

Series In Popular Science Vol. 5

William J Croft

# under the microscope

a brief history of microscopy

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## **SERIES IN POPULAR SCIENCE**

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under the microscope  
a brief history of microscopy



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**UNDER THE MICROSCOPE**

**A Brief History of Microscopy**

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Frontispiece: Antony van Leeuwenhoek (1632–1723)

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## Dedication

To the ladies in my life

Inga, Karin, Susan, Briar and Justine,

with thanks for your patience while this work was being created

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## Foreword

From earliest times man has been interested in the world of the small. In the earliest written literature there exists speculation on man's place in the universe somewhere between the atoms and the stars. In a non-rigorous way I will trace the examination of the "small" from earliest times; from the glass bead and water droplet that acted as a magnifying lens to the high energy particles that act as waves and can be used to image that portion of the world that is beyond the close vision of even the most near-sighted eye.

Legend has it that Democritus conceived of the atom as the smallest building block of matter. Today we know of the infinitely smaller particles that make up atoms and are now able to visualize with modern instruments images of how atoms arrange themselves in space. I will trace the history of this endeavor up to the development of the wonderful new microscopies using electrons and other sophisticated techniques to give us a vision of the "small world". Along with the history I will present some background to help create an understanding of the phenomena leading to these new technologies.

It has been recently observed that van Leeuwenhoek's microscopes were capable of very high resolution. Instruments of that period (~1700) that have been considered "low-power dissecting instruments" were capable of creating very high quality images. Although not the first creator of microscopes, van Leeuwenhoek constructed instruments of remarkably high quality which laid the foundation of modern biology. These "simple" microscopes consisting of a single lens were used by early scientists such as Hooke and Robert Brown for whom Brownian motion is named.

Brown was probably the last scientist to do creative work with a simple microscope but his observation of the cell nucleus stands as one of the great scientific discoveries.

A step in the improvement of the optical microscope was the addition of other lenses to create the compound microscope. This development is hidden in obscurity as is the development of the simple microscope, but such names as the family Jansen (~1595) appear in the tradition. The best work using simple microscopes overlapped the compound microscope by a long period of time.

The nineteenth century was a time of great creativity in the development of the optical microscope. Optical craftsmen created masterpieces of artistic creation as well as great technical achievements in the advancement of the compound microscope. During Leeuwenhoek's time physicists began to understand the properties of light in terms of wave theory. More than 100 years ago the German optical scientist Ernst Abbe showed that diffraction phenomena would obscure the details of any object smaller than one half the wavelength of the light being used for illumination. A few attempts were made to use light of smaller than visible wavelengths but these presented many difficulties and were never really carried through.

In the early part of this century the duality of waves and particles was being considered with de Broglie first theorizing the wave-like characteristics of electrons. This led to the concept of the electron microscope by Knoll and Ruska who created a practical electron lens. In a short time commercial electron microscopes were on the market and the wavelength limit of visible light was exceeded. This led to the visualization of surfaces by scanning electron microscopy.

In the early 1980's the exciting achievement of scanning probe microscopy was introduced to the world in which real space images of atoms were demonstrated. In this work I will attempt to develop the concepts behind the various microscopes and show how

one led to another as well as describe how some of the instruments work and a few of their applications.

As the development of microscopy over the centuries has proceeded, the ability to see smaller objects has vastly increased. This ability has required smaller and smaller units to quantitatively describe the early naked eye observations and later on photographic images. Magnifications in microscopy are based on the metric system whose basic unit is the meter. One centimeter (cm) is 1/100 of a meter and one millimeter (mm) is 1/1000 of a meter. As magnifications increase the micron ( $\mu$ ) which is one millionth of a meter comes into use. As sizes approach the dimensions of atoms the Ångstrom unit comes into use. One micron is 10,000 Ångstrom units. The radii of atoms vary between 0.3 and 10 Ångstroms. The Ångstrom unit is not recognized as a standard metric unit, but it is widely accepted by scientists because of its close correspondence to such items as atoms, grains of intergalactic dust, and optical wavelengths.

The current scientific interest in the creation of small objects, grains of materials as well as devices, has generated the use of the prefix nano, as in “nanomaterials”. One nanometer is a billionth of a meter or ten Ångstrom units. Micrometers (microns  $\mu$ ), nanometers, and Ångstrom units are the measuring scales of the modern high resolution electron microscopes and scanning probe microscopes. On an even smaller scale a typical distance between the centers of two atoms is about 1.5 nanometers. The helix of DNA has diameter of about 2 nanometers and it twists full circle 1 every  $3\frac{1}{2}$  nanometers.

To put these units into perspective, a human hair is about 200 microns in diameter and a sheet of ordinary paper is one million Ångstroms thick.

William J. Croft  
Harvard University



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# Chapter I

## Light and the Ancient Greeks

In this small volume I will attempt to describe the history of microscopy as well as introduce you to the functions of microscopes from early times to the ultra sophisticated techniques of the most modern laboratory. In order to do this I will describe some history of the earliest observations of effects of light and how magnification of small objects developed. In doing this you will be introduced to many new terms which I will try to explain as well as show how they were observed. Since this is a history of microscopy I will get ahead of the story to explain that the term “microscope” was coined in 1624 in Italy by members of the first Accademia Dei Lincei a group which included Galileo who is credited with being one of the early scientific users of the microscope. The word itself is derived from two Greek words small and to view.

Getting back to the Greeks there is a passage from that comic author Aristophanes (who lived in the 4th century B.C.) that describes globules of glass being sold in the grocery shops of Athens. They were called “burning spheres”. This may be the earliest mention of applied optics.

To learn about magnification one must look to a few simple principles of optics. Light always travels in straight lines. It seems obvious to us and has been taught since the time of Euclid around 300 B.C. Euclid has been called the founder of the science of optics.

If you put a small stick in a glass of water it appears bent (Figure I-1). This seems peculiar since we know light travels in straight lines but it is clear that it can also appear bent as when you look at a stick dipped into a pond or the stirrer in your afternoon

cocktail. This bending is called refraction and it occurs whenever light shines into a medium where it travels at a different velocity. An analogy is a line of equally spaced soldiers marching across a field and reaching a ploughed area.

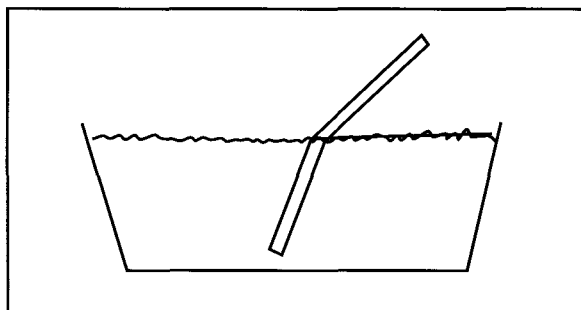


Figure I-1. Apparent bending of stick in water.

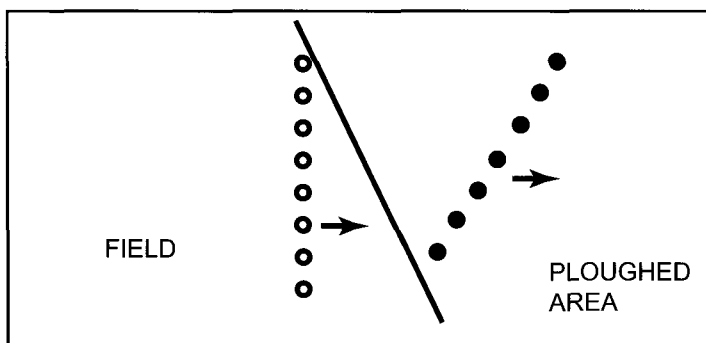


Figure I-2. Changing direction of a line of soldiers moving into a ploughed field.

The first soldier reaching the ploughed area slows down. The next one does also but if the soldiers maintain a straight line their directions will change as the wave front of a light beam does when it passes into a material of different density which changes its velocity (Figure I-2). This is the refraction caused by a difference in speed.

Effectively if a beam passes from a lower to a higher density medium it will be bent toward the normal (this is a line perpendicular to the surface.) (Figure I-3).

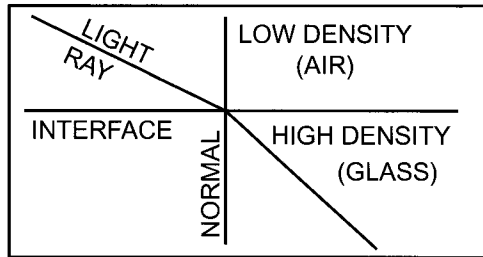


Figure I-3. Changing direction of a light ray moving into a dense medium.

As the sketch shows (Figure I-4), when a beam of light enters glass from air it changes directions. If the glass surface is curved then the further along the surface the light beam passes through the greater will be the angle that it is bent. This is basically how a lens works.

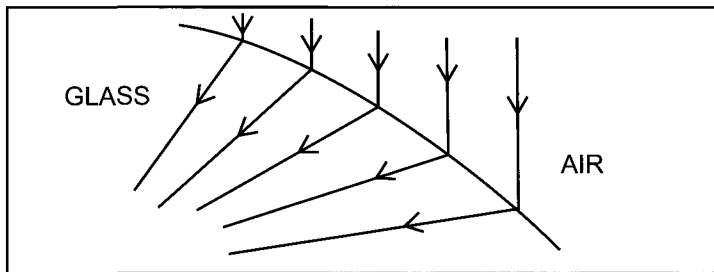


Figure I-4. Bending of light rays at a curved glass surface.

The first real lens is still a mystery but a discovery was made at Nimrod in the seventeenth century by Lanyard of a “polished” plano-convex piece of rock crystal which could be dated no later than 721-705 B.C. This was believed to be a magnifying lens of sorts.

Later examination showed it to be cloudy so it would not have very good optical properties and the “convex” surface is really the worn results of many irregular ground facets, so that it was probably an object of jewelry separated from its finding. Many older bits of polished glass or semi precious stone exist in museums but upon close examination they are usually remnants of jewelry.

There is a story in Pliny referring to a concave emerald used by the Emperor Nero to watch gladiator contests. Maybe it was the first monocle and could have helped his old-age myopia or maybe just eased his eyes from the noonday Roman sunshine.

After a hiatus of twelve centuries spectacles were “reinvented” around 1280 in Florence Italy. Credit was taken by a man named Amati who died in 1317 and had carved on his tomb credit for the invention that he only shared with a few of his close friends.

# Chapter II

## Early Microscopies

In the first century A.D. Seneca described actual magnification by a globe of water. He wrote that letters could be enlarged and made clear by viewing through a globe of glass filled with water. A thousand years later the Arabian scholar Alhazan wrote a major work on optical principles and described the anatomy of the eye and how the lens focuses images.

The first modern application of optics occurred in Florence around 1280 A.D. with the use of eyeglasses as an aid to vision. Although credit for the invention is given to a man named Amati it is really not known for sure who the inventor was. However considering the large number of people with visual problems there was great enthusiasm as spectacles came into common use in Florence and then spread very rapidly into the rest of the world. Up to this point optical science was the domain of a few learned scientists but with the spread of the knowledge of spectacles it became widespread.

The early history of the microscope and its inventors is shrouded in much confusion and probably lots of misinformation. It was only a matter of time with the widespread distribution of spectacle lenses toward the end of the sixteenth century that people noticed the magnifying properties of multiple lenses.

Since a microscope can be created by reversing a telescope Galileo is often credited with inventing the microscope shortly after the telescope. However the first telescope appeared about 1607 when microscopes were already being sold by instrument makers.

Credit for the first compound microscope (two or more lenses) is generally given to Hans Jansen or his son Zacharias of Middleburg,

Holland, around 1595. No early Jansen microscopes survive, but there is a description of a microscope the Jansens made for the Archduke Albert of Austria which was preserved until the early 1600's and described by a friend of Zacharias Jansen named Cornelius Drebbel. The royal instrument consisted of three sliding tubes measuring 18 inches in length when fully extended and two inches in diameter. It was very ornate and supported by 3 brass dolphins forming the feet of a tripod.

The diagram shows the optics of a Jansen microscope as described (Figure II-1). It contains two lenses and diaphragms to cut glare. A reputed copy of a Jansen instrument in the Middleburg museum was said to have a magnification of 3 x when closed and 9 x when fully extended.

After the Jansen's microscope word spread rapidly and many instruments were being made throughout Europe and used by early scientists. At this point we ought to look at some of the terms that will be used as we go further into describing instruments.

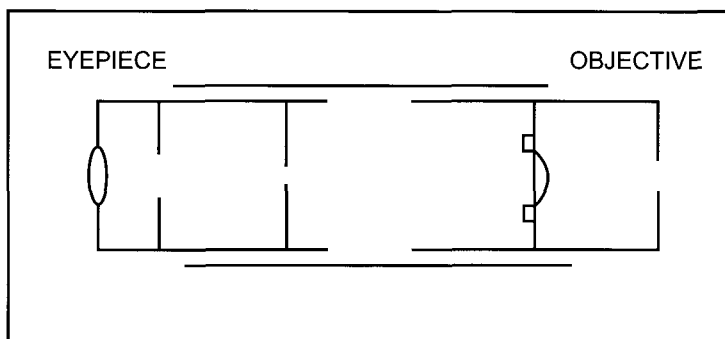


Figure II-1. Sketch of the optics of Jansen's microscope.

The smallest distance that we can resolve with our eyes is about 0.1 to 0.2 mm depending on how good our eyes are and if there is enough illumination to see by. This distance is the resolution or resolving power of our eyes. Any instrument that can show



us images finer than 0.1 mm is a microscope and its highest useful magnification is governed by its resolution. By successively enlarging photographs taken with a microscope one may get enormous magnifications but without good resolution. This is referred to as “empty” magnification. Microscope makers generally term magnification power by linear proportion e.g. an object 1 mm long is magnified to 10 mm. This is 10 x magnification. On the other hand a magnifying glass is rated in terms of areal properties so that a 10 x magnifier only magnifies the square root of 10 or a little more than three times.

In the eighteenth century many mechanical improvements were made in the microscope but the images had colorful haloes and were blurry. In fact the distortions become multiplied with multiple lenses. The best simple microscopes (one lens) attain about two micron resolutions but the best compound microscopes only had five micron resolution. The microscopist uses a convent term “micron” to describe the size of small objects. The micron is one thousandth of a millimeter. Microscope makers generally term magnification power by linear proportion e.g. an object 1 mm long is magnified to 10 mm. On the other hand a magnifying glass is rated in terms of areal properties so that a 10 x magnifier only magnifies the square root of 10 or a little more than three times.

The color haloes are due to a problem called chromatic aberration. When light is transmitted through a substance it will be bent different amounts depending upon its wavelength (color). This means that a lens will have a different focus for different colors of light. Since white light is composed of all colors if we focus on an object it will appear to have a set of colored haloes around it. The solution to this problem came not from microscopes but from telescopes in the 1730's when Chester More Hall noticed that a new glass “Flint glass” dispersed colors more than the “crown glass” being used. He tried this out by ordering a flint glass lens from one shop and a crown glass lens from another. Unfortunately both glass makers sent the glass to the same lens maker George Bass who

realized what was happening but although he made the first achromatic telescope he never patented it or realized from it. Bass kept this a secret for twenty years until he met another telescope maker John Dolland. After hearing about the two kinds of glass Dolland experimented and by 1759 succeeded in making an achromatic lens and patented the idea. He became very wealthy as a result because of the royalties paid by English telescope makers. His patent rights were disputed but finally upheld in court since the original discovery was kept secret and did the world no good.

Although achromatic lenses were very successful for telescopes the tiny objective lenses of microscopes were much more difficult to fabricate and it wasn't until after the beginning of the nineteenth century that they became available.

Another cause of optical distortion that had to be overcome is called spherical aberration. This distortion is due to the fact that light from the object that hits the edge of the lens does not have exactly the same focal distance as light which comes through the center of the lens. There were two approaches to solving this problem. One is to use lenses of less curvature which limits magnification. The other is to insert a small aperture to limit the light angle. This will limit resolution. If you use multiple lenses to increase magnification the problem as with chromatic aberration gets worse because of the synergism of the errors in multiple lenses.

The problem was solved in 1830 by Joseph Jackson Lister (father of Lord Joseph Lister who discovered antiseptic techniques in surgery). He demonstrated that if multiple low magnification lenses are placed a certain precise distance apart you still get spherical aberration from the first lens but do not get the additional component from the other lenses. Lister published this work but it was ignored so he arranged to have a microscope built to his specifications. He did this in the 1830's but it was not until later that it became widely used. Even so a new problem arose as the microscope objective had to be adjusted for the thickness of the cover glass on the slide being examined.

These complications in microscope magnifications led to a Dutch amateur working with very simple single lens instruments of his own construction to make enormous scientific contributions even when more complex instruments were available and being used.

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# Chapter III

## Early Microscopists

One of the earliest books on microscopy was by Robert Hooke (1635-1703). He was curator of experiments at the Royal Society of London. In 1663 he was asked to deliver a weekly series of lectures on his work with microscopes. His earliest work involved looking at the structure of a piece of cork. He cut thin sections with a razor blade and was able to see in his microscope the tiny compartments of which it is made. He names these “cells”. In a single experiment he had invented a basic technique of the microscopist and named the fundamental unit of life. In 1665 he published his results in a book “Micrographia”. He examined natural and man made objects from the point of a sewing needle to the eyes of insects. He also reported on the construction of the microscopes he used.

Micrographia gave details of the instruments Hooke used. He liked microscopes with two lenses which would be a compound microscope. He did suggest the use of a single lens instrument which he thought would give better results but would be inconvenient to use. The working distance, the distance between the lens and the object would be very short at higher magnifications. He even described how such a microscope would be built. In spite of the aberrations, he preferred the compound microscope.

Hooke made an enormous number of major contributions to science including the law of elasticity known as Hooke’s Law. As well as microscopy he worked in astronomy, being the discoverer of new stars and the first observation of the planet Jupiter rotating on its own axis. He suggested the use of a pendulum to measure the force of gravity and in a major contribution to optics described the

phenomenon of diffraction (the bending of light around edges) and offered the wave theory of light to explain it.

One of the all time great contributors to microscopy was an amateur. A Dutch draper whose name has come down to use in various forms but whose name is best known today in its anglicized version "Antony van Leeuwenhoek" (1632-1723). Little is know of his early life. Born in Delft his stepfather sent him to Amsterdam to apprentice with a linen draper. At twenty he returned to become a tradesman of Delft, Holland. Descended from a family of tradesmen, he had no fortune or higher education. He did not even know another language than his native Dutch but with great diligence and skill he made some of the most important discoveries in biology in its earliest days. He discovered and described bacteria, sperm cells, blood cells and much more. His researches were widely circulated and brought his discoveries to the attention of scientists. Although he set himself up in business as a draper (fabric merchant) he worked as a surveyor, a wine assayer and a minor city official. In 1676 he served as trustee of the estate of his good friend Jan Vermeer the famous painter. At some time before 1668 he learned to grind lenses and construct simple microscopes and began observing with them.

There is still a question about how Leeuwenhoek spelled his name, least of all how it was pronounced. In 1932 to mark the 300th anniversary of his birth the Philosophical Transactions of the Royal Society published a biography by Clifford Dobell; "Antony van Leeuwenhoek and his 'Little Animals'". This paper notes nineteen different spellings of his surname. His given name also has a variety of spellings. His given name Anthony is pronounced with the accent of the middle syllable and if you pronounced his surname Lay wen hook most Dutchmen would not be too annoyed.

Three years after Micrographia was published Leeuwenhoek visited London where he became acquainted with members of the Royal Society. During his lifetime he built over 500 microscopes of the single lens type that Hooke regarded as impractical. It is believed that each instrument was used for one type of specimen only. His

drawings are unambiguous and a testament to his skill as an observer. He made the first descriptions of microorganisms including bacteria. His most powerful lenses could magnify about 300 times and could resolve about 1.4 microns. He was a regular correspondent with the Royal Society describing his discoveries until he died at age 90. Figure III-1 shows a working replica of one of his instruments and Figure III-2 shows it being used.

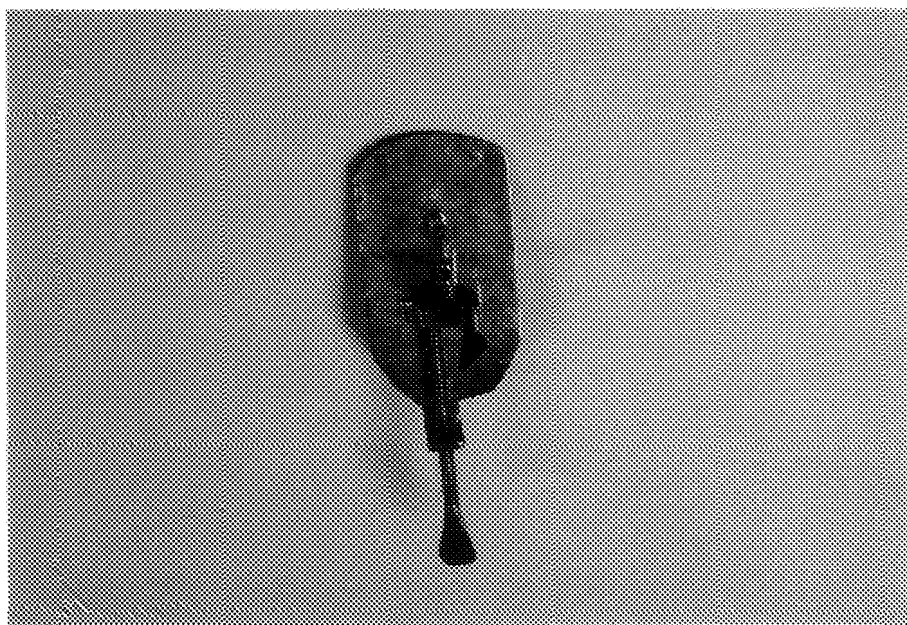


Figure III-I. Replica of Leeuwenhoek single lens microscope, prepared by Alan Shinn of Berkeley, California.



Figure III-2. Technique of using the single lens microscope. The lens is a sphere approximately 2 mm in diameter giving a magnification of about 180 x.



# Chapter IV

## Polarized Light And Crystals

Up to this point we have considered light as a wave motion which vibrates in space in all directions. Looking at a beam of light the vibration directions are in all radial directions perpendicular to the direction of propagation.

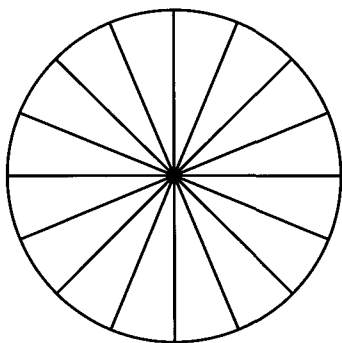


Figure IV-1. Vibration directions in a beam of unpolarized light propagating perpendicular to this page.

When the vibration directions line up with each other the beam is said to be polarized. This situation gives the beam some unique properties which we will examine after seeing how to create polarization. First we must look at some other properties of light.

In 1621 Willobrodrd Snell the Dutch astronomer observed the difference in paths between an incident and refracted ray and determined that  $n_i \sin i = n_r \sin r$  when  $n_i$  and  $n_r$  represent the indices of refraction of the two media and  $\alpha_i$  and  $\alpha_r$  are the angles of incidence and refraction that the ray makes with the normal to the

boundary. The path of the light ray is bent toward the normal when the ray enters a substance with an index of refraction higher than the one from which it emerges. This is another way of saying as in Chapter I that the ray enters a higher density space from a lower density space. The converse is that when the path of the ray is from a higher index to a lower index the ray is bent away from the normal.

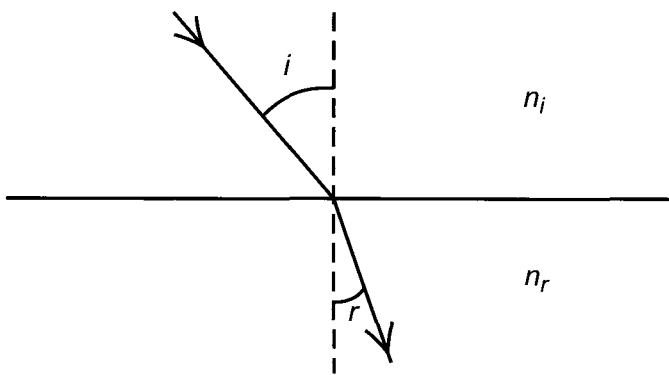


Figure IV-2. Light path between two media of differing refractive index.

The refractive index of a substance is the ratio of the speed of light in a vacuum to the speed of light in the substance. This index will vary with the wavelength (or color) of the light. The spreading of a mixture of wavelengths producing a spectrum is called dispersion. This of course is the reason for the color fringes produced in early microscopes before Hall conceived the concept of the achromatic lens. Later lenses corrected both for chromatic aberration and spherical aberration were called apochromatic.

When light is reflected from a surface the angle of incidence ( $i$ ) equals the angle of reflection ( $r$ ) however at a certain angle (the polarizing angle) the reflected light becomes almost completely polarized. Sir David Brewster in 1799 began investigating this phenomenon and determined a simple relationship between the polarizing angle and the refractive index of the reflective substance.

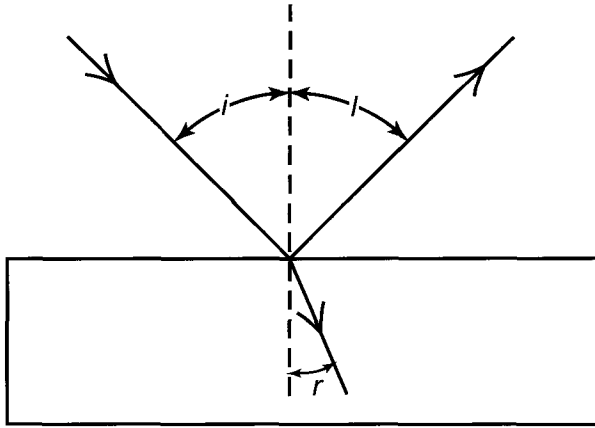


Figure IV-3. Brewster's angle; the angle of maximum polarization.

