening Tests: Screening tests are used to look for potential problems and to identify those who are at high risk of having a baby with a genetic disorder.

The triple screen and the alpha-fetoprotein plus, and more recently, the quad test measure the amounts of certain hormones and proteins in the blood including alpha-fetoprotein, human chorionic gonadotropin, unconjugated estriol and inhibin. The results of these tests together with the woman's age, will provide an estimate of her risk of having a child with Down syndrome. These tests are usually performed between the fourteenth and sixteenth week of gestation. Approximately 60-80% of fetuses with Down syndrome can be identified prenatally by considering the mother's age and employing these screening tests.

In addition ultrasound examinations are almost always performed. During an ultrasound examination the physician looks for "markers", such as a thickening of the skin at the back of the neck (nuchal fold), bright spots on the kidneys or heart, short arms or legs, reduced head size, congenital heart disease, and gastrointestinal problems. If any of these "markers" are observed, diagnostic testing is generally recommended.

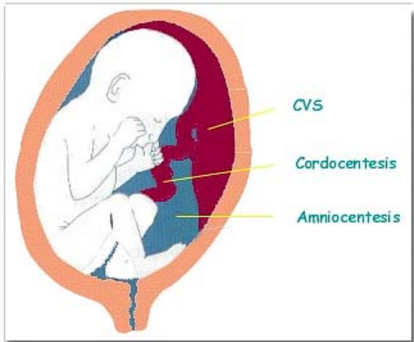
Diagnostic Testing: Diagnostic testing tells whether or not the baby has the condition. However, there is no diagnostic test that is 100% reliable. Amniocentesis and chorionic villus sampling are the two diagnostic tests most often used to determine whether the fetus has Down syndrome.

Amniocentesis is typically performed around the 16th week of pregnancy. Before the procedure, an ultrasound examination is done which shows the location of the placenta, the amniotic cavity, and the fetus. During the procedure, a needle is inserted into the amniotic cavity through the mother's abdomen. A small amount of amniotic fluid is obtained and analyzed. The amniotic fluid contains cells from the fetus, which are cultured and then examined to determine whether or not the fetus has Down syndrome. It generally takes 12 to 20 days to obtain results. Amniocentesis has about a two percent rate of miscarriage as well as other side effects, such as infection, bleeding, cramping and needle puncture of the fetus.

Non-invasive procedure

Amount of fluid behind the neck of the fetus (Nuchal translucency) scan

Three invasive procedures



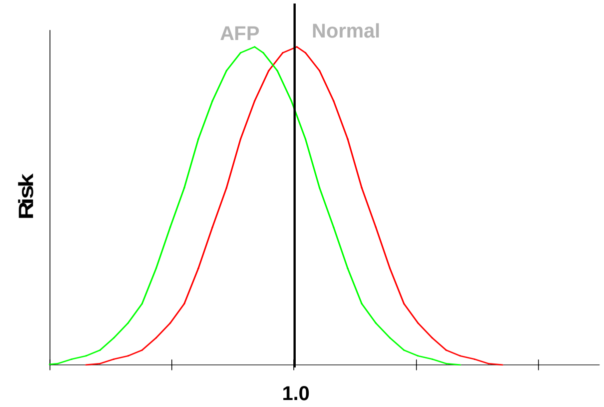
chromosomal abnormality

Chorion villus (placenta) sampling (11-14 wks)

Amniocentesis (>15 wks)

Cordocentesis (>20 wks)

Theoretical AFP Distributions

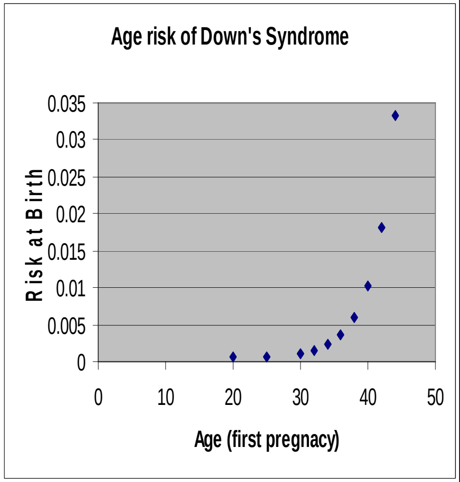


Other Biochemical Markers

	Pregnancies	MoM	SD from Normal
AFP	823	0.75	0.5-1.0
hCG	559	2.05	1-1.5
uE3	363	0.73	0.5-1.0
NAP	76	1.66	>1.5
DHEAS	77	0.88	<0.5
HPL	24	1.79	1-1.5
Free B-hCG	58	2.13	1-1.5
Free a-hCG	30	2.05	1-1.5

Down’s Syndrome (Trisomy 21)

MATERNAL AGE	RISK AT BIRTH
20	1/1527
25	1/1352
30	1/895
32	1/659
34	1/446
36	1/280
38	1/167
40	1/97
42	1/55
44	1/30



http://www.fetalmedicine.ac.uk/antenatal.htm