By 1812 Brewster observed that the incident ray gives rise to two partially polarized rays. The reflected ray whose vibrations are perpendicular to the plane of incidence and the refracted ray whose vibrations are mainly within the plane of incidence. He reported that these two rays obtained their maximum degree of polarization when the angle of incidence and the angle of refraction were complimentary, that is when:  $\cos i = \sin r$ . This is one way of producing polarized light by reflection. There are several other ways that we will now look at.

In 1669 a Danish scientist Erasmus Bartholin discovered that a ray of unpolarized light incident on a plate of the mineral calcite is split into two rays. One ray called the ordinary ray is in the plane containing the incident ray and the normal to the surface. When the angle of incidence is varied this ray obeys Snell's law. The other ray called the extraordinary ray is usually not coplanar with the incident ray and the normal and for it the ratio of sines (Snell's law) is not constant.

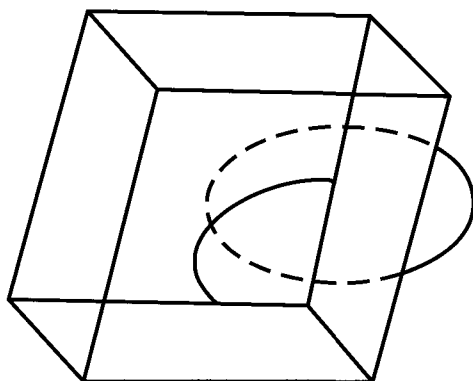


Figure IV-4. Calcite rhomb showing double refraction.

The fact that Snell's law is not obeyed in certain directions implies that the velocity of light in such a medium depends on the direction of travel in it. The two rays are polarized in mutually perpendicular planes. This medium is called anisotropic and will be discussed later along with other properties of crystals. A medium that does not exhibit double refraction is called isotropic and is typically a glass or a very high symmetry crystal.

William Nicol made use of the polarizing properties of calcite to construct a prism that transmits a single beam of plane polarized light. This prism is made by cutting a calcite crystal in a suitable plane relative to the crystal axis and cementing the two parts together with Canada balsam. The critical angle for total reflection is less for the ordinary ray than for the extra ordinary ray. The crystal is cut at such an angle that for a cone of incident rays of about  $24^\circ$  the ordinary ray is totally reflected and the extraordinary ray is transmitted.

The extra ordinary ray has an index of refraction of 1.516 at the angle of incidence of the prism. The ordinary ray has an index of 1.658. The material used to cement the prism is Canada Balsam which has an index of 1.537. The index of the extraordinary ray is close to that of the balsam but the index of the ordinary ray is

considerably greater. Both rays strike the cementing plane of balsam obliquely but the angle of the ordinary ray exceeds the critical angle and it is reflected out through the side of the prism. The ordinary ray which does not exceed the critical angle passes through the prism and therefore light emerging from the prism is polarized in only one plane. Crossed Nicols means, two nicol prisms are superimposed with their planes of polarization at right angles. A beam of unpolarized light entering one prism will not leave the second prism producing darkness at the exit of the second prism. If an isotropic material is placed between the prisms the situation does not change and there is still darkness. If an isotropic material is placed there then interference colors will be seen leaving the second prism. This effect will be discussed later after the discussion of crystals.

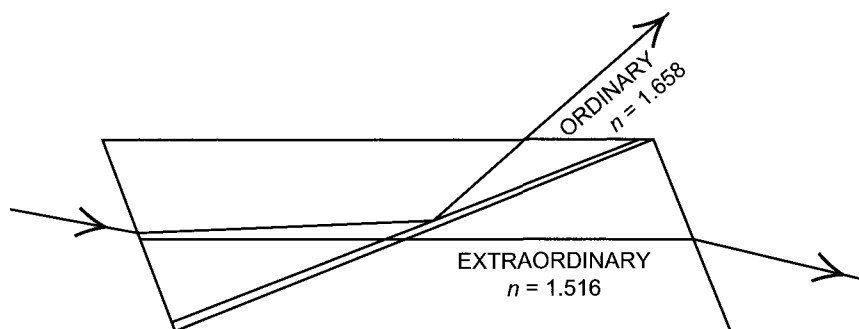


Figure IV-5. Nicol prism which transmits a single beam of plane polarized light.

The polarizing prisms in use in modern microscopes are referred to as “Nicols” although they may be made differently and may not even have any calcite inside.

A third means of polarizing light makes use of polarization by absorption. Many crystalline substances have different absorption coefficients for the ordinary and extraordinary rays but this is usually too small to be useful. In 1852 William Bird Herepath, a physician described crystals of a strongly absorptive compound

iodo-cinchonidine-sulfate. In recent time methods have been developed for producing thin transparent sheets containing small crystals of this material in parallel orientation in a polymer binder. The orientation is achieved by stretching the polymer. This in effect acts like a single crystal of large area and is known commercially as “Polaroid”. This material will transmit about 80% of plane polarized light which is vibrating in one plane and less than 1% vibrating in a perpendicular plane. This “Polaroid” material offers a convenient way of obtaining a beam of plane polarized light and has many applications. Since we have seen that reflected light is polarized to a greater or lesser degree Polaroid finds use in sunglasses that are efficient in cutting glare by cutting out all the light vibrating in directions other than the plane of polarization of the filter.

Some years before Nicol invented his polarizing prism he had used Canada balsam to cement pieces of fossils and minerals onto a glass plate and then ground them down thin enough to see through them on a microscope. He observed such things as bubbles in minerals and the cells in plant fossils that showed the kind of plant that had been fossilized. In 1827 he observed, without a microscope, slices of materials between a pair of Nicols whose planes of polarizations were at right angles to one another. This positioning of the prisms is referred to as “crossed nicols” and demonstrated interesting optical effects such as anisotropy which we will see later in this chapter. It was Henry Fox Talbot, one of the fathers of photography who was the first to equip a microscope with a pair of polarizers in 1834. By 1949 Henry Clifton Sorby began to prepare thin sections of rocks (about 0.025 mm thick) for microscopic study and began to report the optical properties of minerals. During the next few years the demand for special polarizing microscopes was low. People who wanted to study thin sections of rocks and minerals had to make do with adding polarizing prisms to their own microscopes. In 1885 Ernst Leitz in Wetzlar, Germany introduced a special polarizing microscope which soon became an important tool for mineralogists, geologists and chemists.

In order to understand the images seen in a polarizing microscope we have to look at some of the properties of crystals. A crystal is a solid made up of atoms periodically arranged in space. This periodicity is what gives a crystal its properties in contrast to a glass whose atoms are randomly arranged. This randomness is also true of a liquid except for special cases to be discussed later. This brief definition has been arrived at over several hundred years of investigation and really does not fully describe all of the observed properties of crystals. The external form of many crystals is characterized by plane faces arranged in a symmetrical manner. Crystals with faces have been observed since the times of the ancient Greeks. In 1669 Nicolaus Steno, a Danish anatomist measured the angles between similar kinds of faces on a large number of quartz crystals from many different places. He made the remarkable discovery that the angles between similar faces on many different crystals of the same material were the same. This was one of the first concepts about crystals to be recorded and is now called the “law of constancy of interfacial angles”. It took almost a hundred years more for this to lead to the concept of a crystal’s properties being the result of the regularity of its internal structure.

A sense of symmetry is an important tool to develop a classification of crystals. This use of symmetry can be a key to organizing the endless array of atoms that make up the crystalline solids. Symmetry is the result of operations that bring an object or a pattern into coincidence with itself. There are only a few symmetry operations.

For instance if the object in Figure IV-6 is rotated  $120^\circ$  around an axis perpendicular to the page then it will occupy a position which is indistinguishable from its original position. This operation is a symmetry operation and since it occurs three times during a full rotation the axis is called a 3-fold symmetry axis. Consider an axis lying in the plane of the paper that passes between two of the circles and through the center of the third. Rotation around this axis for  $180^\circ$  will bring the circles into coincidence. This is a 2-fold symmetry axis.

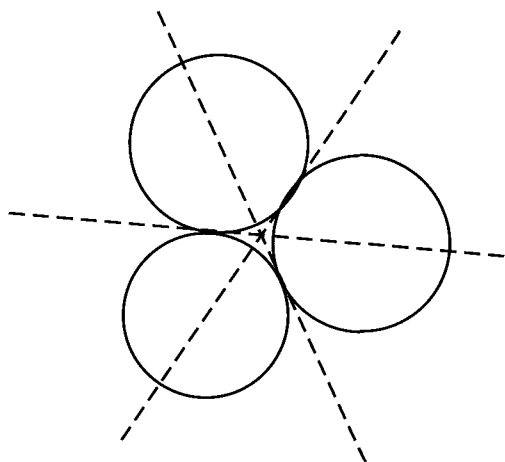


Figure IV-6. Three fold symmetry.

Looking at real crystals another axis that is observed is 4-fold. This really includes a 2-fold axis but the higher symmetry has preference. In addition to axes of 2-fold, four fold, three fold and six fold, further observation of real crystals demonstrates the existence of planes of symmetry. A plane of symmetry acts like a mirror. A demonstration of planes of symmetry in a simple crystal form, the cube, is shown in Figure IV-7.

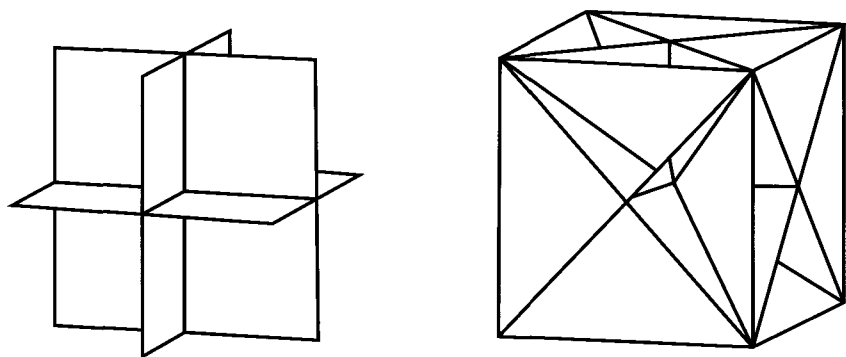


Figure IV-7. Symmetry planes in a cube.



In addition to planes and axes there is a third kind of symmetry element. We can examine this by looking at an orthogonal block that has no square faces.

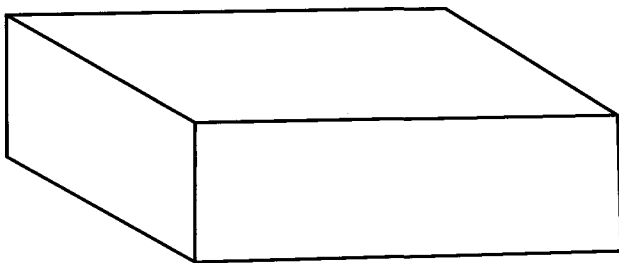


Figure IV-8. This block has a center of symmetry.

This block has symmetry planes and axes. Whatever feature of this block we look at, e.g. a corner or an edge, at an equal distance from the center of the block the same feature occurs. If we draw a line from the center to a corner continuing the line through the center; at the same distance from the center it will meet a copy of the original corner. This feature is the element called a center of symmetry, and the operation is called inversion through the center.

Symmetry operations were first observed in the relationships of faces in real crystals. Many thousands of crystals were measured and described. Based on the axes of symmetry their relative length and the angles between their crystals can be divided into a simple classification of 6 crystal systems.

- 1) The cubic system also called the isometric system has three crystal axes at right angles to each other and of equal length.
- 2) The tetragonal system has three crystal axes at right angles to each other two of them, the horizontal axes, are equal the third, the vertical axis is longer or shorter than the other two.
- 3) The Hexagonal system has four crystal axes three of which are equal and lie in the horizontal plane making angles of  $60^\circ$  and  $120^\circ$  with each other while the fourth axis is vertical and has a

length different from that of the horizontal axes. In the hexagonal division of this system there is a principal axis of hexagonal symmetry which is the vertical crystallographic axis; at right angles to it are six binary axes. In the trigonal or rhombohedral division of this system there is a vertical principal axis of trigonal symmetry and three horizontal axes of binary symmetry.

- 4) The orthorhombic system has three crystal axes at right angles to each other, all of different lengths.
- 5) The monoclinic system has three crystal axes of unequal length having one of their intersections oblique and the other two intersecting at  $90^\circ$ . One plane of symmetry contains the two axes that have the oblique intersection.
- 6) The triclinic system has three unequal crystal axes with mutually oblique intersections. It has no plane and no axis of symmetry but may have symmetry with respect to the center point.

In Figure IV-9 we see a drawing of a crystal form called a sphenoid.

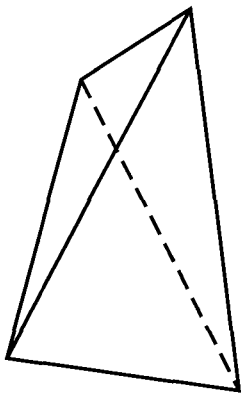


Figure IV-9. Sphenoid.

This is a solid bounded by faces which are isosceles triangles. It has a 2-fold axis through the center of the short edges and two symmetry planes that intersect this axis and are at right angles to each

other. If you rotate this sphenoid  $90^\circ$  around its two fold axis and then perform the operation of inversion through the center it will occupy its original position again. This combined rotation and inversion is indeed a fourth symmetry operation and it is called an axis of rotary inversion. Note that this sphenoid does not have a center of symmetry. All of the symmetry operations that have been described always leave one point unmoved during the operation. (This may or may not be a center of symmetry). The various combinations of these four elements make up the 32 point groups. These point groups can only describe the morphology of crystals. The point groups cannot describe the possible arrangement of atoms that make up a crystal.

Because of the similar relationships between the interfacial angles in real crystals early naturalists were convinced that crystals were made up of simple building blocks. One of the earliest ones to write about this was J.B.L. Romé de l'Isle in 1772. The Abbé René Just Haüy carries this idea further in an essay published in Paris in 1781. The Abbé Haüy was bothered by the fact that the edges of the building blocks could not be detected at the faces of a crystal even with the strongest microscope available to him. Nevertheless he was convinced by the geometry of crystals of the existence of the basic building blocks. It wasn't until the nineteenth century that it was realized that the size of these building blocks would have to be on the order of the wave length of light in order even to scatter light like ground glass.

The actual arrangement of atoms in space is known as the crystal structure. The smallest and simplest unit of structure is repeated in space on a lattice which fits the symmetry of one of the crystal systems. There are 14 of these lattices known as the Bravais lattices. Bravais had been a teacher of astronomy but by 1845 had become professor of Physics in the Ecole Polytechnique in Paris. In 1846 he derived mathematically the 14 possible arrangements of points in space that make the crystal lattices. It is remarkable that this was still more than half a century before von Laue demonstrated the

periodic arrangement of atoms in space that make up a crystal with his remarkable X-ray diffraction experiment.

At the beginning of his career Haüy was a botanist and had a friend whose interest was minerals. One day Haüy was being shown a calcite specimen which he dropped and broke. The specimen broke along a beautiful smooth surface. His friend gave Haüy part of the specimen to keep. At this point he wondered if the smooth surface could be reproduced and he deliberately broke the specimen again. He observed that there were three directions in the crystal that were directions of easy breakage and gave bright plane surfaces. These were called cleavage planes. This demonstrates the tendency of crystals to come apart between planes of atoms that are weakly bonded. For the same reason the mineral family Mica comes apart in sheets. Haüy observed that the angles between the cleavage planes were the same in every fragment and concluded that by breaking the crystal into finer and finer fragments he could reach the ultimate building blocks of the crystal. This was an early idea of the concept of the repeat unit that it takes to build a crystal. He also observed the constant angular relationship of the cleavage planes to the crystal axes. This was also the point at which Haüy gave up botany for crystallography.

Cleavage is only one of many physical properties that is related to crystallography. If we squeeze a cubic crystal in the direction of one cubic axis; for a given stress the deformation will be a certain value. When the stress is removed the material will revert to its original dimension. This is elasticity. Above a certain stress material will fail. This is the elastic limit. Because of symmetry, applying the same stress in any of the other crystal axis directions will give the same deformation, so that we can say that the elastic constants have the same symmetry as the crystal. If we stress the crystal in a direction parallel to a cube face diagonal the stress will affect a different sequence of atoms than in the axial direction and will have a different value. For crystals of lower symmetry than cubic the elasticity will be the same in equivalent crystallographic

directions. From the center of a crystal we can draw vectors representing the directions and value of any property of the crystal. This will form a vector surface. In many cases the vector surface will have a higher symmetry than the crystal because most material properties are centro symmetric. An example is thermal expansion. If we cut a sphere from a cubic crystal and raise its temperature it will expand equally in all directions. The vector surface from thermal expansion in the cubic system is a sphere. In crystal systems where one axis is unique e.g. hexagonal or tetragonal, but the other axes normal to it are symmetrically equivalent to each other the vector surface for linear thermal expansion is an ellipsoid of revolution which is a solid figure formed by rotating an ellipse around one of its two axes. In crystals where no two axes are crystallographically equivalent the vector surface for thermal expansion is a triaxial ellipsoid. In a uniaxial ellipsoid there is one circular section which is perpendicular to the unique axis. In a triaxial ellipsoid there are two circular sections perpendicular to axes of intermediate length.

When the vector surface of a physical property of a crystal is spherical as is thermal expansion in the cubic system the crystal is said to be isotropic for that property. For other properties whose vector surface is not spherical the crystal is anisotropic. The vector surfaces describe all of the physical properties of crystals. If for example the growth rate of cubic crystals were isotropic the crystals would all be spherical.

This discussion leads us to examine the optical properties of crystals and what the vector surfaces for the propagation of light looks like. Consider the vector surface for the velocity of light in a crystal. The simplest case, a cubic crystal with a source of light at the center of the crystal. After an extremely short period of time the ray surface of the light will form a sphere. This is the vector surface of the velocity of light in a cubic crystal. Cubic crystals are called optically isotropic as are most glass, air and vacuum. All other crystals are called optically anisotropic. Isotropic materials do not polarize light by transmission. In hexagonal, trigonal and tetragonal a

light beam traveling in a given direction is broken up into two rays polarized at right angles to each other and traveling at different velocities. One has a spherical vector surface. This is called the ordinary ray. The other is an ellipsoid of revolution and is the velocity vector of the extraordinary ray.

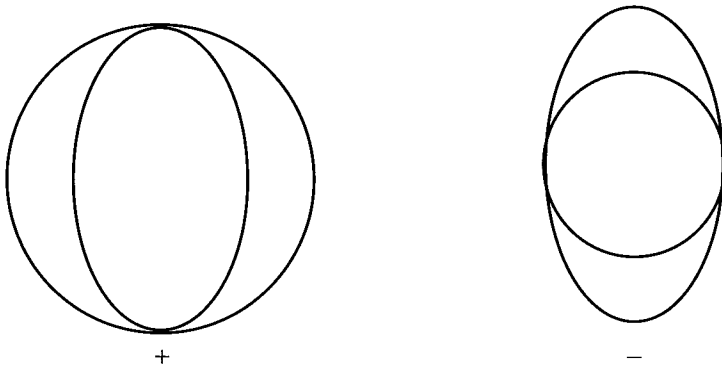


Figure IV-10. Ray surfaces sections in a uniaxial crystal.

The sphere and the ellipsoid touch at only two places. The line connecting these touching points are the ends of the axis of revolution of the optical ray velocity surfaces and represent the unique axis of the hexagonal, trigonal and tetragonal crystal system. If the extraordinary ray is the slow ray relative to the ordinary ray then the ellipsoid will lie within the sphere. This is by convention called positive. The converse where the ellipsoid lies outside of the sphere is negative.

In the case of uniaxial crystals the light beam traveling along the unique axis will only have a single velocity. This direction is the optic axis. In this direction light passing through the crystal acts as if the crystal were isotropic. In every other direction the light is broken into two polarized rays. The further in direction from the optic axis the angle between the ordinary and extraordinary ray increases. It reaches a maximum in the direction at right angles to the optic axis.

The maximum separation of the ordinary and extraordinary rays is called birefringence and is equal to the difference in refractive index of the two rays. In orthorhombic, mono-clinic and triclinic crystals there are two directions along which the light travels with zero birefringence. These are the optic axes and the crystals are called biaxial. The angle that the optic axes make with each other may vary from zero to  $90^\circ$  but is constant for a given material at a given temperature. The ray vector surface for the extraordinary ray is a triaxial ellipsoid which touches the sphere of the ordinary ray at four points. These are the ends of the two optic axes.

In the next chapter we will see how these properties will allow us to characterize materials in the polarizing microscope.

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# Chapter V

## The Polarizing Microscope

The polarizing microscope, also known as the petrographic microscope because of its use in studying rocks, is more than just an image magnifier. With this instrument can be determined optical properties of both crystalline and non crystalline materials that transmit light. Using various accessories one can determine such properties as refractive index, phase transformation temperature and melting point. Looking at thin sections of rocks one can identify the mineral constituents and determine all kinds of information about their relationship to one another.

Essentially a polarizing microscope consists of three major component sets (Figure V-1). First is a stand which can be a heavy base or foot to which the body tube and stage are attached. The most modern instruments consist of a frame-work structure composed of many modules. Second, is the optical system consisting of a source of illumination, usually a lamp which is an integral part of the stand. Older instruments use a tiltable mirror and an external artificial light source or even a window exposed to a good “North light”. The instrument should have various substage lenses including condensers and iris diaphragms to control the illumination, the objective lens at the lower end of the body tube and the eyepiece or ocular at the top. Third are devices for producing plane polarized light. These consist of a polarizer mounted below the microscope stage and an analyser mounted on the body tube above the objective. The polarizer almost always remains in place but can be rotated. The analyser may be removed by sliding it in and out of the body tube. It also usually has the facility to be rotated. Every kind of microscope has a choice of eyepieces of different magnifications but the key to the real quality of the instrument lies in the objective lens.

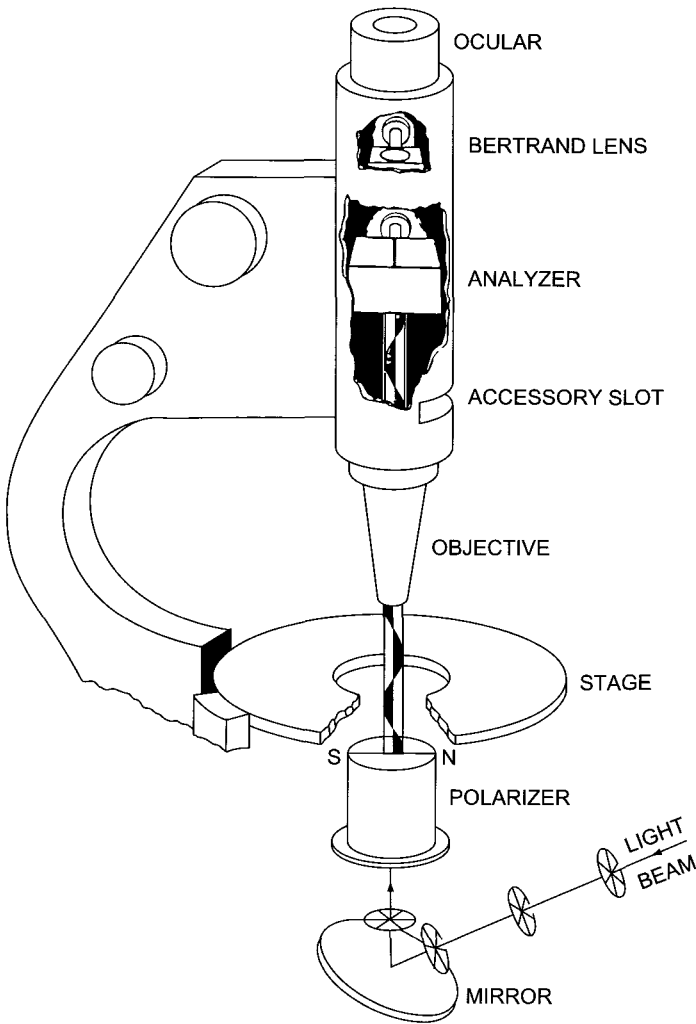


Figure V-1. The polarizing microscope.

Generally the objectives have their magnification engraved on them. They may be changed by means of a clamp or by rotating a nosepiece. A rough idea of the magnification can be gained from the outermost lens. The smaller the diameter the higher the magnification. A good rule of thumb is that the total magnification

is the product of the eyepiece magnification and the objective magnification. A measure of the quality of the objective lens is a value called the numerical aperture (N.A.). This is derived from an earlier measure that was long used as a quantitative means of comparing objectives. This early measure called the angular aperture (A.A.) is the angle between the most divergent rays which can pass through the objective to form the image. With the development of “immersion” objectives used with liquid between their front surfaces and the object being examined it became evident that angular aperture was not the main factor that affected the resolution of a lens. An immersion objective of only moderately high angular aperture could reveal finer detail than dry objectives of greater angular aperture. This discrepancy was examined by Ernst Abbe who showed that the index of refraction of the immersion medium must be considered. He created the term numerical aperture (N.A.).

$$\text{N.A.} = n \sin 1/2 \text{ A.A. } ,$$

where  $n$  is the lowest refractive index of the system from the object through the first lens of the objective.

Using the polarizing microscope the first examination of an unknown material can be made on fragments or a thin section. The earliest thin sections of hard materials were prepared for microscopic examination by William Nicol. They were fossilized wood. He examined them to determine the tree identity from which they were derived. It wasn't until after William Henry Fox Talbot (also arguably the inventor of photography) added the two Nicol prisms to a microscope that the optical properties of transparent material were recognized.

It was Henry Clifton Sorby who showed that minerals in thin section could be identified by their optical properties. Sorby was one of those amateurs of science who contributed much to its advance. He was of independent means and devoted his life wholly to science. He was descended from a long line of master cutlers and his income derived from the family business. He was a pioneer who followed a

particular subject as long as it interested him and then went on to another field. His formal education was at the Sheffield Grammar School followed by private tutoring in mathematics, chemistry, anatomy and crystallography. Sorby was inspired enough to devote his entire life to a scientific career but he never bothered to pass any examinations. His first work was in agriculture but he quickly became interested in Geology. A chance contact with W.C. Williamson who was skilled in the preparation of thin sections of wood, teeth and bone led Sorby in 1849 to apply the method to rocks. He used polarized light microscopy and determined how to distinguish quartz, calcite and chalcedony by methods that he described in 1850. In 1852 on a journey to Germany he met the German mineralogist Ferdinand Zirkel who was so inspired by Sorby's conversations that he devoted his entire life to the microscopy of rocks. He gathered many students around him creating a new formal discipline. Shortly after his return from Germany Sorby revived an old interest in meteorites and went on to the general subject of metallurgy.

The properties used for identification by Sorby and later by Zirkel were such things as crystal form, color, pleochroism, anisotropy, birefringence refractive index and optic sign. These are criteria that are used today for minerals, inorganic and organic materials and even biological materials.

In most polarizing microscopes the polarizer is fixed in place below the stage and can be rotated but is not easily removed from the axis of the microscope. The analyses may be rotated but is removable by means of a hinge or slides.

The first examination of an unknown material is made using plane polarized light. Shape and color are first determined. This is done with the analyser removed from the microscope axis. Certain minerals and materials change in color as the stage is rotated. These materials are pleochroic. A good example is a dark colored mica called biotite. A thin section of biotite cut transverse to the platy cleavage may show a change of color from light yellow to dark

brown. The reason is that biotite being anisotropic is doubly refracting. The light passing through it is split into two rays polarized at right angles to each other.

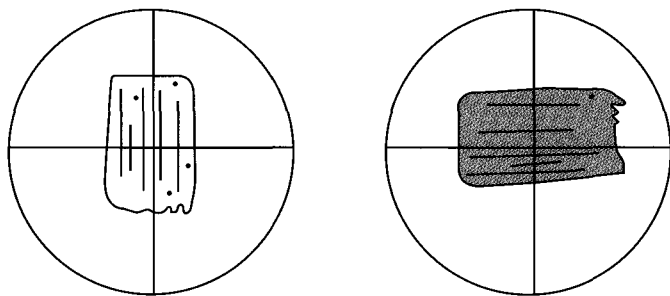


Figure V-2. Pleochroism of mica.

The two rays undergo different amounts of absorption and give different colors when viewed separately. This is possible because each vibration direction in the mica is viewed separately since the plane of polarization of the light coming from below the stage is fixed. As the stage is rotated one vibration direction at a time is parallel to the vibration direction of the polarizer and can be observed. When the mica section is rotated so that the cleavage is oblique to the vibration direction of the polarizer both rays and therefore both colors are seen simultaneously.

The color of transparent materials can be very dependent upon impurity level. However the relative intensities of corresponding directions in differently colored crystals of the same major composition will not change much.

When the analyser is inserted and the planes of polarization of the polarizer and analyser are at right angles the observed field will be black. This configuration of the microscope is also called “crossed Nicols”.

All colors produced by materials between crossed nicols are due to double refraction. Anisotropic directions in sections or

fragments under the microscope are capable of producing colors, but isotropic materials will remain black between crossed polarizers. This includes glasses as well as cubic materials. Continuous blacking out of a grain of a given material during rotation does not necessarily mean that the material is isotropic. It could mean that you are looking down the optic axis of an anisotropic material. If most or all of the grains of a particular material remain black then it is certain that the material is isotropic.

As you rotate most anisotropic materials on the stage of the microscope, from time to time during one rotation they become dark. This is known as extinction. It takes place at intervals of  $90^\circ$  exactly. The extinction is often related to some physical characteristic of the fragment such as cleavage, crystal shape or elongation of crystals. In some materials extinction takes place when the cleavage (as a reference direction) or an elongated crystal is parallel to either of the microscope crosshairs. This is called parallel extinction. If the extinction occurs when the reference direction on the fragment is oblique to the crosshairs this is called oblique extinction and the angle between the crystal or cleavage and the North-South crosshair is called the extinction angle. It can be measured by using the scale on the rotating stage. In lower symmetry crystals the maximum angle of extinction should be determined on several crystals or fragments in order to get a value that can be used for identification.

Extinction occurs whenever one of the two planes of polarization in an anisotropic crystal lies parallel to the vibration plane of the polarizer. When this happens the light from the polarizer passes through the crystal without any deflection and is eliminated at the analyser. This is why extinction occurs four times in a complete revolution of the stage.

When an anisotropic fragment lies with its two vibration planes inclined to the vibration planes of the crossed polarizers it appears colored. The colors reach their maximum intensity midway between two extinction positions. The colors vary according to the properties, thickness and orientation of the material. These colors,

called interference colors are caused by the double refraction of the material. The greater the difference between the highest and lowest refractive index the higher the order of color produced. This difference between the indices is known as birefringence and can be distinctive in a material identification.

The order of color also depends upon the thickness of the section or grain. Petrographers have adopted a standard thickness for thin sections of rocks or minerals of  $0.03\text{ }\mu\text{m}$  or 30 microns so that all minerals may be viewed under similar conditions.

By making use of the substage condenser and high magnification it is possible to get further information about the optics of a crystal. If you look down the optic axis of a uniaxial crystalline material in polarized light that has been made convergent by the substage condenser and using the Bertrand lens to focus upon the back focal plane of the objective lens you can see a dark shadowy cross (Figure V-3a). This is called an interference figure. In the case of a biaxial crystal looking down the line that bisects the two optic axes will produce a figure as in Figure V-3b.

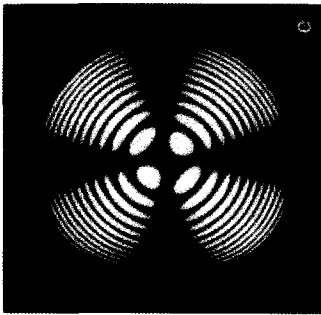


Figure V-3a.  
Uniaxial interference figure.

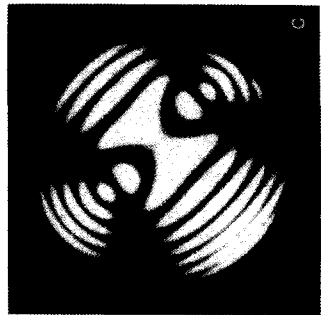


Figure V-3b.  
Biaxial interference figure.

This convergent light technique is called conoscopic observation. By using a polarizing microscope accessory such as the quartz wedge the optic sign can be determined.

In addition to these effects certain materials possess the property of rotating the plane of vibration of polarized light which passes through them. The amount of angular rotation is a property of the material and is also proportional to the thickness of material traversed. This phenomena is “optical activity” and is the property of many organic and inorganic materials as well as liquids. Some materials are optically rotary in the solid state but not in solution. In some cases both the crystals and its solution are optically active. The rotary effect may be observed in isotropic as well as anisotropic materials.

One of the early observers of this effect was the great microbiologist Louis Pasteur. In 1848 at the age of 26 he reported to the Paris Academy of Sciences a remarkable discovery. He found that certain chemical compound were capable of being separated into components that were mirror images of each other. One component rotated polarized light as a right handed screw and the other as a left handed screw. This discovery was from his crystallographic study of tartaric acid which forms during the fermentation of grapes and racemic acid, a product of an industrial process. Tartaric acid was previously shown by the German chemist Mitscherlich to be optically active where racemic acid was not. Both acids had the same composition and seemed to have the same structure. Pasteur showed that he could separate a racemic acid salt into two materials both optically active. One was a tartaric acid salt and the other

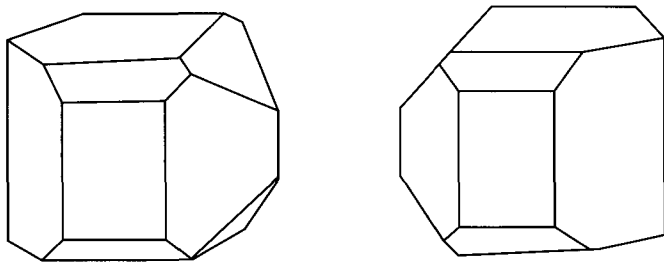


Figure V-4. Crystals of left-handed and right-handed tartaric acid.



a crystallographic mirror image. One salt rotated the plane of polarization clockwise and the other counterclockwise. Both salts had the same chemistry and seemed to have the same structure but they had different properties. One of the two crystal forms of racemic acid was identical to tartaric acid. It turned out that tartaric acid could be used for nutrition by micro-organisms while the mirror image salt was not assimilable by living organisms.

The existence of mirror image forms of the same compound is also called chirality. Many biologically important chemical compounds exist in left and right handed forms each of which may differ greatly in their biologic activity. For example humans can only metabolize right handed glucose and not the left handed form. All amino acids in living organisms occur as left-handed but never right-handed. This early work of Pasteur on the chirality of tartaric acid was the beginning of one of the most varied and valuable careers that eventually led to showing that microorganisms cause fermentation and disease. Later in his career he originated and used vaccines for rabies, anthrax, and chicken cholera and made major contributions to stereochemistry. His was a scientific career that began with a study using polarized light microscopy.

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# Chapter VI

## Reflected Light Microscopy

Early in the history of microscopy scientists looked at metals and other opaque materials as eagerly as they examined the transparent objects that came under their view. Biological materials had a greater impact than the inorganics because parts of living structures were more clearly visible without treatment and because the resulting knowledge had such a tremendous impact on biological science.

Microscopists, early on, exhausted their interest in metals because they could only see surface features and these were more dependent on accidental circumstances than on significant internal structure. In 1664 Henry Power made observations on mercury, gold, silver, iron, copper, tin and lead. On polished pieces of these he observed “fissures, cavities, apertures and irregularities”. On mercury he reported that even the tiniest sphere seemed like a globular looking glass. Power also observed the fused globular nature of sparks struck by flint from steel, but credits the explanation of their origin to Robert Hooke, of fame for his *Micrographia*.

Although Hooke was certainly not the first to use the microscope and did not invent the compound microscope he is often given credit for it. His writings on its use surpass all others of his time in meticulous observation and careful draftsmanship. His microscopic observations are illuminated by discussions of a variety of physical, chemical and biological topics, however his metallurgical observations are somewhat disappointing. His first subject is the point of a needle which he regards as very gross compared with naturally occurring points. He next examines a razor which at high magnification “I could not find that any part of it had anything of sharpness in it”. When he set the razor between lens and light so that

reflections occurred from its very edge he observed scratches perhaps caused by dust falling on the hone. The parts of the razor that had been finished on a grinding stone appeared rougher, “looking almost like a plow’d field with many parallels, ridges and furrows”.

Hooke observed, as did Power, the vitrification of sparks struck from steel by flint. He explained this as due to the violence of the concussion working on a very small fragment of the steel which became heated. The globular form came from the natural tendency of all fluids to acquire this shape. Hooke observed the interference colors that occurred during the hardening and tempering of steel and following this study considered the composition of steel. During this time Hooke’s contemporaries considered steel to be the purest form of iron. Hooke believed steel to be iron in which certain salts had been intermixed with the iron during melting. Hooke’s observations were published in 1665 in *Micrographia*.

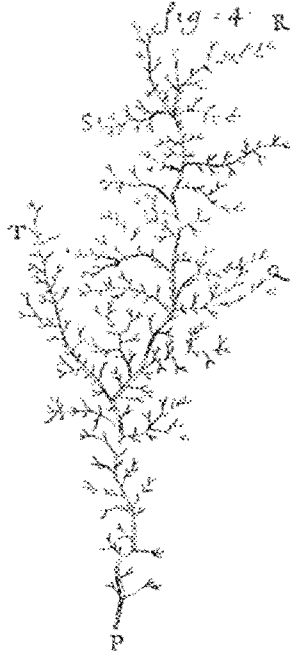


Figure VI-1. “Silvertree”, observed by Leeuwenhoek.

Leeuwenhoek using his simple microscope observed the fine structure of so much of nature but really did not see much to elucidate the structure of metals. His first observations were in 1702 where he described the notches and holes in the edge of a razor. Leeuwenhoek observed a “silver tree” grown by placing a copper fragment in silver nitrate solution. He did not seem to recognize the crystallinity of the tree.

By 1708 Leeuwenhoek did observe the crystallinity in a fracture surface of a razor and concluded that cast iron was composed of coarse particles and as the iron “crystals” became smaller the iron or steel became more valuable. He also observed the joining of particles in forged metal. By the latter part of the eighteenth century observations of fracture of iron and steel had been made and chemists found the distinction between hardening carbon and cement carbon in heat treated steel. This shows how much could be learned about steel without a knowledge of its microstructure.

As the eighteenth century went on microscopes became common but there are very few references to metal structure before the work of Sorby. In 1850 after his trip to Germany, Sorby again started examining meteorites and then continued on to smelted metals. During this time and indeed for most of his life Sorby lived quietly and uneventfully. He frequently traveled to London and elsewhere to attend scientific meetings and was a member of numerous scientific societies. He was made President of the Geological Society, The Royal Microscopical Society and the Mineralogical Society. In his later years he became active in many civic organizations eventually becoming President of Firth College which later became the University of Sheffield. For most of his life he was a member of a local organization the Sheffield Literary and Philosophical Society. This was a common local focus for intellectual activity in nineteenth century industrial England. This small society brought many professional scientists to Sheffield to lecture and had papers of high quality presented by its own local members. There were talks on local geology, chemistry, archeology and other topics particularly

metallurgy as would be natural in the city of Sheffield. In 1860 John Holland presented a paper "On Ornamental Etching in Sheffield". He discussed the forms of etching in use for ornamentation of manufactured goods, for making plates, for printing trade books and for producing pictorial works. The art of ornamenting polished steel articles by etching had been practiced in Sheffield for seventy years or more on such items as razors. An ornamented saw was exhibited at the 1860 meeting manufactured by the firm of Messrs. Sorby. Although Sorby probably did not pay much attention to the operations of his ancestral cutlery firm it seems likely that his evenings at the Literary and Philosophical Society planted the seeds of what would be his studies of the action of acid on metals.

Sorby had a good friend, William Baker, who in 1863 presented a paper entitled "On the Processes Employed in Refining Iron and Steel". It is believed that since Baker and Sorby were friends and Sorby had shown etched samples of steel to Baker before his lecture that this paper led to Sorby's microscopic studies.

Fortunately for the history of microscopy Sorby kept a diary over the years 1859-1908. In many ways this would be the equivalent of a research notebook; the keystone of all scientific research. During the early years of his diary Sorby recorded experiments on the equilibrium between liquids and solids at high pressure, probably because of his geological interest. He also referred to an examination of meteorites. In 1863 and for the year after the diary has many references to the etching of iron. The diary shows that Sorby had many visitors and did not hesitate to talk freely about his work on iron. Sorby's first representation of microstructures were sketched by hand but he soon had the idea of printing without magnification directly from the etched surface. By this time (1864) he had already identified some of the constituents of steel. In answer to a critic Sorby later reported that although others had taken prints from meteorites as early as 1843 Sorby's work on iron and steel was certainly a first. This method of printing was inadequate because it allowed no magnification.

In early 1864 Sorby was experimenting with projecting images of sections on a screen. His diary also shows that during early August 1864 he was making almost daily visits to his friend Charles Hoole. Hoole was listed in Sheffield directories as a commercial photographer. At that time photographic plates were very slow and artificial illumination was very undeveloped. Unfortunately Sorby kept no records of how his early photomicrographs were prepared but it is probable that he used wet collodion plates which were in vogue at the time. He was known to have used oxyhydrogen lamps for projection so he probably used such a lamp for his photographs. His diary in 1864 reports many good photographs of which some were finally published in 1883. Figure VI-2 shows a photomicrograph of wrought iron from a print still preserved in London at the Science Museum in South Kensington.

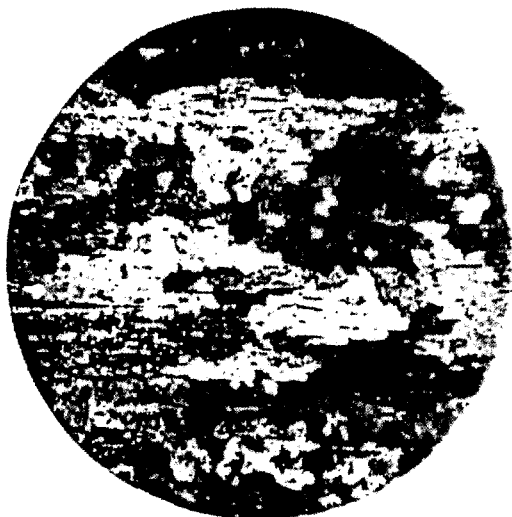


Figure VI-2. Photomicrograph of wrought iron armor plate (9 x) (Sorby, 1864).

Sorby's interests changed very often and he drifted back to meteorites by 1865. His diary didn't show any interest in iron and steel until 1884 after he had already become president of Firth

College. By 1887 Sorby published a definitive paper covering his work on iron and steel up to that time. This paper contains some of the most fundamental aspects of our present knowledge of iron and steel. He refers to seven well marked constituents and to the recrystallization of iron upon annealing.

Sorby's great contributions were based on his accurate observations in the microscope. He was able to relate the properties of iron and steel in various forms to visible structural changes. He showed that metals were undoubtedly crystalline. He observed distorted crystals and realized they were unstable and would recrystallize under suitable treatment as annealing. Sorby realized that as increasing amounts of carbon were added to iron a sequence of constituents appeared. He observed the "pearly constituent" and determined it to be a lamellar structure composed of alternate layers of pure iron and layers of a compound of iron and carbon. This structure resulted from the decomposition of a constituent that was homogeneous at high temperatures. Both iron and the iron carbide are stable at all temperatures. The high hardness of quenched steel is due to the suppression of this decomposition and tempered steel (cooled slowly from a high temperature) becomes softer because of the separation of the two phases.

The seven microscopically distinct phases that Sorby identified in steel have become commonplace names to today's metallurgist.

Sorby's photographs must have had a disturbing influence on the steel manufactures of his day as they show the lack on uniformity of their products.

An important part of Sorby's contribution was his polishing technique. His techniques are described in detail in his publications. Because of his previous experience in polishing rock slices he used the same slicing technique on iron and steel. He cemented the slice to a glass microscope slide and covered the polished and sometimes etched sample with a canada balsam cemented cover glass. Thanks to this technique some of his slides are still preserved in their original state and of a quality up to the best of today's metallographer.



It is interesting that Sorby's work did not become very well known when it was first presented in 1864. He enlarged and rewrote the work due to the influence of his friends in 1887 with photomicrographs and higher magnifications because of the availability of improved vertical illuminators. The years between the two papers showed a slow development of Sorby's ideas and observations and followed by a rapid outpouring of studies. It is interesting that such an early study on steel, a very complex material, preceded by many years studies of very much simpler materials which lead to many of the fundamental principles of metallurgy.

From the earliest days of microscopy observations were made on transparent objects. Opaque materials were occasionally observed but certainly not in any detail. As early as Leeuwenhoek's time the "Lieberkühn" reflector existed. This was a concave reflector which was mounted on the microscope with the lens in its center and the concave reflecting surface mounted facing away from the observer. When the object was held up to the light the rays were reflected from the Lieberkühn to the solid specimen. This gives a very evenly illuminated specimen. Leeuwenhoek himself published a design for such a device. It is not clear why the name Lieberkühn was attached to the device since he lived more than a century after the device was first described. Dr. Johann N. Lieberkühn was a celebrated 18th century German biologist and microscopist. In 1740 he demonstrated a device consisting of a cup shaped mirror surrounding the objective lens of a microscope for illuminating opaque objects to the Royal Society. This and his ability to popularize such a device is probably why his name became attached to it.

Sorby used a parabolic reflector manufactured to his design by R. Beck in London. Sorby designed improvements in the illuminator as shown in Figure VI-3.

When Sorby placed a small reflector (g) over the objective lens of his microscope he was able to distinguish slag from the hard constituent of steel. He considered such a device indispensable to the study of iron and steel. The oblique illumination produced by the

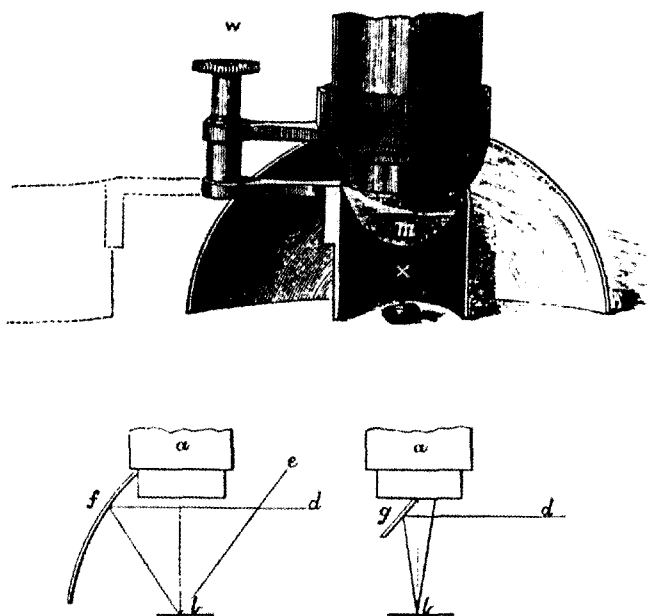


Figure VI-3. Sorby's microilluminator using Beck's parabolic reflector and an added flat mirror (from Quarterly Journal Microscopical Society, 1865).

parabolic reflector caused a polished surface to appear black while a roughened surface appeared light colored due to diffuse reflection.

When the small flat reflector is half over the objective lens a polished surface appears bright and a rough surface comparatively dark. This distinguishes a slag from the very hard constituents of some steels which are slightly etched by the acids he used. This was an indispensable technique in these early studies of iron and steel.

The microscopic study of opaque minerals followed slowly after the studies of meteorites, iron and steel. Sorby had laid the groundwork of microscopic petrology in his early work on the properties of the transparent minerals. However it was a Swedish chemist Jöns Jacob Berzelius who suggested the microscopic examination of polished surfaces of opaque minerals. In 1813 he observed the mineral pyrrhotite, an iron sulfide and noticed areas of

different color. It was later determined by chemical analyses that he observed different chemical phases. Berzelius' great contribution to mineralogy was the idea of a classification of minerals based on chemistry. He based this on his early development of analytical chemical techniques.

Following Sorby there was a long and intermittent growth of knowledge of the opaque minerals. By 1860 A. Reuss had microscopically examined native arsenic from Příbram in Bohemia and discovered the presence of inclusions of native antimony. Step by step the properties of opaque minerals in the microscope began to appear in the literature. In 1905 Waldemar Lindgren one of the better known economic geologists of this century described the microscopic relationships of the copper minerals of Morenci Arizona. A major contribution was the study of J. Königsberger in 1913, on the effects of minerals on the reflection of polarized light. The fact that

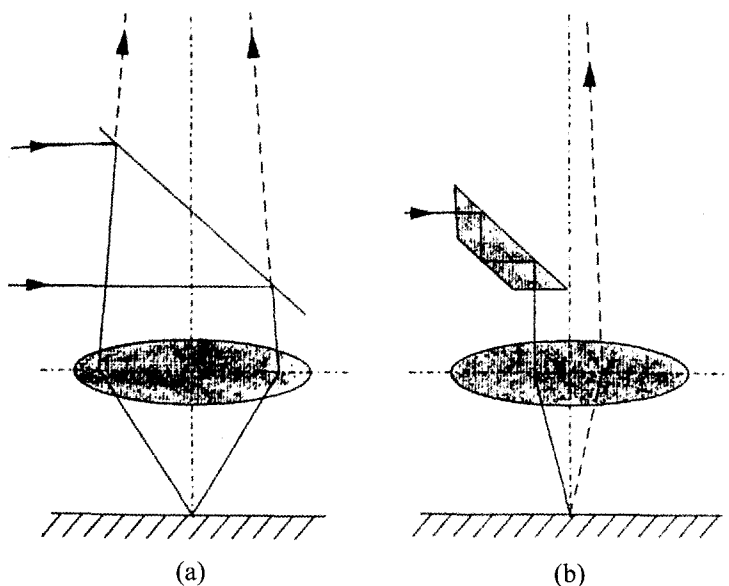


Figure VI-4. Illustration of reflection microscopy a) Semi reflecting mirror and b) Berek prism.

anisotropic minerals could affect a beam of polarized light created a major tool for identifying opaque minerals. At this time the Lieberkühn began to be replaced by microscopes where the incident beam went straight through the objective lens and was reflected back up through the same lens to the eyepiece.

Over the period 1920-1934 H. Schneiderhöhn and P. Ramdor produced the definitive work of the properties of ore minerals.

Reflected light methods yield fewer data useful in identifying minerals than polarized light microscopy by transmission. The determination of opaque minerals has depended on properties such as relative hardness, comparison of color and brightness and micro chemical tests. Newer techniques are now available for measuring micro hardness and quantitative reflectivity. Chemistry can be directly measured on a polished surface by means of the electron-probe micro-analyser.

In modern reflecting microscopes, fitted into the instrument is a vertical illuminator which reflects the entering beam on to the specimen. Since the beam reflected by the specimen must pass through the tube to reach the eye the reflector must be transparent or only occupy part of the tube. A simple reflector consists of a thin glass plate inclined at  $45^\circ$  to the tube axis (Figure VI-4a) so that the entering beam is deflected on to the specimen. Some of the light is lost by being transmitted through the glass plate and then absorbed on the wall of the instrument. The light reflected forms the beam incident on the specimen. The beam is reflected by the specimen back up the tube to the eyepiece. Some instruments replace the reflecting plate with a total reflection prism which covers half of the tube and the light from the specimen goes through the other half of the tube to the eyepiece.

Figure VI-4 illustrates the optical arrangement in the body tube of a modern reflecting microscope. It will also include a polarizer for the incident beam, first outside of the tube and an analyser in the body tube for the reflected beam.

As previously described, with transmitted light the chief optical effects seen in the polarizing microscope are due to refraction and birefringence. Refractive indices can be easily measured as well as the distinction between isotropic (cubic and amorphous), uniaxial and biaxial crystalline materials. With reflected light microscopy it is more difficult to collect data to identify materials.

An obvious optical property is the amount of light reflected by a particular material. For a given surface this depends also on the quality of polish and the cleanliness. If these things are close to perfect then you can measure quantitatively the proportion of incident light that is reflected. This is an intrinsic property and is also a function of the crystallographic orientation of the reflecting surface. As with transmitted light if a polished surface remains uniformly dark during rotation it is cubic or amorphous or you are looking at the basal plane of a uniaxial crystal. In the case of an anisotropic crystal you can measure a maximum and minimum reflectivity and determine their difference which is known as bireflectance. Instrumentation to perform this measurement is now routinely available for polarizing microscopes. In the case of minerals extensive tables are available to use this measurement as an aid in identification. Another significant measurement is the variation of color with the incident light wavelength. This is known as dispersion and is more pronounced in reflected light than transmitted.

In the case of metals as well as minerals, hardness is a significant measurement. In the hardness instrument a diamond point of pyramidal shape is pressed into a specimen under a known weight and the area of contact is measured. The hardness is defined as load (g) over contact area ( $\mu^2$ ) which gives a scale from tens, for very soft material to over 2000 for very hard materials. The simplest type of indentation hardness tester can be fitted to a microscope and its use is easily learned. Tables of hardness are available for minerals and metals.

It very often becomes necessary to determine the chemical composition of a material as a means of identification. One approach

is the microchemical use of specific reagents for the various elements. These reagents need to have great sensitivity for the element since the quantity attacked is very small. The compounds produced with the element must have a characteristic color and the test must be specific and not give a similar reaction with other elements. These reagents have been developed over many years by chemists for use as spot tests. Due to the corrosive nature of these reagents many laboratories will reserve a particular microscope for doing chemistry. In recent times these chemical procedures are less in use because of the availability of the electron probe micro-analyser.

Although the methods described in this chapter are applied to metals and minerals, particularly ore minerals, the principles involved have carried over to a large number of fields including Materials Science, Forensic Science, and study of art materials for both historical purposes and authentication.

# Chapter VII

## Particles and Waves

In 1801 a British Scientist Thomas Young conducted an experiment in which he let filtered sunlight pass through a pin hole and then on to a surface with two more pinholes. The light then fell on to a screen where he observed alternating strips of brightness and darkness. These are interference fringes. The bright strips are where both waves from the pair of pinholes are at a peak or trough and the dark strips the result of one trough overlapping the other peak. This kind of interference can be seen whenever two waves overlap in space and time. It doesn't matter if the waves are light, water or any other observable form. This experiment as well as the experiments of Fresnel would have convinced most scientists of the wave nature of light except for the problem that the great Isaac Newton in 1704 described his corpuscular theory of light. The politics of that day being what it was kept Young's work from being accepted for a long time. Eventually his work was realized to be right until the issue was confounded again in 1900 by Albert Einstein. He explained the photoelectric effect; where light landing on certain metal surfaces causes electrons to be released. This makes sense if light is considered to be traveling as discrete particles. Einstein called these light quanta and are now known as photons. Instead of a continuing argument this has led scientists to the concept of wave-particle duality. It turns out that the duality also applies to such "particles" as electrons and neutrons. At the same time as Einstein was explaining photoelectric effect Max Planck was developing the quantum theory which also helped to turn the physicist's world upside down.

L.L. Marton one of the early pioneers of electron microscopy has pointed out that electron optics started long before the electron

was discovered. The earliest observations of electron optics go back to the observations of the northern lights; the aurora borealis.

Many early scientists contributed to the studies of the aurora among whom are Joseph Henry (1797-1878) Karl Friederick Gauss (1777-1855). Benjamin Franklin (1706-1790) and Edmond Haley (1656-1742). Marton believes that Haley (the discoverer of Haley's comet) was the first to show the relationship between disturbances of the earth's magnetic field and occurrences of the aurora.

Interesting light phenomena resulting from an electric current passing through a partially evacuated glass tube has been known since 1706 (Francis Haukbee). The propagation of the rays from the cathode (negative electrode) was observed by Julius Plücker in 1859. Focusing of the rays was demonstrated by William Crookes who used a concave cathode and showed that the beam appeared to be normal to the cathode surface. This certainly pointed the way toward using an optical analogy to describe the focusing of electrons.

Early users of cathode ray oscilloscopes were the first to use magnetic or electrostatic fields to concentrate their beams. This occurred at the beginning of this century. Even twenty five years later Dennis Gabor another of the pioneers of electron microscopy and developer of an early magnetic lens was only trying to concentrate the beam in the oscilloscope he was building. Gabor points out that, after the fact, the electron microscope is an obvious invention. After all if you combine the fact that an axial symmetric electric or magnetic field is an electron lens with wave mechanics you can see the possibilities. In particular you can break through the Abbe barrier of resolving power. It was suggested to Gabor in 1928 by Leo Szilard to make an electron microscope. Gabor gave an answer that would probably have been given by most physicists, "Everything under the electron beam would burn to a cinder".

Fortunately as Gabor points out the origin of the idea of creating a microscope using electrons controlled by electromagnetic fields is still somewhat of a mystery but in 1931 several coincidences occurred that might be considered the true birth of electron



microscopy. On the fourth of June 1931 Max Knoll and his student Ernst Ruska reported their experiments over a period of several years with magnetic lenses. During this time they built and demonstrated an instrument which magnified such a simple object as a coarse wire screen. Coincidentally the same year on the thirtieth of May Gunther Rüdénberg of the German company Siemens Schuckert applied for a patent on the idea of combining several electron lenses for the purpose of using them as an electron microscope.

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# Chapter VIII

## The Electron Microscope

Physicists learned that since electrons are smaller than atoms it might be possible to see detail well below the atomic level. In Chapter II we saw that the smallest distance we can resolve with our eyes is about 0.1 to 0.2 mm. This is the resolving power of our eyes so any instrument that can exceed that is a microscope. The best optical microscopes can resolve about 2000 Ångstroms and the best transmission electron microscope in the single Ångstroms range. It must be remembered that the highest useful magnification obtained in an instrument is governed by its resolution. We cannot “see” electrons. In fact as has been pointed out earlier a beam of electrons would probably destroy your vision so an integral part of an electron microscope is a viewing screen to convert electron intensity to light intensity that can be looked at or photographed. The viewing screen for a long time was a layer of a fluorescent compound. In the most modern instruments it may be an electronic device.

Since electrons are charged particles they can be deflected by a magnetic field. This was known to the people who had been working on oscilloscopes in the early 1930's. The actual “invention” of the electron microscope is surrounded by controversy but there are several notable landmarks in the development of the instrument. On June 4, 1931 Max Knoll gave a talk at the Technische Hochschule in Berlin in which he and his student Ernst Ruska reported on their experiments with magnetic lenses and the actual building of a two lens microscope which was demonstrated earlier that same year.

Coincidentally on May 30, 1931 Günther Rüdénberg of the Siemens-Schukert company filed with the German Patent office for the idea of the combination of several electron lenses used as an

electron microscope. The same year there were two other efforts presented. One is by E. Brücke written in November 1931 and published the following January in which he reported on the development of an electrostatic emission microscope and in June of that same fateful year at an American Physical Society meeting in Pasadena, California two physicist from the Bell Telephone Laboratories (C. Davisson and C.J. Calbick) presented their work on the development of an electron lens. They gave the focal length of a circular hole in a flat plate which is basically an electrostatic aperture lens.

Marton points out in his “Early History of the Electron Microscope” how many people contributed to the invention and development of the electron microscope as well as how much work depended on previous reported results. Marton feels that although legally Rüdénberg is the inventor of the electron microscope because his patent documents were filed to say so but practically the electron microscope is the creation of many people. In 1986 the 79 year old Ernst Ruska received the Nobel Prize for physics.

The instrument to be discussed here is called a Transmission Electron Microscope (TEM). The reason is that the beam passes through the sample and the interaction between the beam and sample are observed on the side of the sample where the beam exits.

Essentially the TEM has three major parts: (1) the source of electrons, (2) the image producing system, and (3) the image recording system.

### 1) The source of electrons

The source of electrons is the electron gun which produces the electron beam. The electrons originate in the cathode which may be a heated V shaped tungsten filament or in many advance instruments a sharply pointed single crystal of lanthanum hexaboride which is heated.

The filament is surrounded by a control grid which is also known as the Wehnelt cylinder. This has a central aperture lying on the axis of the microscope column. The cathode point lies just above

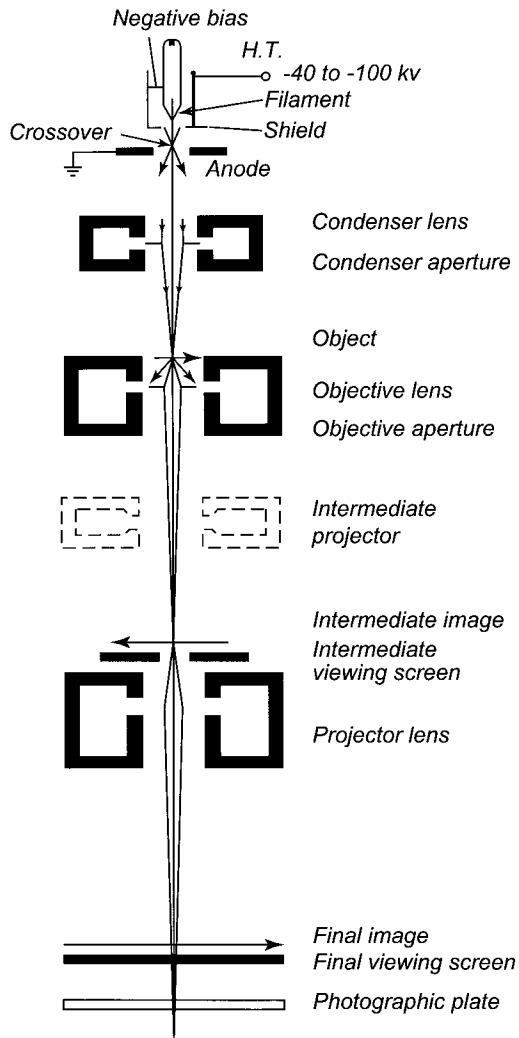


Figure VIII-1. Schematic of a typical magnetic lens transmission electron microscope (TEM).

or below this aperture. The cathode and Wehnelt are at a negative potential equal to the desired accelerating voltage and are insulated from the rest of the instrument. The final electrode of the electron gun is the anode which takes the form of a disc with an axial hole.

Electrons leave the cathode and shield, accelerate toward the anode and pass through the anode at a constant energy. This energy is related to the effective wavelength of the electrons which determine the “optical” properties of the instrument. From classical mechanics deBroglie showed in 1924 that the wavelength of an electron  $\lambda$  is equal to  $h/mv$  where  $h$  is Planck’s constant,  $m$  is the mass and  $v$  is the velocity. Shortly after that Davisson and Germer in their classical experiment demonstrated that when electrons were reflected from the surface of a nickel crystal the resulting diffraction pattern (to be discussed later in this chapter) was consistent with the wavelength given by the deBroglie expression. Since Einstein showed the variation of mass with velocity it is realized that the de Broglie wavelength is only a partial description of electrons. One must also take into account the momentum associated with electrons at rest. After considering these things, at high velocities the expression for wavelength becomes:

$$\lambda = \frac{12.3}{\sqrt{V + 10^{-6} V^2}}$$

where  $\lambda$  is in Ångstroms and  $V$  is in volts.

Some typical values obtained from de Broglie’s expression and then corrected for relativity are shown in the following table:

Table IX-1. Wavelength of electrons as a function of accelerating voltage.

Accelerating voltage (kV)	Nonrelativistic wavelength (nm)	Relativistic wavelength (nm)
100	0.00386	0.00370
120	0.00352	0.00335
200	0.00273	0.00251
300	0.00223	0.00197
400	0.00193	0.00164
1000	0.00122	0.00087

This relationship demonstrates the very important feature that by increasing the accelerating voltage the wavelength of the electrons is decreased. Also as the accelerating voltage increases to above 100 kV the velocity of the electrons becomes half the speed of light and even greater with increasing voltage. All of this becomes very important when we consider the resolution of the electron microscope.

An integral part of the electron source is the condenser system which focuses the beam on to the object. The intensity and angular aperture of the beam are controlled by the condenser lens system which is between the gun and the specimen. Most instruments now use a double condenser. The first lens is very strong and produces a reduced image of the source which is then imaged by the second lens on to the object.

This is a good arrangement because the final size of the illuminated area on the specimen may be varied by controlling the first lens. The use of a small spot size minimized disturbances in the specimen due to heating and irradiation.

## 2) The image producing system

For many materials such as biological slices or inorganic grains supported by a thin cellulose film the specimen may be mounted on a copper mesh grid and held in a holder mounted on a moveable stage. This stage motion may be lateral, rotary or tilt.

The objective lens (Figure VIII-1) is usually of short focal length (one to five mm) and produces a real image of 20 to 200 x. This image is then magnified by the projector lens. Modern instruments use two projector lenses. One of this is called the intermediate lens which permits a greater magnification without making the column longer. The microscope is often operated to give a magnification of from 1000 to 250,000 on the image screen. Higher magnification may be obtained by photographic enlargement. The quality of the final image in the electron microscope depends on the accuracy of the various electrical and mechanical adjustments of the

lenses and the illuminating system. The lenses of a modern instrument require power supplies whose stability is better than one part in a million.

### 3) The image recording system

Electrons are not detectable by the human eye so the electron image must be converted to visible light. The oldest means is a screen of fluorescent material fitted at the base of the microscope column. This screen is used for image focusing and for alignment of the microscope components. A low power binocular optical microscope is fitted to the outside of the viewing window — to allow closer inspection of the screen. Because of the inherent grain of the screen this image lacks good resolution and contrast. By allowing the electrons to fall on a photographic emulsion (plate or film) a high resolution negative may be created which can be enlarged by printing to show all of the detail available in the electron image.

Imaging systems are now available using television type detectors with the final image presented on a cathode ray tube monitor. These systems are electronically amplified so that images can be examined using very low beam current which permits looking at all kinds of materials that would suffer radiation or thermal damage.

## **Interactions with Matter**

Electrons are a form of ionizing radiation. This means that they can remove a tightly bound inner-shell electron from around the nucleus of an atom. The advantage of this process is that a wide range of secondary signals is produced by the specimen and gives all kinds of information that can be used to characterize the material being examined. Figure VIII-2 summarizes some of the signals generated when a high energy beam of electrons interacts with a thin specimen.



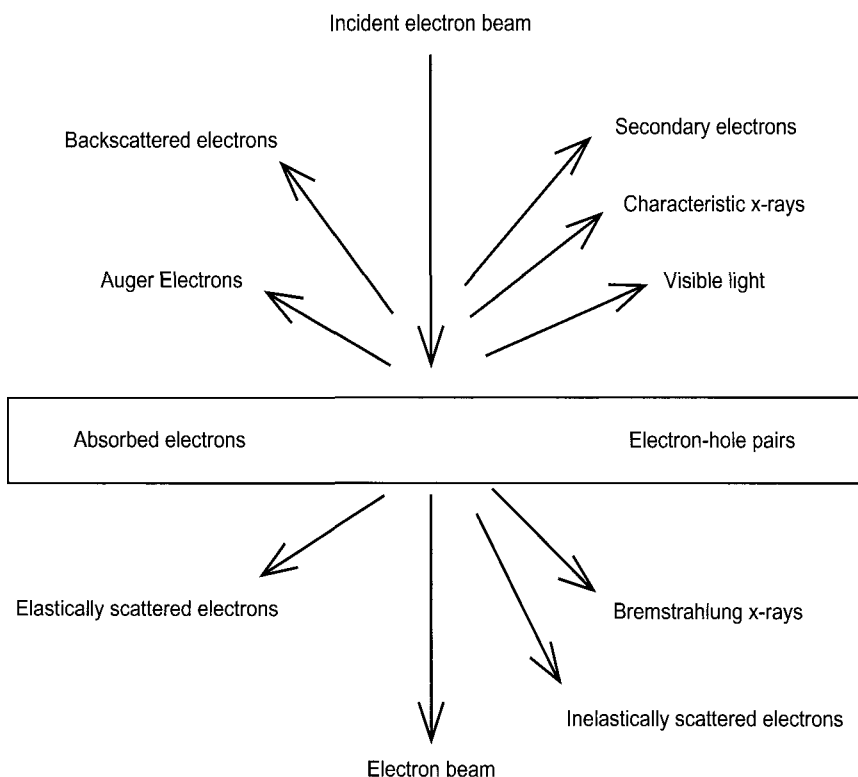


Figure VIII-2. Summary of some signals generated by interaction of the electron beam with a thin specimen.

Many of these signals can give chemical information as well as much other information about the sample. The incident electron beam will interact and remove inner shell electrons from the atoms they hit. This will produce secondary signals from the specimen some of which are shown schematically in Figure VIII-2. An example of this are the characteristic X-rays produced from each element in the sample. These X-rays have a wavelength characteristic of the element producing them. Using energy dispersive spectroscopy it is possible to analyze materials based on the wavelengths of the secondary X-rays produced. A typical spectra is shown in Figure VIII-3.

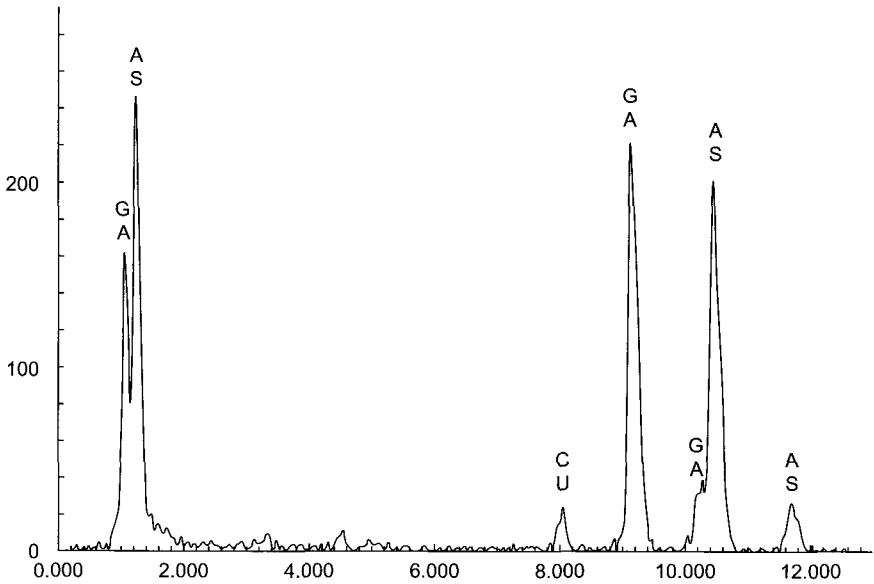


Figure VIII-3. Typical X-ray spectra of three elements.

This type of spectra can be analyzed and give quantitative information about such things as elemental changes associated with changes in microstructure. Such changes may reflect heating, cooling or stress affects on materials. This kind of signal (the spectra) is typical of the signals used in “analytical electron microscopy” (AEM). This type of microscopy may use X-ray energy dispersive spectroscopy and electron energy loss spectroscopy. In energy dispersive spectroscopy the signal is analyzed by a semi-conductor device usually held at very low temperatures. Each element has its own distinctive signature which can be quantified using modern digital techniques.

## **Biology**

By far the application of the electron microscope to biological materials has exceeded its use in all other fields. There is hardly a hospital without a transmission electron microscope used for medical

diagnostic purposes as well as biological research. The early interest in looking at biological materials was first stimulated by L.L. Marton, a physicist member of the faculty of the Université Libre in Brussels. After his early construction of an electron microscope he concluded that biological materials would be an interesting and valuable subject for the new instrument in spite of Gabor's comments that everything under the electron beam would burn to a cinder. Fortunately one of Marton's friends, a biologist, gave him a microtome slice that had been fixed with osmium tetroxide. This created a kind of replica of what he thought would be heat sensitive material and allowed the production of what was the first biological electron micrograph. In 1934 he presented this work at a scientific meeting and published it in a scientific journal later that same year. He then attempted to protect the slice from heating by placing it on a thin aluminum foil in the electron microscope. This caused a loss of contrast but gained considerable detail. In addition to the cell walls observed in his first micrographs he was then able to visualize cell nuclei. These pictures were presented later that same year 1934. Marton's further contributions at the same time consisted of the use of a thin supporting film of collodion and of being able to prepare much thinner microtome sections. He also had idea of looking at living materials but the vacuum in the microscope and the heating affect of the electron beam make short work of this idea. It was not until very recent times that Marton's ideas would come to fruition in the Environmental Electron Microscope. Some examples of the use of staining in a modern electron microscope are shown in the following photographs.

In Figure VIII-4a which is rat bone marrow the contrast in an unstained section would be the result of elastic scattering of the primary beam as it passes through the specimen. Biological material is primarily composed of low atomic weight elements which are weak scatters of electrons making the contrast very low. Scattering is enhanced by staining sections using solutions of compounds of heavy elements such as uranyl acetate, lead acetate or osmium tetroxide.

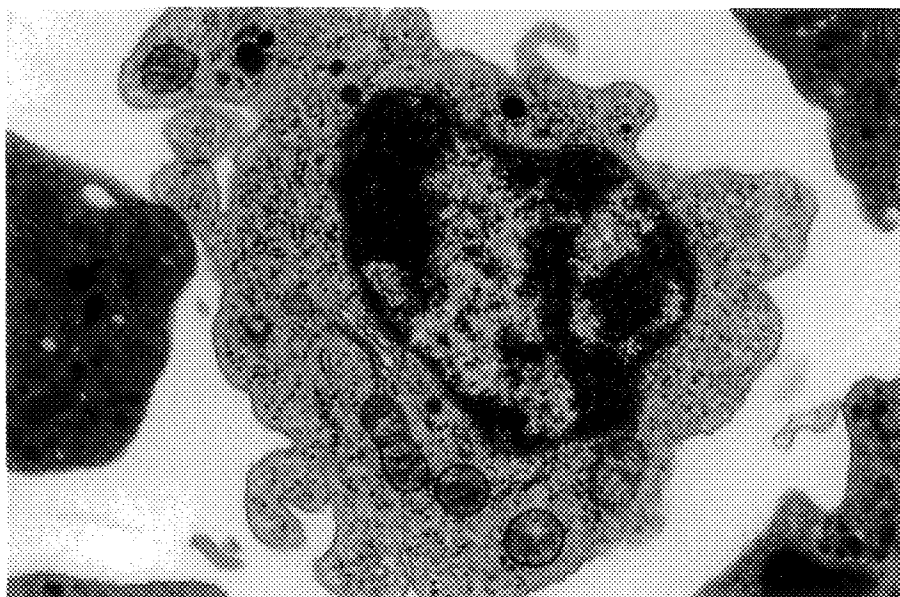


Figure VIII-4a. Rat bone marrow fixed and stained (16,500 x).



Figure VIII-4b. Rat nerve showing fine detail of myelin sheath around nerve (53,500 x).

Individual compounds have particular affinities for specific areas. Uranyl acetate for example has an affinity for DNA containing nucleoprotein and phosphate groups. In Figure VIII-4b the rat nerve has been fixed with glutaraldehyde and then osmium tetroxide. Because the osmium is slow to penetrate the specimen it imparts contrast mainly to membranes. The tissue was then stained with uranyl acetate and lead acetate.

## **Resolution**

The resolution of a TEM can also be thought of in terms of the Rayleigh criteria for light microscopy where the resolution is directly proportional to the wavelength of the radiation and inversely proportional to the product of the refractive index of the viewing medium and the size of the semi angle of the collecting lens. This latter product called the numerical aperture is approximately unity so introducing the proper factors the resolution is equal to approximately half the wavelength of the light.

For light in the middle of the visible spectrum the resolution of a light microscope is about 300 nm. This is about 1000 atomic diameters which is well above the ability of the optical microscope to observe the things that control the properties of materials. This is why with the possibility of being able to image down to the atomic level has led to such great use of the TEM.

Since we have seen how de Broglie's equation relates the wavelength of electrons to their energy for a 100 kV electron. The wavelength is approximately 0.004 nm which is much smaller than the diameter of an atom. Because we are nowhere near building electron lenses that approach this resolution but we are getting closer. Since the mid 1970's many commercial TEMs have been capable of resolving individual columns of atoms in crystal. This is the relatively new field of "high resolution transmission electron microscopy. A typical HRTEM image is shown in Figure VIII-5.

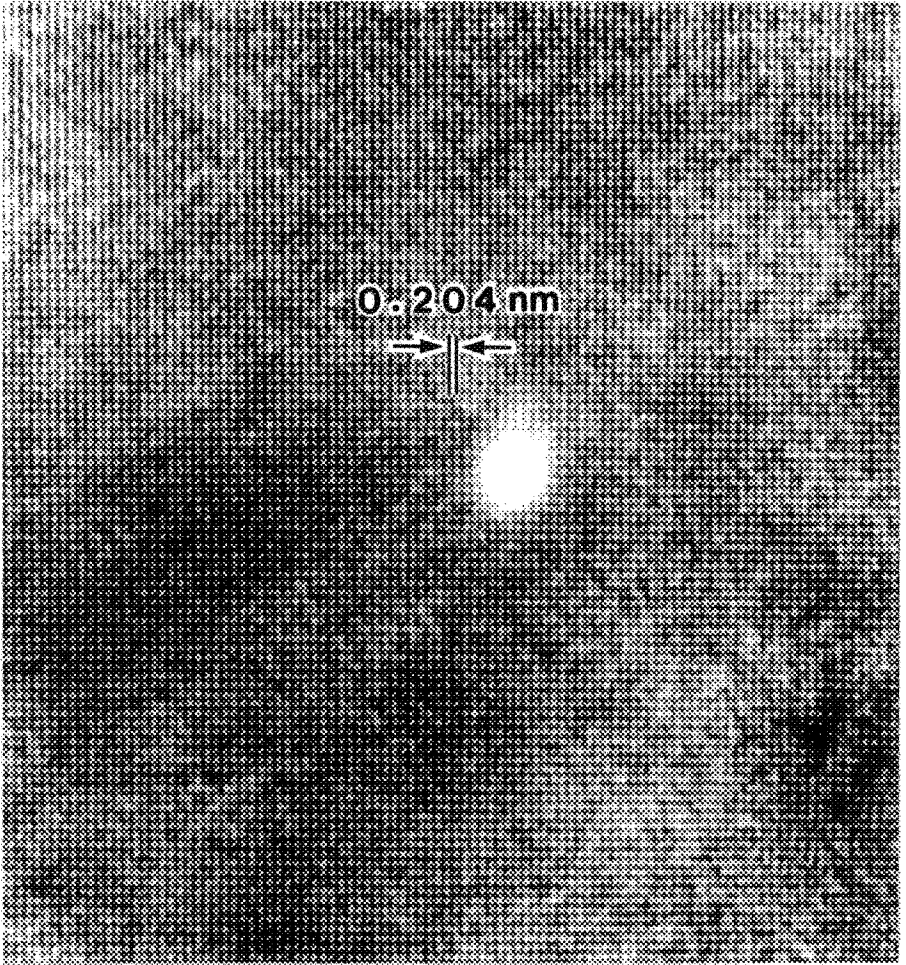


Figure VIII-5. Lattice image of Gold (100) direction (courtesy of David Harling at JEOL Corp.).

### **Electron Diffraction**

When a beam of electrons passes through a crystal it will be scattered into a series of beams whose geometrical distribution depends on the geometry of the atomic arrangement in the crystal. Max von Laue demonstrated the diffraction of X-rays by a crystal in 1912 and since

an electron beam has wave properties it should also be diffracted. G. Davisson and Lester Germer at Bell Laboratories in 1927 diffracted an electron beam passing through a thin nickel crystal. By 1939 Kossel and Mollenstadt showed that electron diffraction could be combined as an integral part of electron microscopy.

A crystal is composed of a periodic array of atoms which von Laue showed would scatter X-rays in a periodic manner. Davisson and Germer showed the same type of scattering, called diffraction, for electrons. This diffraction effect periodicity is related to the periodicity of the symmetry of the crystal. If the crystal is larger than the beam the diffraction pattern will consist of a periodic array of images of the beam as in Figure VIII-6. This diffraction effect is one of the most powerful tools in the TEM for both identifying materials and determining many of their properties.

Von Laue theorized and his two assistants Frederick and Knipping demonstrated that the diffraction waves are in phase if the path difference between waves scattered by adjacent scattering centers are related by whole numbers of wavelengths. This leads to a set of equations to cover the three dimensional case of a real crystal. This generally holds for electrons as well as X-rays. For this work in 1912 von Laue received the Nobel Prize in Physics. These complex equations were simplified by W.H. Bragg who showed that the waves behaved as if they were reflected off of planes of atoms in the crystal. From this simplification he developed the expression now known as Bragg's Law:

$$n\lambda = 2d \sin \theta ,$$

where

$\lambda$  = the wavelength of the radiation (electrons, or X-rays),

$d$  = the interplanar spacing of the reflecting planes,

$\theta$  = the angle between the planes and the incident beam when the equation is satisfied.

The angle  $\theta$  is called the Bragg angle and is the most important scattering angle in the electron microscope. W.L. Bragg

and his son W.H. Bragg received the Nobel Prize for this work. Many properties of the material under study may be deduced from the diffraction pattern. The symmetry of the pattern will reflect the symmetry of the crystal. Knowing the wavelength of the electrons the size of the unit cell of the crystal may be determined. The shape of the spots if different than the shape of the beam may indicate such things as lattice strain or disorder.

In the case where the size of the crystals is very much smaller than the beam the diffraction from a set of the same planes in different orientations will produce a set of coincident cones. The film image that intersects these cones will appear as a set of concentric circles of different diameters. This is called a powder pattern (Figure VIII-7). Since we know the wavelength of the electrons and the effective distance between the sample and the film, measuring the circle diameters and calculation the  $\theta$  values will allow the calculation of a set of  $d$  values. These values and value of the relative intensity of each circle can be tabulated and referred to an index called the PDF (Powder Diffraction File) where a search for identification can be made. Data for several hundred thousand inorganic and organic materials are in this file which is accessible and can be searched by computer.

All of the advantages of a TEM have one great drawback. You really are only studying a very small part of the specimen at any one time. Around 1970 Peter Swann estimated that up to that time all the TEMs in the world since they became available had only examined a total of  $0.3 \text{ mm}^3$  of material. Extrapolating to today this volume may double or even triple which makes the TEM not a very good sampling tool. This emphasizes the thought that before you examine a material by TEM it is worth examining it by a technique with less resolution but better sampling such as your eyes, an optical microscope or a scanning electron microscope.



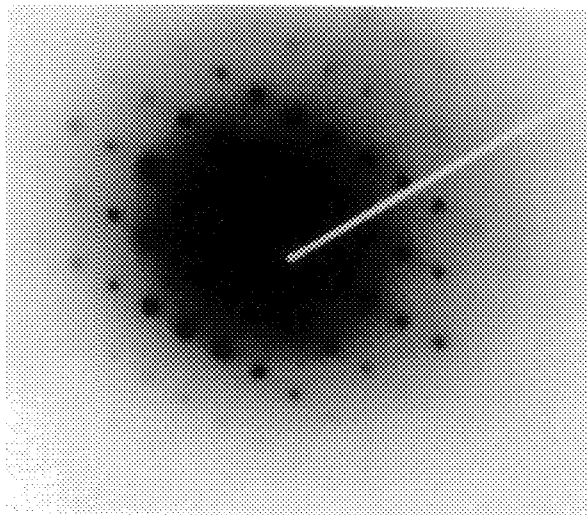


Figure VIII-6. Electron diffraction pattern single crystal Titanium Diboride (200 Kev). Image on film.

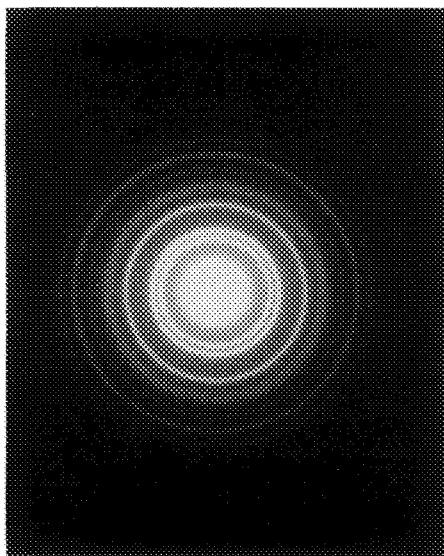


Figure VIII-7. Electron diffraction pattern polycrystalline nickel oxide (powder pattern). Electronic image.

**Endnote**

It is generally acknowledged that L.L. Marton, a physicist, was the first to use an electron microscope to examine biological specimens. It is unique that the microscope was one of his own designs. From this beginning is derived the field of biological electron microscopy with its enormous consequences in diagnosis and prevention of disease.

In the early development of the TEM it was known that as the accelerating voltage was made higher the effective wavelength became smaller giving the possibility of higher and higher resolution as well as an increase in penetrating power of the beam into the sample. In the last thirty years because of the contributions of many engineers and physicists, microscopes with accelerating voltages of over a million volts have been created. These were mainly produced by commercial instrument manufacturers and paid by governments. As time went by the engineering improvements in electron lens quality began to bring microscope resolution closer to the theoretical value of  $\sim 0.7 \text{ \AA}$  (0.7 nm). Unfortunately, the vast cost of these high voltage instruments has prevented their construction in recent times. Since the early high voltage instruments were built improvements in lenses have allowed lower voltage ( $\sim 200 \text{ kv}$ ) commercial instruments to achieve  $\sim 2 \text{ \AA}$  resolution. The progress made in specimen thinning techniques almost insures that such high voltages will no longer be necessary just for sample penetration.

# Chapter IX

## The Scanning Electron Microscope

The scanning electron microscope is probably the most widely used electron beam instrument. At the low end of its operating range its images can be compared to those of optical microscopes while at the high end it is comparable to such instruments as the scanning tunneling microscope. Its resolution now approaches 0.5 nm, close to a transmission electron microscope and many models can handle very large specimens such as industrial gears and silicon wafers. In recent years pictures made with the SEM have been widely published in large circulation magazines as well as scholarly journals. These photographs are notable for their remarkable three-dimensional quality. They convey a sense of reality usually lacking in micrographs made by other means. One example is shown in Figure IX-1. A micrograph made with an ordinary light microscope or a TEM is a two dimensional image. You see a pattern of light and dark areas produced by the transmission of light or electrons through a thin specimen. The light microscope image is two dimensional because of its limited depth of field. It can only be focused sharply in one limited plane. It works best with very thin samples viewed by transmitted light or flat opaque samples by reflected light. The TEM requires even thinner samples for reasons to be discussed.

The SEM had been thought of in the early days of TEM. An early electron microscope pioneer Manfred von Ardenne added scanning coils to a transmission electron microscope. In 1938 he mechanically scanned a photographic plate beneath the sample in synchrony with the beam to produce an image which was really a scanning transmission electron microscope image. The first recognizable, modern, SEM was described by Vladimir Zworykin

and his colleagues at the RCA Laboratories, who in 1942 described many of the features of current instruments such as a cathode ray tube to display the image and a secondary electron detector. Zworykin is remembered mainly for his development of the modern iconoscope or television camera tube and the modern kinescope or television picture tube. He was always disappointed in the use of television for entertainment rather than for educational and cultural enrichment.

By 1948 Prof. C.W. Oatley and his student Dennis McMullan at Cambridge University were working on the development of an SEM which led by 1965 to the first commercial machine the Cambridge Scientific Instruments Mark I "Stereoscan". By this time

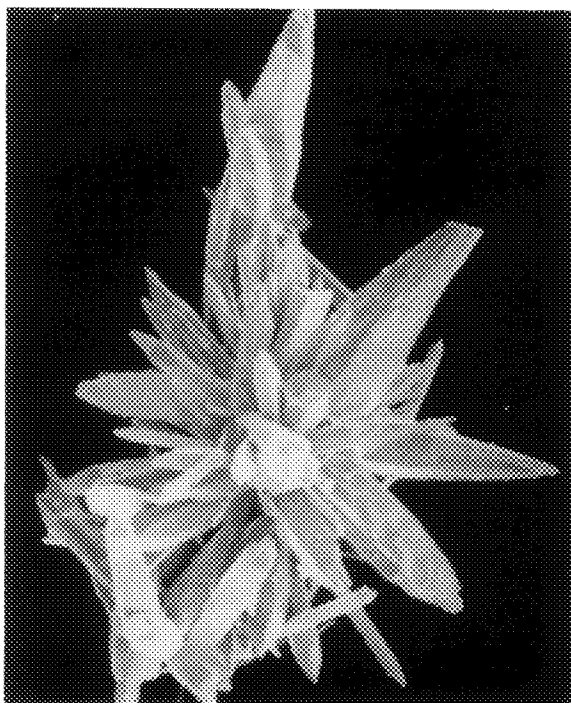


Figure IX-1. SEM image of crystals of the mineral Plattnerite (PbO<sub>2</sub>) magnified 170 times. The sample was carbon coated and imaged at 20 kV on a JEOL scanning electron microscope.

some of Oatley's students were in America and instruments were beginning to be manufactured here as well as in Europe and Japan. At present there may be as many as a dozen manufacturers and many tens of thousands of instruments in current use.

The SEM is a mapping rather than an imaging device. This makes it a member of the same class of instruments as the fax machine, the scanning probe microscope and the confocal optical microscope. The SEM sample is probed by a beam of electrons scanned across the surface. Radiation from the specimen which is stimulated by the incident beam is detected, amplified and used to modulate the brightness of a second beam of electrons. This second beam is synchronized with the first beam and used to excite an image on a cathode ray tube display which may then be photographed. In a transmission electron microscope a very thin sample is required because only those electrons that emerge from the specimen with a single energy can be focused in a single image plane by the magnetic

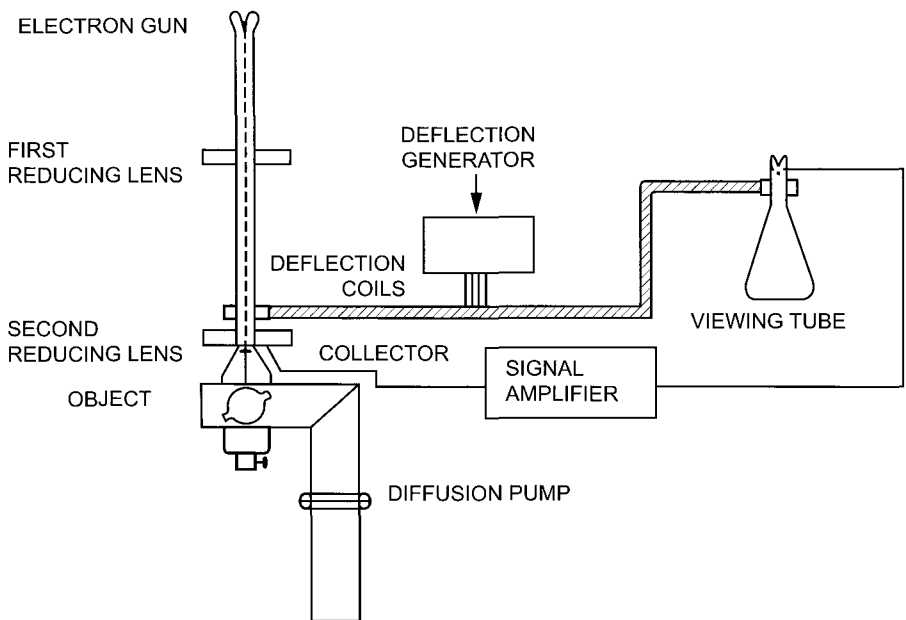


Figure IX-2. Schematic of an SEM; after Zworykin (1942).

field of the objective lens. If the electrons emerged with a wide spectrum of energies they would be bent by varying amounts in passing through the magnetic lens and the image would be blurred. If a reasonable proportion of electrons are to emerge with their energy unaltered the sample must be very thin usually about 500 Ångstroms or about a thousandth of the diameter of a typical living cell. The SEM provides images of three-dimensional objects because in its normal mode it records not electrons passing through but the secondary electrons that are released from the sample by the beam impinging on it.

### **The Electron Source**

The best resolution in the SEM is limited by the final electron beam diameter called the spot size. The current in the final probe determines the intensity of emitted signals such as secondary electrons, backscattered electrons or X-rays. Usually the smaller the probe size the smaller the current can be. The microscope controls are adjusted to produce the most desired result, either high resolution or higher depth of field. The source of electrons is the electron gun or cathode. The purpose of the electron gun is to provide a small electron beam with a large stable current. There are several types in use on SEMs. Most common are the tungsten thermionic types. The tungsten filament has served the TEM-SEM community for over 50 years. It is reliable, its properties are well understood and it is relatively inexpensive. A typical tungsten cathode is a wire about 100  $\mu\text{m}$  in diameter bent to a V shaped tip with a radius also about 100  $\mu\text{m}$ . This cathode is heated resistively to a typical temperature of 2700 K and it may last from 30 to 100 hours before failing. A high brightness source now coming into more common use is a block of lanthanum hexaboride ( $\text{LaB}_6$ ) heated to thermionic emission. This source may be 5 to 10 times brighter than tungsten and has a longer lifetime but it requires a much better vacuum. The first practical  $\text{LaB}_6$  sources used pressed and sintered polycrystalline material. The

present ones are single crystal and about 100  $\mu\text{m}$  in diameter and resistively heated by its support which may be carbon or rhenium. In spite of being ten times the cost these  $\text{LaB}_6$  electron sources are extensively used on both TEMs and SEMs.

The electron sources described to this point depend on high temperatures to enable free electrons in the cathode material to overcome the energy barrier and escape into the vacuum. Field emission is a way of generating electrons that is free from thermal drift and evaporation of cathode material as well as being much brighter. A field emission cathode is usually a wire of single crystal tungsten formed into a sharp point and spot welded to a tungsten hair pin. The significance of a small tip radius (less than 100  $\mu\text{m}$ ) is that an electric field can be concentrated to a high level allowing electrons to leave the cathode without requiring any thermal energy to lift them over the energy barrier. A field emitter may have a current density as high as  $10^5 \text{ A/cm}^2$  compared to  $3 \text{ A/cm}^2$  from a tungsten filament. Electrons emitted from a very small virtual source behind the tip result in a very high brightness. A cold field emission cathode must operate in a clean high vacuum in order to be stable. The tips are vulnerable to catastrophic failure from high voltage discharge. Field emission sources provide high current into small electron probes and this produces excellent SEM images but these sources are not suitable for all applications particularly when larger probe sizes are required. At an Electron Microscope Society of America meeting in 1964, Albert V. Crewe of the University of Chicago suggested using the field-emission source for scanning electron microscopy. By the late 60's using an ultra high vacuum environment Crewe was able to achieve a 5 Ångstrom resolution in the scanning transmission mode. In special preparations he was able to resolve individual uranium atoms.

## **The Lens System**

The electron lenses outside of the electron gun are used to demagnify the image of the point where the electrons from the gun coverage.

This is called the crossover. For a thermionic gun the crossover diameter may be 10-50  $\mu\text{m}$  and the final spot size on the specimen should be from 1nm to 1  $\mu\text{m}$ . This is a demagnification of as much as 10,000 times. Since the source in a field emission gun is so small the demagnification only has to be 10 to 100 times to give a spot size of 1-2 nm.

As in the lenses of a TEM electrons can be focused by either electrostatic or magnetic fields. Most electron microscopes use magnetic focusing in the column outside of the gun because of their lower aberrations. SEMs use one to three condenser lenses to demagnify the electron beam crossover diameter in the gun to a smaller size.

The final lens in the column is the objective lens and focuses the image by controlling the movement of the probe crossover along the optic axis of the column.

## **The Electron Collector**

In transmission electron microscopy the specimen is illuminated everywhere at once. The image is created from the electrons that pass through the specimen and appear on a fluorescent screen which can be photographed. Most commonly the photograph is a direct electron image on film. In the scanning electron microscope a three dimensional image can be observed because it records the secondary electrons that are released from the sample by the electron beam. These secondary electrons do not have to be focused but only collected. The SEM has a great depth of field since the envelope of the impinging beam is like a sharp needle. Under bombardment from the electron beam a variety of radiation leaves the specimen. We have been calling it secondary electrons but it consists of back-scattered electrons, secondary electrons, characteristic X-rays, light from cathode luminescence and many others. The detector must efficiently convert the particular radiation of interest that strikes it to a useable signal. Backscattered electrons are beam electrons which escape the specimen as a result of multiple elastic scattering and



secondary electrons are specimen electrons given a small amount of kinetic energy by inelastic collisions with beam electrons. These have widely differing properties and detection may be selective for one or the other or both.

The secondary electrons are low energy that only escaped from a few atomic layers of the specimen surface. They are easily attracted and collected and are produced in great quantities. They are the source of such memorable SEM images as the dust mite (Figure IX-3) and micro machined gears (Figure IX-4).

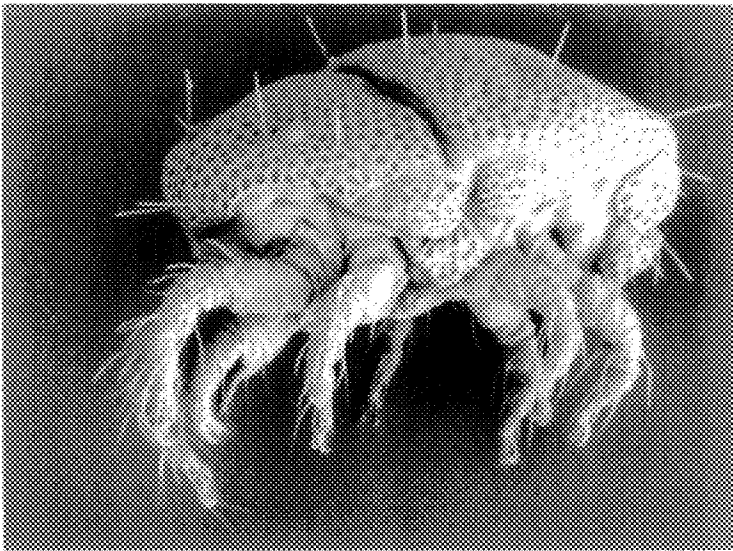


Figure IX-3. Dust Mite. Secondary electron image.

The back-scattered electrons are energetic and can escape from several nanometers beneath the sample surface. Back-scattered electrons travel in straight lines and carry such information about sample chemistry as average atomic number. They are not produced in very large numbers and may be difficult to detect except with the newest technology.

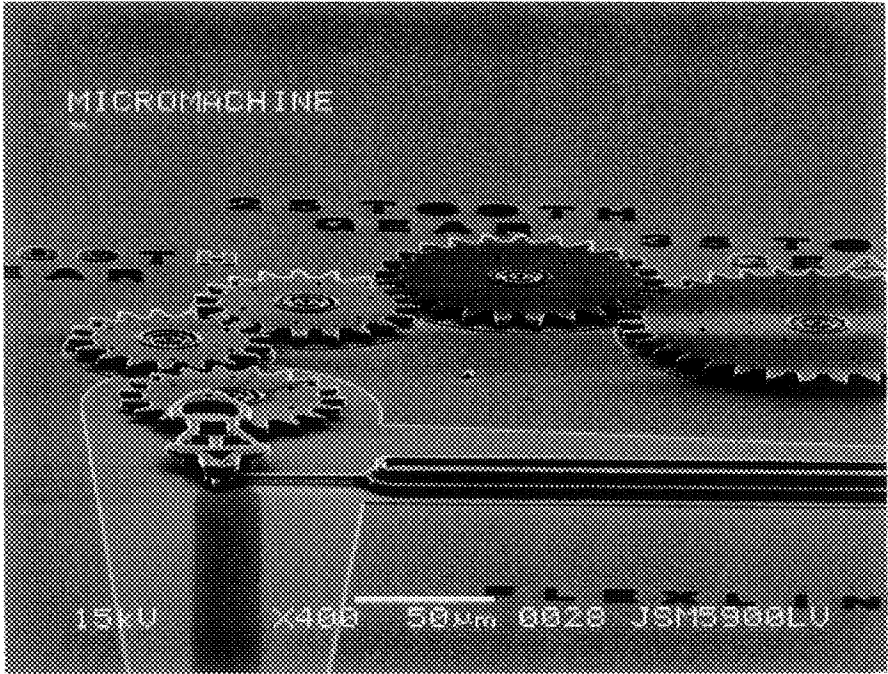


Figure IX-4. Micro mechanical gears secondary electron image.

In the SEM the specimen is scanned in a raster pattern by the focused electron beam. The secondary and back-scattered electrons are collected and used to form the image. The image that results is formed by a technique similar to that used in present day commercial television. There are some differences. The standard TV of today consists of 525 horizontal lines but the SEM can be varied from 100 lines to more than 1000 depending on the required resolution. The coarse scan is used to create a visual image but a finer scan is used for a high-resolution micrograph. The scan of the beam in the microscope is coordinated with the scan in the output oscilloscope.

One of the more commonly used detectors in SEM was developed by Everhart and Thornley in 1960. This came from the work at Cambridge done by Prof. Oatley and his students. This detector creates a signal from both the backscattered and secondary

electrons. It works when an energetic electron strikes a scintillator material, which may be a doped plastic, glass or crystal. A photon is produced. The photons are conducted by a light pipe to a photomultiplier which converts the light flux into an electric current. The electrons are accelerated in the photomultiplier on to successive electrodes and cascade producing a very large gain, typically  $10^5$ . This response is fast enough to operate at the TV scan rate. The detector is surrounded by a Faraday cage which is charged to attract the secondary and back scattered electrons from the sample. The bias on the Faraday cage can be used to distinguish back scattered from secondary electrons.

## **The Image**

As suggested in Zworykin's diagram from 1942 (Figure IX-2) the SEM image is produced by synchronizing the beam in a cathode ray tube with the rastered primary electron beam in the column of the SEM. At a TV scan rate a visual image can be observed on the fluorescent screen of the oscilloscope. The usual technique for photography is to have a very slow raster rate in the microscope as well as the oscilloscope. Two classic examples of secondary electron images are shown in Figures IX-3 and IX-4. Because the insect is insulating it is coated with an evaporated film of gold. If this were not done the surface would build up a charge and not come into sharp focus. The gears are silicon and conduct well enough so that they do not have to be coated.

## **Backscattered Electrons (BSEs)**

These electrons are energetic and can escape from several nanometers below the sample surface. BSEs travel in straight lines and contain such information about sample chemistry as average atomic number. They are produced in large numbers but are not easy to detect. Images produced are usually not sharp and not easy to interpret. Early BSE images were collected by turning off the bias of the

conventional Everhart-Thornley detector and raising the gain. This produced very noisy images.

The real value of the BSE image is the ability to distinguish areas of different average atomic number. This has been of great value to geologists, metallurgists and materials researchers who can observe and select appropriate targets for further analysis. Biologists use it to image heavy metal distributions in cells where proteins have been labeled with compounds containing heavy elements. Biological tissue usually requires special preparation. The tissue must be fixed to hold cells and other parts in position. The water must be removed and the sample dried, mounted and coated with a conductor so that it does not build up a static charge. When an E-T detector is negatively charged it can provide a signal composed of backscattered electrons but it has a very low geometric efficiency and is highly directional in its collection. Because of the large differences in energy between back scattered and secondary electrons, detectors have been developed which are dedicated to back scattered electrons. The earliest and still very widely used detectors are solid state photodiodes. They take advantage of the fact that the BSEs travel in straight lines and that the intensity of the image increases with mean atomic number. Two photodiodes face the sample on either side of the SEM beam. This arrangement allows the user to differentiate between a compositional image and a topographic image. Usually the compositional image appears very flat and the topographic image is quite noisy.

Another type of BSE detector consists of a phosphor coated light pipe coupled to a photo-multiplier. These light pipe detectors capture a very large solid angle of BSEs emitted from the sample without interfering with other SEM detectors. Robinson in 1975 made the scintillator of the same material as the light guide. He also designed this combined scintillator-light pipe as an inverted bowl placed symmetrically above the specimen with a hole for the electron beam a great improvement in the detection efficiency for BSEs.

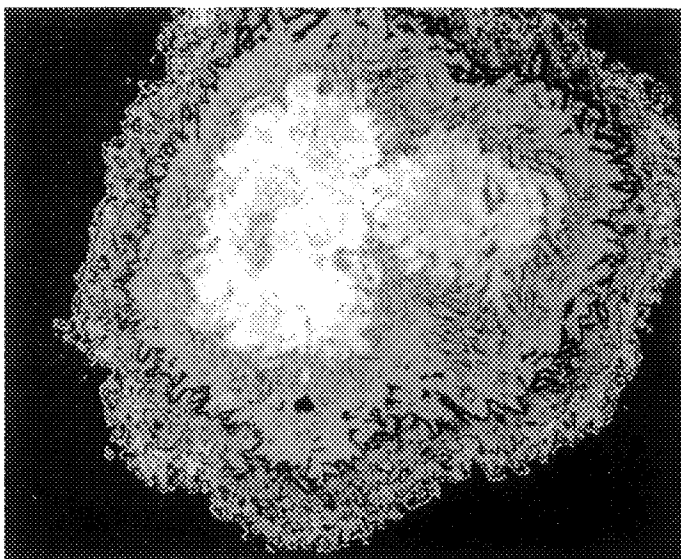


Figure IX-5. Backscattered electron image of a polished section through a polycrystalline nodular specimen of the mineral Duftite  $(\text{Pb,Ca})(\text{Cu,Zn})(\text{As,V})\text{O}_4\text{OH}$  magnification 170 x (David Lange, Harvard University).

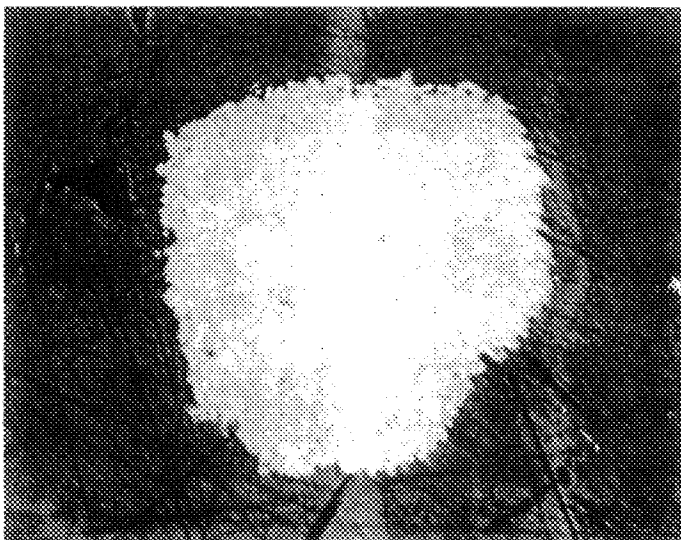


Figure IX-6. Optical image of Duftite specimen. Magnification 130 x.

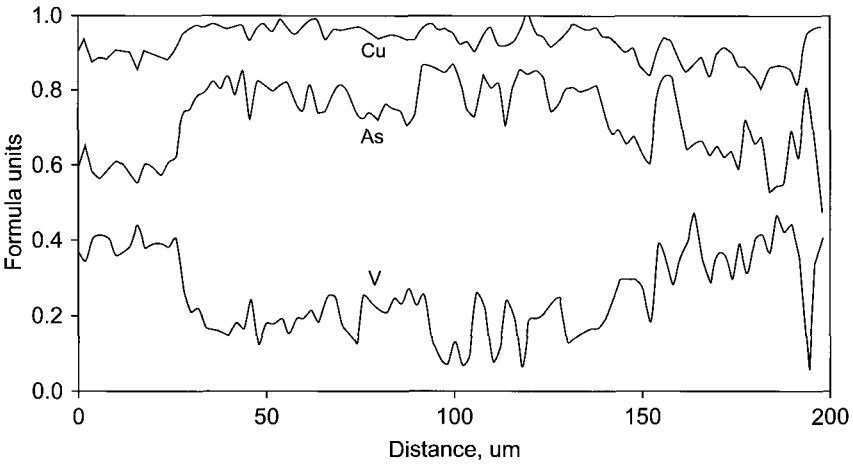


Figure IX-7a. Scan from center of Duftite specimen in Figure IX-5 showing distribution of copper, arsenic and vanadium.

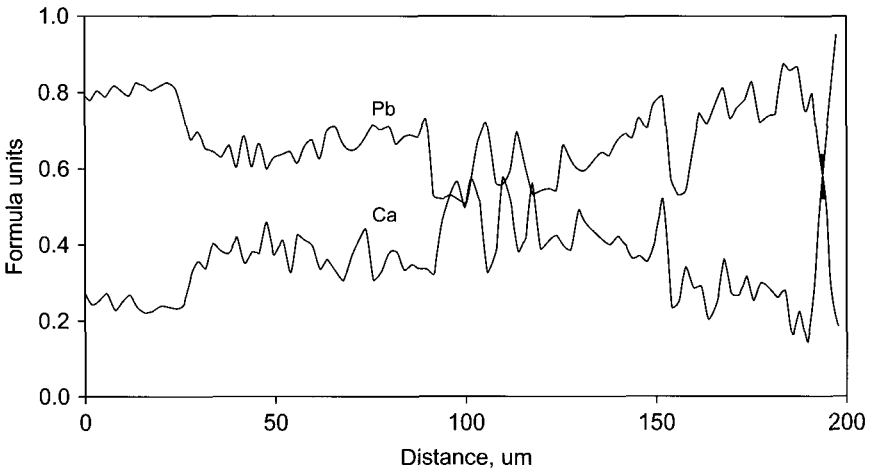


Figure IX-7b. Scan from center of Duftite specimen in Figure IX-5 showing distribution of lead and calcium.

Figure IX-5 made from a polished flat specimen of the mineral Duftite shows how to observe a compositional difference within a single nodule composed of polycrystalline material.

The optical microscope image (Figure IX-6) shows a relatively uniform grain distribution. The back scattered electron image shows a nodular distribution of heavy elements. This kind of information is useful because it tells the investigator to look further and indeed in Figure IX-7 can be seen the variation in chemistry across the nodule which partially explains the results of an X-ray diffraction pattern which did not agree with data for the pure mineral standard. As can be seen from the charts the elements are not uniformly distributed and the intensity of any area of the secondary electron image represents the summation of all the concentrations in the area.

### **Environmental SEM**

The SEM is typically operated with the pressure in the sample chamber below about  $10^{-6}$  torr. A vacuum is measured in terms of atmospheric pressure where a standard atmosphere equals 760 mm of mercury, which is 760 torr. A pressure of  $10^{-6}$  torr is the limit above which the tungsten filament could burn up. The LaB<sub>6</sub> source is more sensitive and must be protected by operating below  $10^{-7}$  torr. The Everhart-Thornley detector operates with a bias of 12,000 volts or more applied to the face of the scintillator. If the chamber pressure exceeds  $\sim 10^{-2}$  torr electrical breakdown could easily occur between the scintillator and the Faraday cage which is close by. A major source of specimen contamination during SEM examination comes from the cracking of hydrocarbons by the electron beam. The higher the vacuum there will be fewer hydrocarbon molecules to interact with the beam. In general the worse the vacuum the more the electrons in the beam will be scattered by collisions. In order to operate the standard SEM in a high vacuum the specimen must be prepared so as not to evolve gases in the vacuum environment. This limits the examination of many important materials such as biological tissues containing water which will rapidly evaporate at reduced pressure distorting the specimen and upsetting the normal operating conditions of the microscope. For normal examination the sample

must be prepared by drying or the water must be immobilized by freezing. Such specimen preparation can lead to sample distortion and creation of artifacts as well as being time consuming.

Environmental scanning electron microscopy obtains the benefits of operating at higher pressure while getting a reasonable SEM performance. Another important application of Environmental SEM is the examination of valuable cultural assets which are not allowed to be coated with metal. This includes such things as coins and art objects. The environmental SEM typically operates between one and ten torr and uses the idea of differential pumping to obtain an elevated pressure in the specimen chamber while maintaining a good vacuum in the electron gun chamber. Differential pumping consists of establishing a series of regions of successively lower pressure; each region with its own dedicated pumping system and separated by a small aperture. These apertures are the electron beam apparatus and are small enough to limit diffusion between the stages.

Since the Everhart-Thornley detector cannot be used at elevated pressure the passive backscattered large area scintillator detectors are commonly used.

An image of cloth with stains is shown in Figure IX-8. In the high vacuum mode the cloth would have to be metallized to prevent charging. In the low vacuum SEM because of the absence of charging the stains are visible.

### **Microlithography**

Modern integrated circuits can contain transistors smaller than 100 nm and gate oxides as thin as 2 nm. SEMs are a useful method of quality control in the integrated circuits manufacturing process. Because of the nature of the process the use of the SEM is employed for failure analysis rather than a continuous inspection system.





Figure IX-8. Environmental SEM image of cloth with stains.

The semi conductor industry makes use of photolithography as a technique for printing conducting images for the production of such things as large-scale integrated circuits.

The masks for lithography are produced by optical means but as the circuits become smaller and more complex electron beam techniques have come into use. The first commercial use came in 1967. It is used for the mask making process as well as for direct writing techniques. Accessories are available to convert an SEM for producing masks and for very large amounts of money there are instruments dedicated to e-beam writing for the production of masks. Typical e-beam writing patterns are shown in Figure IX-9.

The final frontier of e-beam lithography is its use in continuous production. The benefits are the high resolution produced by shorter wavelengths so that more information can be put in a smaller space. This will probably be in the future as engineering reduces the cost to where such instruments could be used in production.

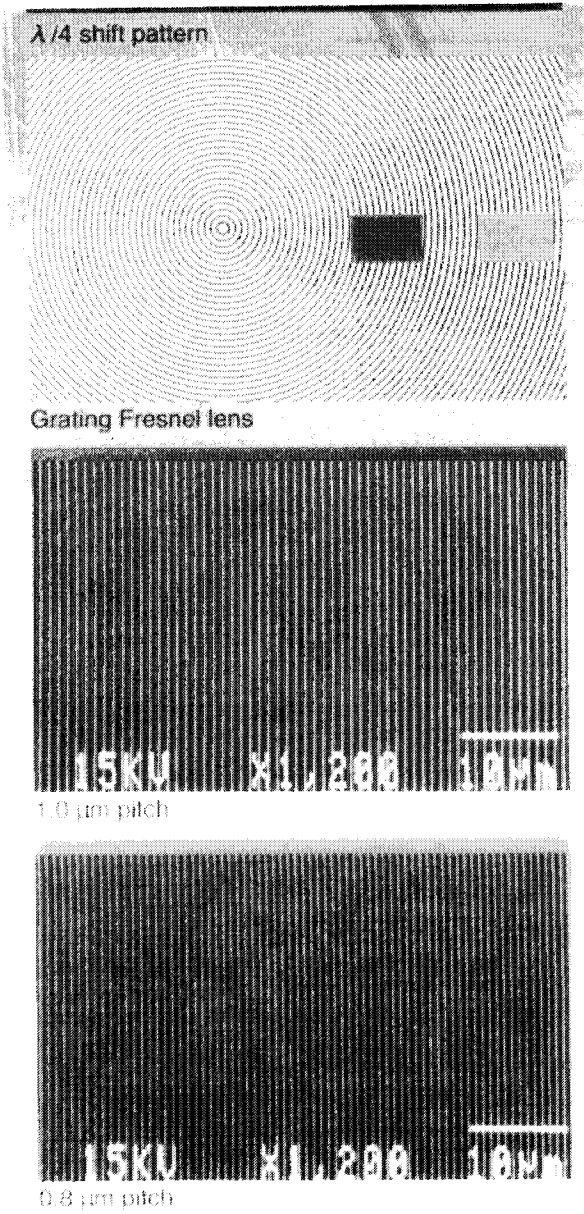


Figure IX-9a. Typical e-beam writing patterns. (Courtesy of JEOL.)

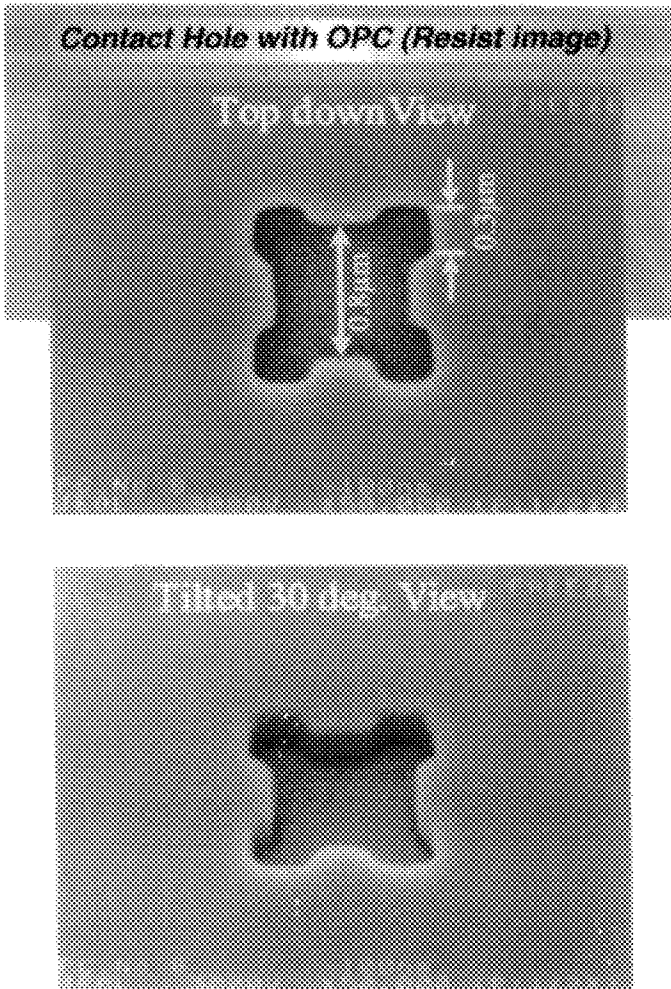


Figure IX-9b. Typical mask for e-beam lithography. (Courtesy of JEOL.)

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# **Chapter X**

## **Chemical Composition From Microscopy**

### **Optical Microscopy**

It is often necessary to determine the chemical composition of a small amount of a material as a means of identification. An early approach was the use of polarized light microscopy as discussed in Chapter V. This technique is amplified by the microchemical use of specific reagents for the various elements. These reagents can have great sensitivity for a specific element since the quantity reacted with is very small. The compounds produced with the element must have a characteristic color and the test must be specific and not give a similar reaction with other elements. Reagents have been developed over many years by chemists for use in spot tests. These are in use in laboratories devoted to such varied fields as forensic science, materials science, mineralogy, fine art conservation, and authentication and metallurgy. With the development of instruments as the Electron Probe Microanalyser (EPMA) optical microchemistry is becoming a lost technology with the notable exception of the McCrone Institute of Chicago. This is unfortunate because of the low cost of optical microscopy as compared to the instrumentation needed for electron beam techniques. Each technique has its place but unfortunately the optical methods are no longer taught at most universities.

### **Electrons, Atoms and X-Rays**

In 1895 Wilhelm Conrad Röntgen discovered a penetrating, uncharged radiation produced when electrons strike a surface in an evacuated tube. This radiation could be observed by the fluorescent

glow of a coated screen or a photographic plate. Within months this discovery had medical applications. In 1905 C.G. Barkla performed experiments showing that scattered X-rays behaved like light and could be plane polarized. This led two years later to his discovery of “characteristic X-rays”. Barkla discovered that when X-rays fell on a material a homogeneous radiation characteristic of that material came from it in addition to the scattered incident radiation. The higher the atomic weight of the material the more penetrating was the radiation it produced.

He found two types of radiation for each element. The harder (more penetrating) is now called K radiation and the softer L radiation. Barkla regarded this phenomena as equivalent to the excitation of a spectrum. By 1907 the scientific world accepted the electromagnetic nature of X-rays and W.H. Bragg proposed that gamma rays and by implication X-rays were particles.

In 1912 Max von Laue with the help of W. Friederich and P. Knipping demonstrated in a famous diffraction experiment the periodic nature of the atoms in a crystal as well as the wave nature of X-rays. This was followed by the work of W.H. Bragg and his son W.L. Bragg who created a spectrograph using crystal diffraction that showed not only the continuum but also the characteristic lines of the target element in the X-ray tube. By 1913 a young English physicist Henry G.J. Moseley determined that the charge on the atomic nucleus revealed more about the atom than its mass. He studied the X-ray spectra of the heavy elements building on the work of Barkla and the Braggs. By using a crystal of potassium ferrocyanide as a grating he examined the X-ray spectra of different metals and could obtain the wave length of the incident X-rays. The X-rays emitted by each element have a unique frequency that differs according to a regular pattern. The difference in frequency was caused not by the mass but by the charge on the nucleus. Mosley called this the atomic number. In 1913 he studied the K series of the elements up to zinc and the following year extended the work up to gold. In a few places Moseley found elements separated by more than one integer and

correctly predicted the existence of new elements which was later corroborated. Because there is only one element for each atomic number scientists could be sure for the first time of the completeness of the periodic table.

Moseley worked in the laboratory of Lord Rutherford at the University of Manchester until the beginning of World War I. He enlisted into the army and was killed at the Battle of Suvlan Bay at the age of 27. The world was deprived of a most promising physicist.

## **EPMA**

At a meeting in Sweden in 1949 Raimond Castaing reported on his ongoing thesis research at the University of Paris and in 1951 he received his doctorate for the development of the Electron Probe Microanalyser which could be used for chemical analysis. The thesis describes the first microprobe built by modifying a transmission electron microscope with electronic lenses. During the following years two prototypes were built and by 1958 the first commercial instrument identical to the prototypes was on the market (CAMECA).

In 1940 Castaing entered the Ecole Normale Supérieure in Paris, the highest academic institution for studying the physical sciences in France. He became the student of Frederic Joliot at the College de France. During the war he was a member of the French resistance and graduated in 1946 with the highest honors. He began his career as a research engineer at the Office National d'Etudes et de Recherches Aeronautiques where he started his doctoral thesis under Prof. A. Guinier. It was this work that he presented at the Swedish meeting in 1949. He received his doctorate in 1951 from the University of Paris which described the first microprobe built by modifying a transmission electron microscope with electronic lenses. This was the prototype of what became the first commercial instrument. The concept of the electron microanalyser is to focus an electron beam on a small area to excite secondary X-rays which can be analyzed. The area of interest is located by a light microscope. This concept first appeared in the patent literature in the 1940's

(Marton, 1941 and Hillier 1947). Castaing in his thesis showed that a localized chemical analysis could be made on the surface of a specimen and outlined the approach by which this could be quantified. Figure X-1 shows schematically what Castaing's "Microsonde electronique" was like.

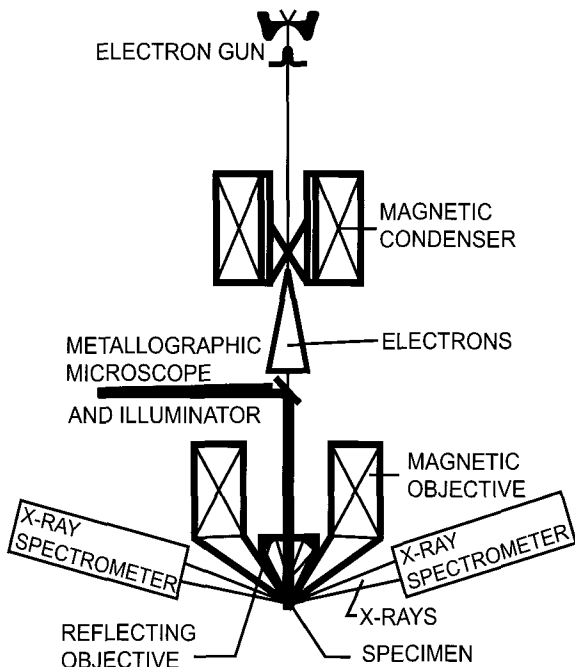


Figure X-1. Schematic diagram of Castaing's "Microsonde electronique".

The electron probe can detect and quantify any atom in the periodic table except for the very lightest ones. For elements with an atomic number greater than ten the detection limit is about 0.1% by weight. The detectability is less sensitive at either the highest and lowest atomic numbers. In general the accuracy will be about 2%. Electron microprobes can be dedicated instruments as shown in Figure X-2 or a system can be an add-on to a TEM or SEM.



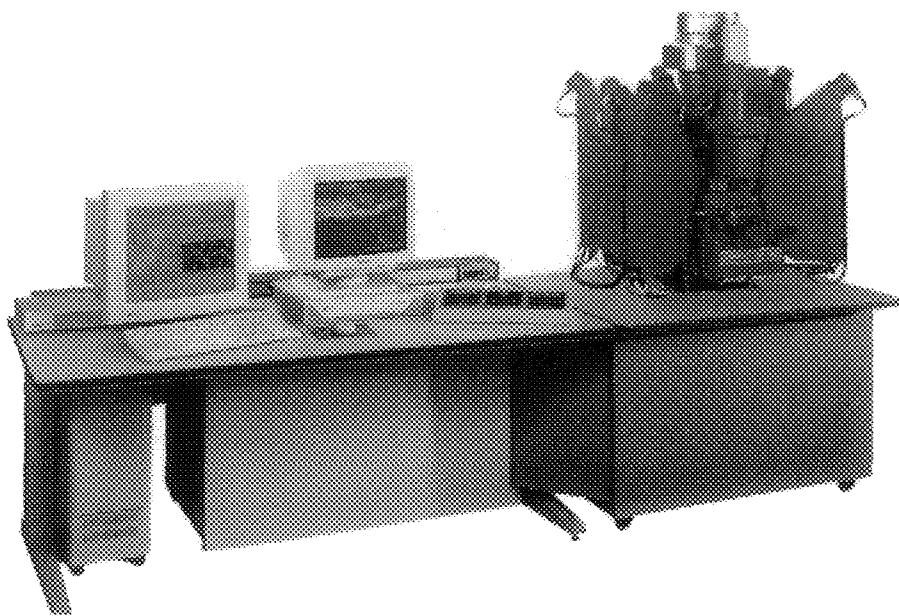


Figure X-2. Dedicated Electron Microprobe (Cameca Instrument Co.)

By 1956 Cosslett and Duncumb at the Cavendish Laboratories of Cambridge University designed and built the first scanning electron microprobe. All previously built instruments had a static electron probe. Cosslett and Duncumb swept the beam across the surface of the specimen in a raster as is now done in SEMs and television cameras. They used the backscattered electron signal to modulate the brightness of a cathode ray tube beam sweeping synchronously with the electron probe. They also used the X-ray signal to modulate the brightness of the oscilloscope image which allowed a scanned image to be obtained showing the lateral distribution of a particular element.

### **X-Ray Detection**

There are two commonly used techniques for determining the wave length and intensity of the X-rays emitted from the sample. These are

energy dispersive X-ray spectroscopy (EDS) and wave length dispersive X-ray spectroscopy (WDS).

In an EDS system the key component is a silicon crystal with lithium atoms diffused into it to create a diode. When electrons in the incident beam collide with the material in the sample they displace electrons from orbitals of atoms in the sample. This interaction creates an excited state for many electrons. When a displaced electron returns to its lower energy state, energy lost during the transition is emitted as x-radiation. The energy of the X-rays depends on the orbitals, their energy and which electron moves down to fill the hole. If the incident electron ionizes an electron in the innermost or K shell an electron from the L shell is most likely to drop down to replace it. This creates a  $K\alpha$  X-ray. The electron from the M shell is less likely to drop down creating a transition giving a  $K\beta$  X-ray. As Barkla discovered in the early days of X-rays each element has its own characteristic X-ray spectral pattern. A typical spectrum is shown in Figure X-3.

The detecting element in the EDS system is the lithium drifted diode which is cooled to liquid nitrogen temperature. When the X-rays enter the detector silicon atoms are ionized producing electrons. These produce electron-hole pairs which are drawn through the silicon-lithium lattice to the detector contacts by an applied field to produce a signal in a field-effect transistor mounted behind the crystal. The signal is electronically amplified and analyzed in a multi channel analyzer. Identifying the elements is carried out by the instruments software which matches emission line patterns to elements. The most common EDS application is qualitative identification of elements. The usual EDS unit can detect elements above sodium in the periodic table. With special windows for the detector the sensitivity may be extended as low as boron.

The early electron probes used wavelength dispersive spectrometers. This type is still in use today because of its superior energy (wavelength) resolution. For example the energy resolution of an EDS is 140 eV at Mn  $K\alpha$  compared with 5-10 eV for a wave-

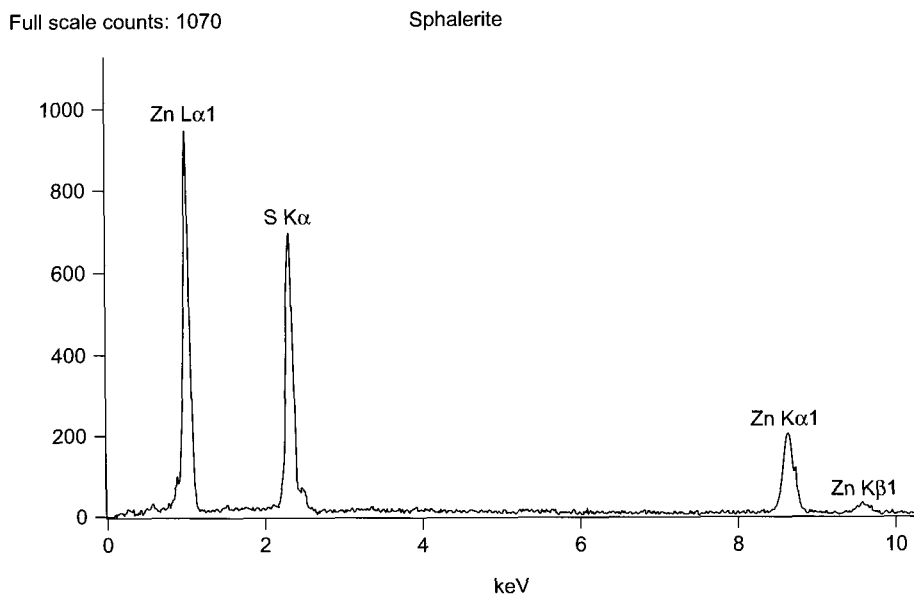


Figure X-3. Typical spectrum of the mineral sphalerite (ZnS) showing intensity of lines as a function of energy.

length dispersive spectrometer. For EDS this leads to frequent spectral interferences and poor peak to background values.

In a WDS spectrometer a portion of the X-ray signal from the sample impinges on an analyzing crystal. As described in the chapter on the electron microscope (section on Electron Diffraction) the crystal acts as a diffraction grating and resolves the spectra according to Bragg's Law:

$$n\lambda = 2d \sin \theta ,$$

where  $\lambda$  is the wavelength of the characteristic X-ray,  $n$  is an integer,  $d$  is the interplanar spacing of the analyzing crystal and  $\theta$  is the angle between the incident X-ray and the planes of the crystal that are diffracting. The diffracted X-rays are generally detected by a gas filled proportional counter which is connected to an amplifier, rate meter and a display device or recorder. The spectra are obtained by

scanning with the analyzing crystal and the detector. The data may be displayed as a plot of wavelength vs. intensity as in Figure X-3.

### **Qualitative Analysis**

The first step in the analysis of an unknown is the identification of the elements present in an area observed in the optical microscope. The instrument is designed so that the electron beam and the optical microscope come to a focus at the same point on the sample. This is qualitative analysis and is straightforward as long as the analyst is aware of all of the artifacts, and spectral interferences that can lead to the misidentification of the elements. This can be a particular problem in the identification of minor or trace level elements. Generally qualitative analysis is greatly aided by the computer software that matches the emission line patterns to the elements. In a large proportion of analyses the most common application is the determination of the elements present and their relative abundance.

### **Quantitative Analysis**

With the proper experimental setup and data reduction procedure the measured X-rays can be used for quantitative analysis with an accuracy and precision approaching 1%. Quantitative analysis requires the use of well characterized standards to measure concentrations. These can be either well polished standards with a composition similar to the sample or pure element standards. The pure elements offer less precision but more flexibility.

Casting's great contribution in his doctoral thesis was the development of the theory enabling the conversion of measured X-ray intensities to chemical composition. A series of measurements are obtained to compare the peak intensity obtained with the standard to that of the sample under a standardized set of conditions of electron bombardment and similar surface preparation. The ratio of the intensity of the same peak in sample and standard is called the  $k$  factor and can be converted to composition. In order to do this the

deviation from a linear relationship with composition for  $k$  must be accounted for. Some of the corrections include an atomic number correction for backscattered electrons, a correction for the absorption of any generated X-rays and a correction for secondary X-ray fluorescence. This is all done by software now an integral part of any EPMA.

In a modern instrument the limit of detectability with EDS X-ray spectrometry is on the order of 0.1 wt% for minor elements compared to 0.01 wt% for a wavelength spectrometer.

A major advantage of doing quantitative analysis in the EPMA as well with SEM is that the analysis is obtained from very small volume of material. Depending on the electron beam energy and atomic number, sizes as small as 1 micrometer are possible to analyze.

## **Elemental Mapping**

Since the SEM and EPMA are very similar instruments manufacturers have constructed instruments capable of operating in both modes. The scanning unit allows both electron and X-ray signals to be measured and displayed on a cathode ray tube screen.

SEM photographs of a back-scattered electron signal provide direct information about compositional differences using atomic number as the contrast mechanism (Chapter X). This is purely qualitative as it is not usually possible to distinguish individual elements.

The use of X-ray signals from a sample to provide an element distribution map came from the work of Cosslett and Duncumb at Cambridge in 1956. This mode of operation called "dot-mapping" although a very slow process became a very popular mode of qualitative X-ray analysis. The X-ray scan is performed by the same technique as conventional SEM imaging. The beam scans the specimen in the usual fashion and the synchronously scanned CRT has its intensity modulated by the signal from the preset energy-dispersive or wavelength dispersive X-ray spectrometer. The signal

is generally very weak. This is taken care of in the dot mapping mode by raising the intensity of the CRT beam whenever an X-ray is detected in a pre-set range. A dot is created on the display to note the detection and location of the event. The image created in this mode appears as a field of white dots qualitatively indicating the presence of a particular element. To obtain a high quality dot map requires a considerable time as well as careful attention to the instrument operating conditions. A typical dot map is shown in Figure X-4.

When carefully used the dot map can produce valuable qualitative information. Unfortunately it is subject to artifacts especially when the elements to be mapped are present in a small quantity. A typical subject for analysis is the iron meteorite Toluca shown in Figure X-4a. The back scattered electron image shows a Plessite structure which is a coarsely lamellar, eutectic-like structure. The dot map of the same area (Figure X-4b) in nickel radiation shows a Kamesite matrix, iron containing only a small amount of nickel and a precipitate of Taenite, a high nickel iron alloy. Although Sorby had studied iron meteorites in the late nineteenth century using the optical microscope it wasn't until the twentieth century that modern microscopic techniques were applied to the understanding of the observed structures. The application of EPMA has increased our understanding by many orders of magnitude. Taenite and kamecite are not easy to distinguish in the optical microscope but are obvious in the EPMA.

To overcome the problems of artifacts and to increase the usefulness of mapping, a technique of quantitative compositional mapping has been developed. This allows a complete compositional determination to be performed at each point in a matrix scan. When displayed the gray or color scale of a particular pixel represents the actual concentration of each element. Figure X-5 shows a typical quantitative image.

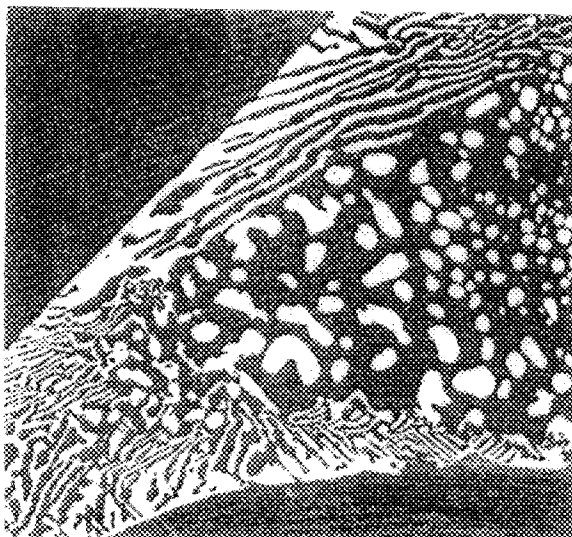


Figure X-4a. Back scattered electron image of an area of the Toluca meteorite showing the Plessite structure. (D. Lange, Harvard University) 400 x.

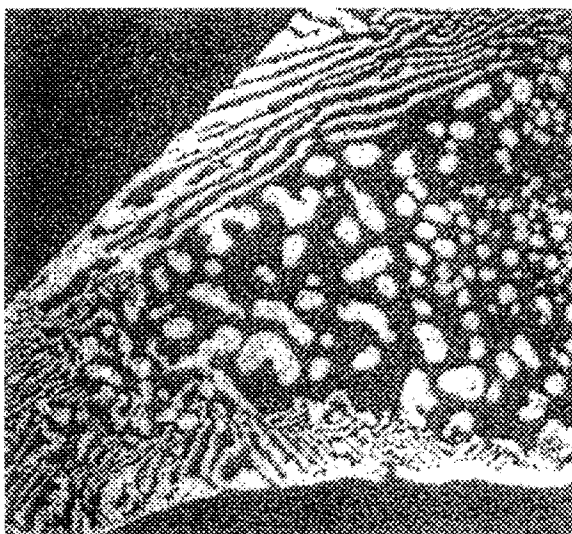


Figure X-4b. Dot map in Ni radiation of the same area of Toluca meteorite as X1-4a. (D. Lange, Harvard University) 400 x.

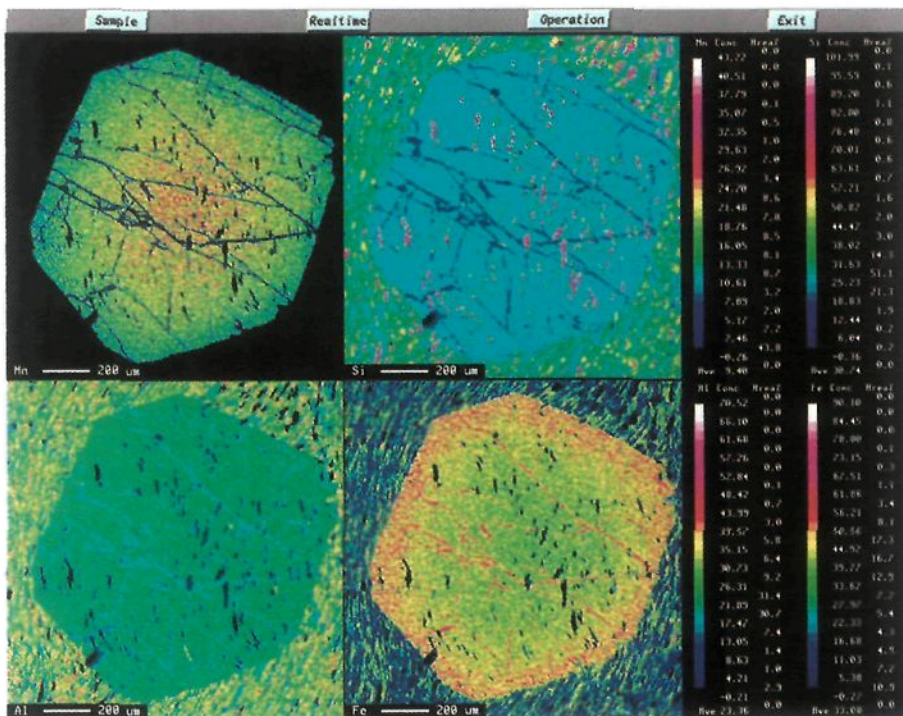


Figure X-5. Quantitative analysis for four elements in same area.

## Materials

The early work done on the EPMA was on material that would not be affected by the vacuum and was resistant to electron damage and changes due to beam heating. Work was done on metals, alloys and ceramic materials all containing heavy elements. An early advance was the development of diffracting crystals having large interplanar spacings by B.L. Henke in 1964. These allowed long wavelengths from light elements to be measured using a wavelength dispersive spectrometer. The detections of elements such as fluorine, oxygen, nitrogen, carbon and boron opened the way to studying many new problems.

Examination of organic and biological materials in the EPMA are presented with similar problems as occur in the SEM. It is



important that the elements being measured in the sample are not moved or lost by the preparative and observational procedure. A key consideration is the preparation of a clean and undamaged surface. Some examples of materials that have been examined with EPMA in addition to metals and ceramics are natural and synthetic fibers, polymers, paints and a variety of ice and frozen biological materials. Techniques are available for rapid cooling of materials to temperatures as low as liquid nitrogen.

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# Chapter XI

## Scanning Probe Microscopies

In the history of microscopy a driving force has been the search for greater and greater magnification with an ultimate aim to visualize individual atoms and their arrangement in space.

In 1986 the Nobel Prize for Physics was given to Ernst Ruska for his contribution to the invention of the electron microscope and was shared with Heinrich Rohrer and Gerd Binnig for their invention of the scanning tunneling microscope. In Ruska's case his recognition came 55 years after his contribution. But for Rohrer and Binnig it came in record time, after their work. Because of the rapidity of modern communication their invention led to a rapid technological spreading of a variety of related techniques which have become known as scanning probe microscopes. In many ways the scanning probe microscopes are a product of considerable evolution. In the 15<sup>th</sup> century simple magnifying glasses were made which evolved into the single lens optical microscope of the late 17<sup>th</sup> century with which Leeuwenhoek revealed the existence of single cells and bacteria; his "Little Animals". Optical microscopy became a sophisticated technique but still had the physical limit of not being able to resolve atomic structures since an average wavelength of visible light is about 2000 times greater than the diameter of a typical atom. An early successful exploration of atomic structures grew out of a basic concept of quantum mechanics that light and other kinds of energy exhibit characteristics of both particles and waves. In 1927 Clinton J. Davison and Lester Germer at Bell Laboratories confirmed the wave nature of the electron. They also showed that a higher energy electron would have a shorter wavelength than a lower energy one. With sufficient energy an electron can have a wavelength

comparable to the size of an atom. As shown in chapter IX this led to the electron microscope and to the observation of rows of atoms.

In Berlin in 1937 Irwin Müller developed the concept of the field emission microscope which worked on the point projection principle and provided magnifications greater than a million. Individual atoms could be seen. In Müller's instrument a wire with a microscopically sharp tip is mounted in a cathode ray tube. Electrons are drawn from the tip by a high electrical field and travel toward the screen on which the image is formed. The magnification is proportional to the ratio of the radius of curvature of the screen on which the image forms to the radius of the metal tip. Only a few very strong metals such as tungsten and platinum can be examined in this way because the high field at the tip exerts a large mechanical stress. A further development of the field emission microscope is the field ion microscope in which the tip is surrounded by a low pressure of helium gas. The gas is ionized at the atom planes on the tip and produces an image that can have a magnification of up to ten million times. This has been applied mainly to the study of metals and semiconductors, but a few biological images have been obtained. Irwin Müller left Germany in 1951 to come to the U.S. He became a professor of physics at Pennsylvania State University where he worked on the field ion microscope with his student R.D. Young.

Another development of the field-ion microscope is the atom probe in which individual atoms are removed from the tip by pulsing the electric field. The atoms pass through a mass spectrometer where they can be identified.

Until recently the observation of atoms and atomic and molecular shapes could only be done by relatively cumbersome and often destructive techniques as electron microscopy and diffraction and X-ray diffraction. The familiar light microscopes are limited by a fundamental problem described by Ernst Abbe over a hundred years ago which is the diffraction effect that obscures details smaller than about one half the wavelength of the radiation. New microscopes that exceed this limit and can resolve features as small as single atoms are

typified by the scanning tunneling microscope (STM) whose inventors worked at the IBM Laboratory in Switzerland. This was followed by a large family of instruments based on similar principals such as the atomic force microscope.

The principle by which the Abbe barrier was overcome was described in 1956 by J.A. O'Keefe at the U.S. Army Mapping Service. He proposed a microscope in which light would shine through a tiny hole in an opaque screen illuminating an object directly in front of the screen. Light transmitted through the specimen or reflected back through the hole would be recorded as the sample was scanned. The resolution of such a "scanning near-field microscope" would be limited only by the size of the hole and not by the wavelength of the light. Theoretically this device could resolve images showing details smaller than half a wavelength. At that time the technology to move and position the sample with the needed precision did not exist. By 1972 Eric Ash at University College, London adopted O'Keefe's idea and used 3 centimeter microwave radiation and a pinhole aperture to capture an image with a resolution of 150 microns. This was one two-hundredth of the wavelength used. At about the same time as Ash's experiment, means of controlling the sample with the precision needed to exceed the resolution of a light microscope became available. Russell D. Young at what was then the U.S. National Bureau of Standards succeeded in manipulating objects in three dimensions with a precision of one nanometer (a billionth of a meter). He used a piezoelectric material, which changes in size by microscopic amounts when an electrical potential across the material is varied. Young's device was called the "topografiner" and led by 1981 to the development by Binnig and Rohrer of the scanning tunneling microscope (STM).

Binnig and Rohrer were interested in studying the local conductivity of surfaces. They made use of the quantum mechanical phenomenon known as tunneling in which the wavelike properties of electrons permit them to "tunnel" beyond the surface of a solid into regions of space forbidden to them under the rules of classical

electrodynamics. The probability of finding such tunneling electrons decreases exponentially as the distance from the surface increases. To measure this current in the scanning tunneling microscope (STM) the detector is a tungsten probe ground so fine that the tip may be only a single atom and have a width of 0.2 nm. Piezoelectric controls maneuver the tip to within a nanometer or two of the surface of a conducting specimen. This distance is so small that the electron clouds of the atom at the probe tip and of the nearest specimen atom overlap. A small voltage applied to the tip causes electrons to “tunnel” across the gap generating a minute tunneling current. The strength of the current is extremely sensitive to the width of the gap. It may decrease by a factor of 10 each time the gap is widened by 0.1 nm, which is half the diameter of an atom.

Binnig and Rohrer had to overcome four instrumental problems: (1) the isolation of the apparatus from the vibrations present in every laboratory, (2) the approach of the probe tip to the sample surface, (3) the scanning of the probe across the sample, and (4) the sharpening of the tip. Binnig and Rohrer elegantly solved these problems using techniques that are still used.

The sample and the scanning probe are protected from vibration by being placed on a stage that is suspended with springs from another stage. This in turn is spring suspended from an outer stainless steel cage. The structure is equipped with magnets and copper plates that will induce eddy currents that damp any motion of the stages.

The placement and scanning movement of the probe are controlled by a tripod made of a piezoelectric material which will expand or contract when a voltage is applied to it and by a piezoelectric plate with three metal feet that can be fastened to a metal plate carrying the sample. Applying a voltage to one leg of the tripod (or the piezoelectric plate) causes the other tripod legs to move slightly. Both the direction and amount of this movement can be precisely controlled.

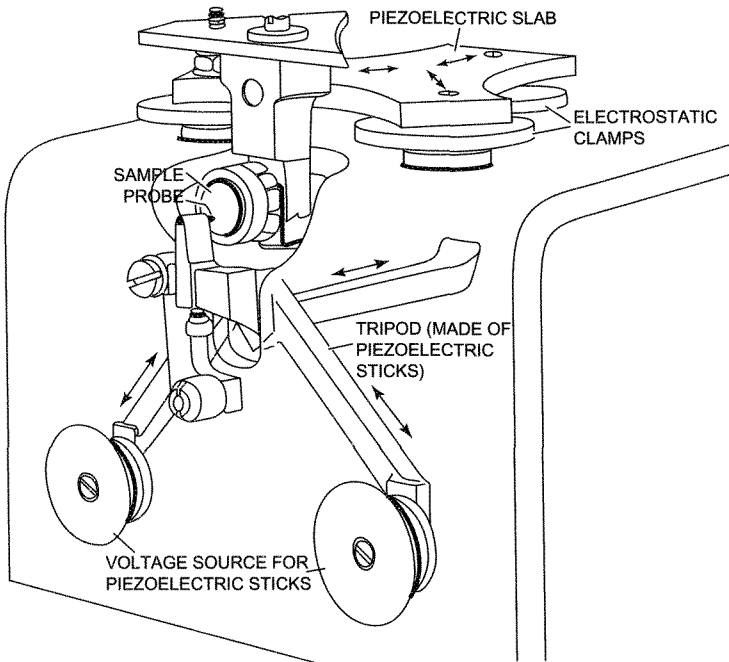


Figure X1-1 Arrangement of the piezoelectric drives for the sample and probe. (after Binnig and Rohrer).

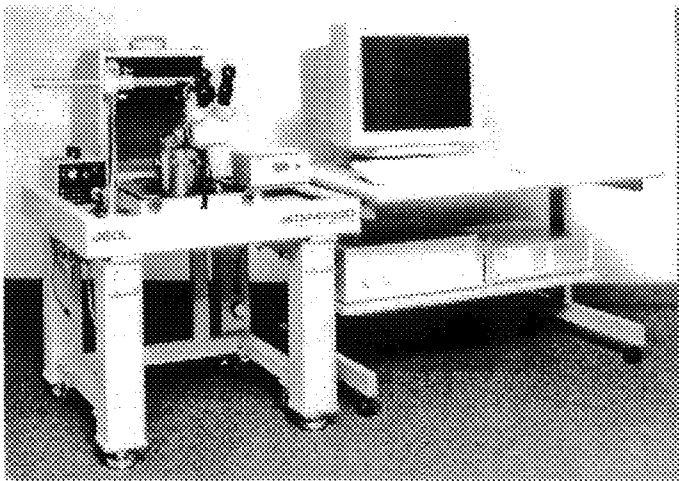


Figure X1-2. Scanning tunneling microscope model JSTM 4200 from JEOL.

The probe is sharpened by applying a strong electric field to the formed point. This dislodges the surface atoms off the tip until ideally only one atom remains. A typical commercial STM (JEOL model JSTM 4200) is shown in Figure XI-2.

A great number and variety of surfaces have been studied with the STM. The arrangement of individual atoms on the metal surface of gold, platinum, nickel and copper have all been documented. In particular the surfaces of silicon have been studied in detail. Measurement has been made in different crystallographic directions both before and after the material has been heat-treated to reconstruct the surface arrangement of the atoms. Figure XI-3 is an example of a typical silicon surface.

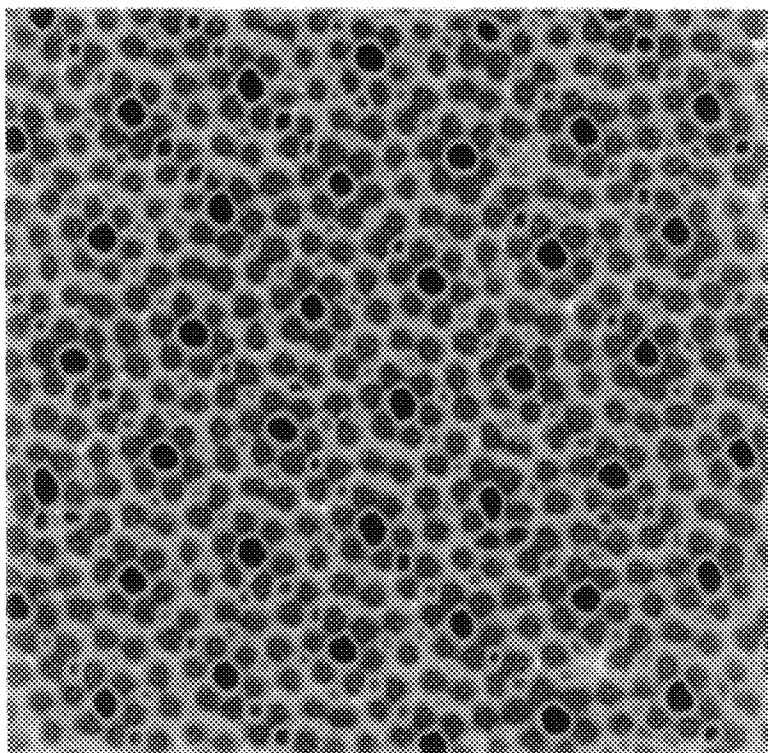


Figure XI-3. Silicon (III) 7 x 7 with 27 Å unit cell. STM image from Veeco Metrology Group.



The formation of the interface between silicon and a variety of metal layers has been extensively studied including molecules that have been deposited on gold or graphite surfaces and liquid crystals. Biological molecules are beginning to be studied and these will certainly become of great importance.

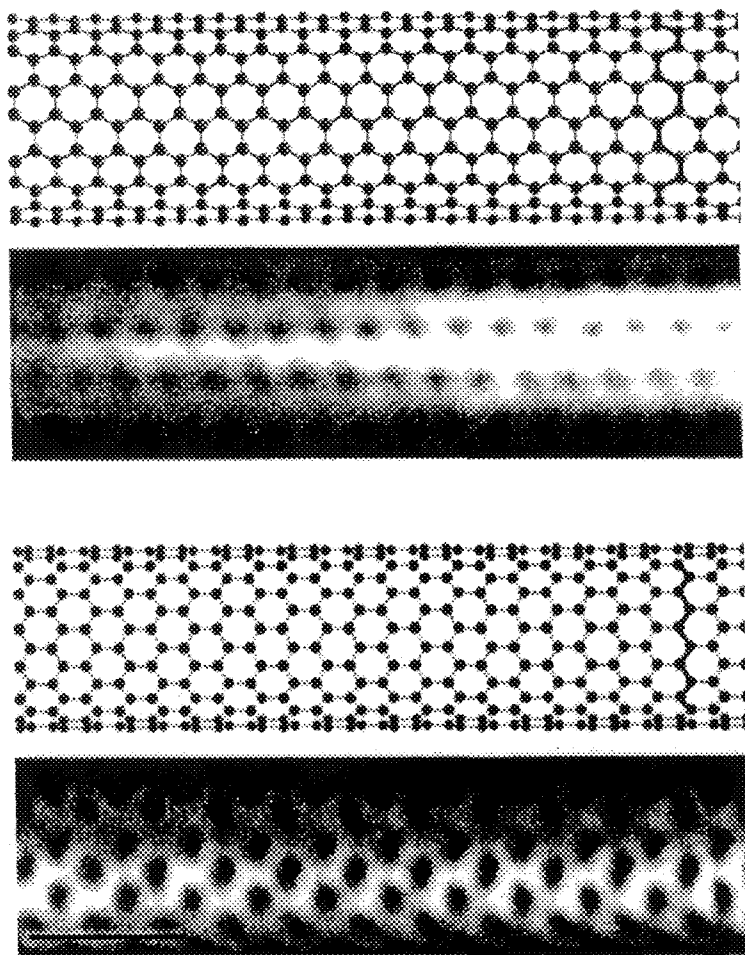


Figure XI-4 STM image at low temperature (40° k) of single wall nanotubes. Two typical structures are shown along with a model (upper diagram) of the atomic arrangement in the tube corresponding to the STM image. STM image from Prof. C. Lieber, Harvard University.

An example of the power of the STM is shown in Figure XI-4 for two types of carbon single wall nanotubes along with a drawing of the atomic arrangement corresponding to the STM image. Two typical structures are shown. The upper photo shows the atomic arrangement known as the armchair arrangement (outlined) and the lower illustrations show the zig zag arrangement.

Although it is possible to achieve high resolving power with an electron microscope the fact that the electrons penetrate surfaces gives the TEM great success in observing the bulk features of materials. Because there are no free particles in the STM there is no need for an electron source or lenses or a vacuum system. In the STM the bound electrons already existing on the surface of the sample are the exclusive source of radiation. After the original description of the STM in 1981 and the Nobel award in 1986 the STM was introduced commercially in 1989 and has had success evidenced by the numbers of manufacturers and by the numbers of instruments derived from it. Scanning tunneling microscopes were originated to measure surface topography. They are still primarily used for this on an atomic level but they can measure many other surface properties. They have the capability of measuring surface features that range from interatomic spacing to 100  $\mu\text{m}$ .

Derived from the STM a large family of scanning probe microscopes (SPM) has been developed over the last few years. The common feature of all of these is that the measurements are performed with a sharp probe operating in the near field scanning over the surface while contacting or maintaining a very close spacing to the specimen surface.

### **Scanning Probe Microscopes (SPM)**

STM's were originated to measure surface topography. They are still primarily used for this on an atomic level but they can measure many other surface properties. They have the capability of measuring surface features that range from interatomic spacing to 100  $\mu\text{m}$ . Derived from the STM a larger family of scanning probe microscopes

has been developed over the last few years. The common feature of all of those is that the measurements are performed with a sharp probe operating in the near field, scanning over the surface while contacting or maintaining a very close spacing to the specimen surface. The STM has atomic resolution capability; however the surface of the sample and the tip must be electrical conductors. This limits the material that can be studied. The first derivative of the STM was developed in 1986 and used a very sharp tip to map the surface morphology. This was the first atomic force microscope (AFM) and worked in contact mode. It is not necessary to measure a current between tip & sample. The tip is positioned on the end of a long cantilever with a low spring constant. The force on the tip is fixed by maintaining a constant and very low deflection of the cantilever pushing the tip against the sample. This force is in the range of interatomic forces in solids which is the origin of the name “atomic force microscopy”. It is derived from Young’s Topografiner and the more recent stylus profiler used in the semi conductor and optical industries. The atomic force microscope (AFM) uses much sharper probes and lower forces to get higher resolution with minimal surface damage. A fundamental component of the AFM is the scanner, a piezo electric device as employed in the STM. The tips are now mass-produced and assembled to the cantilever. When scanning, the interaction between the tip and sample is monitored by an optical lever or a beam-deflection system. A laser beam may be reflected off the back of the cantilever, to a photodiode detector. This is connected to feedback electronics which determines how much and how fast the piezo electric scanner moves out of a plane in the Z direction to maintain a constant tip to sample interaction. The 3D topographic image is a plot of the X–Y coordinates of the scanner and the output of the feedback electronics from the optical system.

The AFM represents only one of a large family of scanning probe microscopes. The AFM that determines topography by sliding a probe tip across a sample surface is called, “Contact Mode AFM”. By lightly tapping the surface with an oscillating probe tip the

shear forces of contact mode AFM are eliminated. This is called “Tapping Mode AFM”. There are also contact mode AFM’s that can determine topography by sensing van der Waals attractions between the surface and probe tip. AFM’s can operate in air, vacuum and even in liquid. Biological measurements are often carried out *in vivo* in biological fluids. By measuring frictional forces between the probe tip and the sample surface one has “Lateral Force Microscopy”. By functionalizing a probe tip with chemical species a chemical map may be created. This is “Chemical Force Microscopy”. It is possible to measure magnetic forces, electric forces, electrochemical forces, capacitance, surface temperature distribution and a host of other properties each of which creates a new type of microscopy. The techniques described as well as many others are being applied to a wide array of applications in biology, semi conductors, advanced materials, data storage devices, optics and measuring forces between particles and surfaces. Other applications lie in such fields as paints and coatings, metals, alloys, plating, polymers, food science, ceramics, thin films, geological materials and environmental studies. Many SPM systems are used for handling semiconductor wafers, CD and DVD bump/pit measurement and in computer production hard disc measurements. Film deposition, nucleation and growth are capable of being studied in the vacuum environment of the deposition system. Systems are now becoming available to study bio-materials and polymers up to 250°C complete with sample and environmental sensing.

### **Scanning Near Field Optical Microscopy (SNOM)**

SNOM is high-resolution optical microscopy, which scans a small spot of light over the specimen and detects the reflected or transmitted light for image formation. In SNOM a light emitted from the location opposite the aperture is used to form the optical image. The resolution of the image is defined by the size of the aperture, which may range from 50 to 100 nm, which is smaller than half the wavelength of typical visible light. Apertures may be prepared in the

metal coating at the apex of an optically transparent sharp tip. Light cannot pass through such an aperture, however an evanescent field, the optical near-field, protrudes from it. The optical near-field decays exponentially with distance and is only detectable in the immediate vicinity of the tip. If the aperture is brought close to a sample surface the pressure of the sample produces a disturbance of the optical near field which causes emissions of light from the location opposite the aperture. Scanning with the aperture or with the sample at the same time detecting in emitted light in reflected or transmitted mode can produce a high-resolution optical image. SNOM instruments are closely related to STM's and SFM's since the probing involves scanning with either the probe tip or the sample together with a tip to sample distance regulation. SNOM extends the range of optical resolution far beyond the limits of conventional optical microscopes. Resolution is achievable well below 100 nm.

### **Confocal Microscopy**

O'Keefe's ideas for overcoming the Abbe barrier led in time to the development of a whole new field of optical scanning near field microscopy known as confocal microscopy.

An interesting sidelight to the development of the confocal scanning microscope is found in a patent by Prof. Marvin Minsky, known for developing the field of artificial intelligence. In 1955 he was a Junior Fellow at Harvard interested in studying the brain but disturbed by the scattered light that diffused the microscope image. He conceived of using a second microscope to image a pinhole aperture on a single point on the specimen. This idea to avoid all the scattered light was not to allow any unnecessary light to enter in the first place. The second microscope images the pinhole on a single point of the specimen. Some initially focused light is scattered by out of focus specimen points but these can be rejected by placing a second pinhole in the image plane that lies beyond the exit side of the objective lens. Using this single point illumination it is only possibly to measure one point at a time. This makes it very slow to collect an

image as lasers were not available in those days. He used carbon arcs and the zirconium arc. The image was reconstructed on the screen of a war surplus long persistence radarscope. The image remained visible for about ten seconds, which is how long it took to make each scan. One question in the design was whether to move the optics or move the specimen. The technology of the time led to moving the stage. Minsky demonstrated his microscope to many visitors but he felt they were never impressed by the image on the radar screen. Fortunately Minsky's brother-in-law happened to be a patent attorney and also liked the instrument. He secured the patent, which was the only printed record of another inventor of the confocal microscope. Thirty years after the 1955 construction the rusty instrument was cleaned up, still worked and the paper finally got published.

Many years after the idea of Minsky the concept was developed extensively by Egger & Navodovits at Yale and by Shepherd and Wilson in Oxford as well as at other universities and microscope manufacturing companies.

The usefulness of the confocal microscope began to be appreciated about a decade ago when biologists attempted to examine the bacteria living in biofilms. Biofilms are created by microorganisms to form living veneers as in dental plaque, the slippery coating on a rock in a stream and the slime that forms inside a flower vase after a few days. Biofilm bacteria are a common source of infection in such implants as urinary catheters which may remain in a patient for long periods of time. Biofilms can be established in metal pipes where they contribute to corrosion even in steam pipes in power plants as well as drinking water supply pipes. For a long time biologists had attempted to examine biofilms using ordinary optical and even electron microscopes. They always saw some bacteria but since they could not get clear images from within the living layers they concluded that the bacteria and cells were mostly dead and jumbled in random clumps. When biologists applied a variety of confocal microscope called a laser scanning confocal

microscope the view changed radically. This technique allowed them to view slices at different depths within a living biofilm and to stack these planes together to create a three dimensional image. By 1991 it was found that in biofilms the bacteria grow in tiny groups called microcolonies. Bacteria are usually less than a third of the film. The rest is a goo secreted by the cells containing water and small particles. Biofilms are extremely resilient and at times not affected by antibiotics and antiseptics. By combining microscopy with other scientific techniques it was found that a variety of chemical environments may arise in a single biofilm which means that one cell may look and act very different from the next even if they are identical. It has been found with biofilms that cells produce certain molecules that act as signals which when reaching certain levels trigger changes in the activity of dozens of genes and change the activity of the films. Now that biologists understand this they are working on drugs for controlling film growth which is implicated in periodontal infection, tuberculosis, legionnaire's disease and other infections.

An important drive for the present interest in the new scanning microscopes is the dwindling size of circuits in electronic chips. These new tools that are capable of imaging as well as manipulating single molecules or atoms are leading us into a new age. A recent issue of the popular journal *Scientific American* (Sept 2001) is entirely devoted to nanotechnology. One common feature of all the articles is the application of the new microscopes as both a tool and a control of the processing of nanomaterials and nano devices.

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# Chapter XII

## Acoustic Microscopy

Sound is propagated in the form of waves. These waves can be reflected and refracted very much as light waves. We normally think of sound as it is detected by the human ear, but sound can travel through liquids and solids. It can also have a frequency both above and below the sensitivity of the human ability to detect. The higher frequency waves are now called ultrasonic. The science and engineering of sound are described by the term acoustics. Acoustic waves very often can propagate through materials impervious to light. In the early 1920's the Russian scientist Sergei Y. Sokolov at the V. I. Ulyanov (Lenin) Electrochemical Institute in Leningrad was working with sound waves using a through-transmission technique to detect flaws in metals. The experimental devices that he fabricated at that time had such poor resolution that they could not be used for a practical device. At about the same time he proposed a different ultrasonic application. He recognized that sound waves could be used to create a new form of microscope based upon a reflective principle. His calculations showed that a microscope using 3000 megahertz sound waves would have a resolution equal to an optical microscope. It was not until the late 1930's that the technology to create such a device was developed. The instrumentation for generating the high frequencies needed for an acoustic microscope were developed for microwave and ultrasonic systems used for radar and underwater navigation.

In its simplest form performing an ultrasonic examination using a reflection technique a pulsed sound wave is transmitted from one side of a sample, reflected off the far side and returned to a receiver located at the starting point. If a flaw or crack is present the

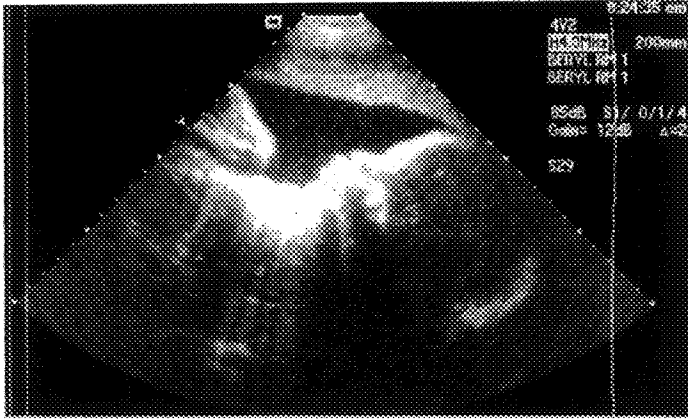
signal is reflected and its travel time altered. Producing an image with sound waves is not a trivial procedure and the resolution is easily surpassed by electron microscopes but there are two advantages to using acoustic waves. First is the ability of acoustic waves to penetrate materials that are opaque to other radiations particularly light. The second is the contrast produced by the mechanical properties of the material under examination.

## **Imaging**

The first of these, imaging, has been exploited in the main uses of ultrasound. The earliest use was for the detection of submarines in the first world war. Since then, two big areas of interest are medical imaging and non-destructive testing. In medical imaging use is made of the ability of sound to penetrate body tissues and be scattered by changes in tissue density and elasticity.

The reflected echoes are detected and can be used to build up a scanned image. The image is created by using a fixed array of transducers and the scanning is performed electronically by means of relays and phase shifts introduced into the signals to and from each transducer. The information is usually displayed as a tomogram corresponding to an imaginary slice in the plane in which the ultrasonic waves are scanned.

Small changes in density show up with remarkable contrast, often equal to or better than created by X-rays. Ultra sound is generally considered not to effect human tissue when used under careful medical control, although a small warming affect has been recognized. The apparent safety and unique contrast has led to extensive use in fetal examination. There have been no injuries reported in 50 years of use. Most ultrasonic images are effectively two dimensional slices, however, the technology exists to create a three dimensional image as shown in Figure XII-1b.



## **Microscopy**

Since Sokolov's work in the 1930's and 40's the technology to put his ideas into practice have been gradually developed. He patented the concept in the U.S. in 1937 as well as the tube used for underwater imaging. By the 1970's several groups of researchers realized his recognition that sound waves with a frequency of 3000 megahertz imaging would have a resolution equal to an optical microscope.

Modern scanning acoustic microscopes (SAM) can be used to produce high resolution internal images of materials and components. As with imaging, an acoustic microscope must also scan since it is not possible to form an extended image simultaneously of a whole object. It is possible to create an acoustic lens that has good focusing properties on axis. Such a lens as is shown in Figure XII-2 can be used to focus acoustic waves onto a spot on a specimen and can also be used to receive the acoustic energy from that spot. If the lens is simultaneously scanned over the specimen and the intensity of the reflected signal sent to a synchronous display an image can be built up as in an SEM. The lens shown in Figure XII-2 can be used to focus acoustic waves to a spot on a specimen and can also be used to receive the acoustic energy from that spot. The lens shown in the figure is typical for a SAM and consists of a disc of single-crystal sapphire ( $\text{Al}_2\text{O}_3$ ) with its c-axis on the axis of the disc.

The earliest scanning acoustic microscopes were in the transmission mode with a separate lens along the "optic axis" acting as the receiver. The specimen was introduced between the two lenses and scanned between them. Simple continuous wave electronics could be used to create an image. The first materials to be examined this way were biological. The sound waves easily penetrated and the contrast scan in the images was derived from the mechanical properties of the tissue being examined. The advantage of this technique lay in the ability to distinguish areas that normally would only be observed by selective staining. Later this technique was applied to optically opaque materials.

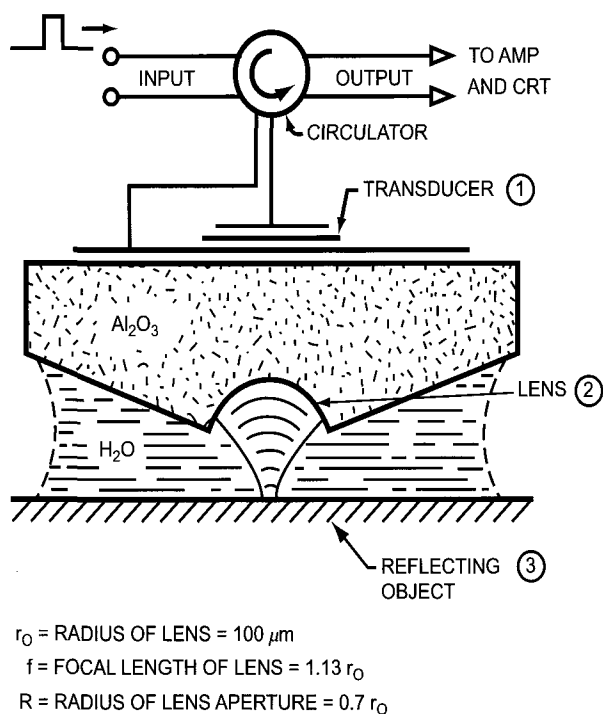


Figure XII-2. Schematic illustration of the acoustic transducer and lens in the SAM (after Lemmons and Quate 1979).

As scanning acoustic microscopy developed, interest shifted from transmission microscopy to reflection with a single lens being used both for the transmitted signal and for receiving the reflected one. This came about because, as the resolution requirements of SAM began to be increased, the difficulty of aligning the two lenses became more apparent. There was also a growing interest in looking at bulk solid materials. Because acoustic waves are attenuated in any medium for high resolution microscopy the fluid path between the lens and the specimen must be kept small, which means that the focal length of the acoustic lens must also be small. This would be fine for a rock thin-section but very difficult for metals or semiconductors which are usually examined microscopically by reflected light.

Because of these problems most current SAM's are used in the reflection mode.

A vital role in the generation of contrast in the SAM is played by surface waves, also called Rayleigh waves. Rayleigh waves may extend about a wavelength below the surface making it possible to image features lying below an opaque surface layer. As shown previously, the heart of the SAM is the lens as shown in Figure XII-3. Such a lens is used to focus acoustic waves onto a spot on the specimen as well as to receive the reflected energy from that spot. The lens is scanned systematically over the specimen and the reflected signal is sent to a synchronous display. A scanned image is built up in a way similar to a scanning electron microscope. The acoustic incident wave is generated by a piezo electric material bonded to the back of the sapphire lens. A typical material is ZnO which may be prepared by sputtering onto a gold film previously deposited on the sapphire. For lower frequencies thin plate transducers such as  $\text{LiNbO}_3$  can be bonded to the lens.

SAM's find their use in the laboratory on a large variety of mainly optically opaque materials. In recent times automated instruments are being used for the rapid non-destructive inspection of such things as integrated circuit packages at various stages of manufacture. They are used in failure analysis situations to give detailed information on internal features showing the presence of manufacturing, processing, and in-service defects.

The following illustrations are typical of the kinds of materials that can be effectively examined by scanning acoustic microscopy. The images were provided by Ultrasonic Sciences Ltd. of England and are reflection scans (pulse echo).

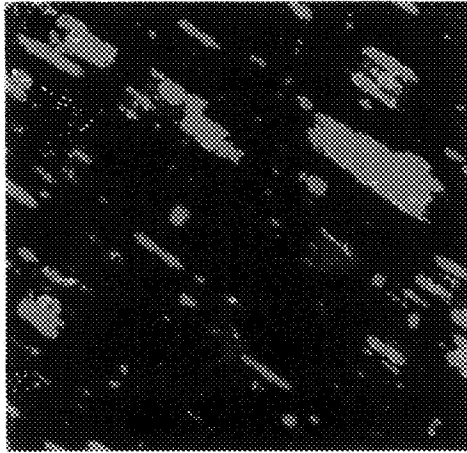


Figure XII-3. 25 x 25 mm scan showing inclusions in steel after being subjected to “hydrogen induced cracking”.

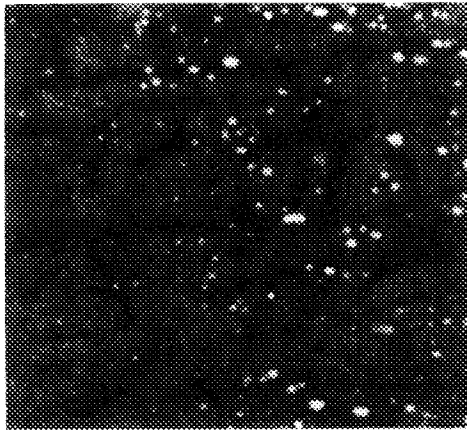


Figure XII-4. 40 x 40 mm scan showing inclusion distribution in a steel sample.

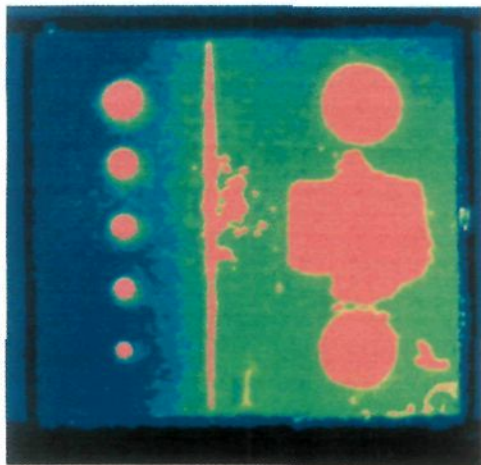


Figure XII-5. Scan on brazed sample 50 mm x 50 mm with some intentional and some unintentional defects.

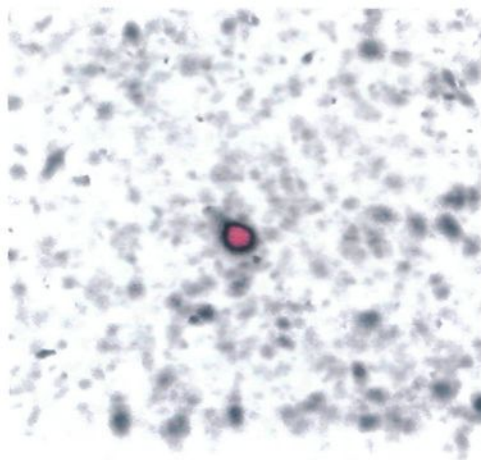


Figure XII-6. Portion of 5 x 5 mm scan on cast aluminum sample showing small void about 30 microns in diameter.



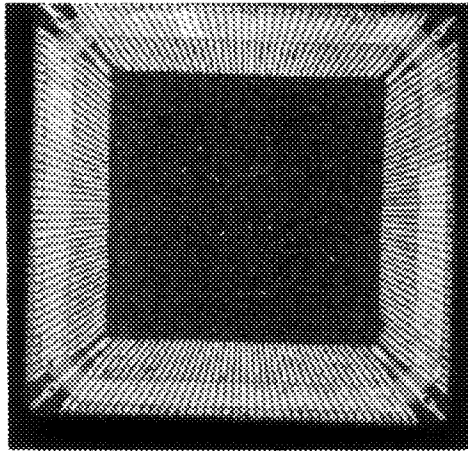


Figure XII-7. Scan of 20 x 20 microelectronic package showing general structure including small voids in the plastic encapsulant near the center.

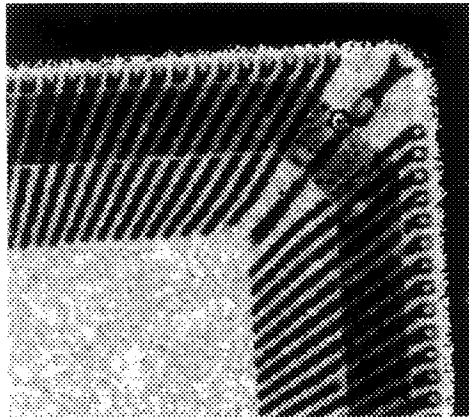


Figure XII-8. 5 x 5 mm scan of previous sample showing detail.

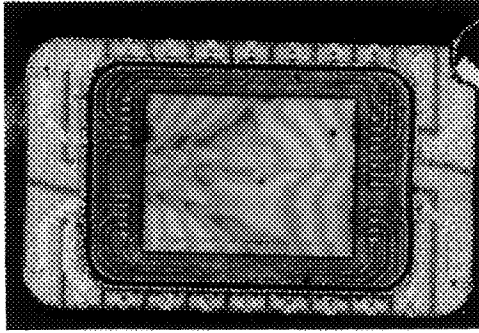


Figure XII-9. Scan on memory chip for digital camera.

The primary application of acoustic microscopy is that it enables the interaction of acoustic waves with elastic properties and features that can be visualized with microscopic resolutions. There is especially strong sensitivity to scattering by surface cracks and boundaries. At lower resolutions and in polymeric materials because Rayleigh waves are less dominant useful interior imaging is possible. This can bridge the gap between high resolution acoustic microscopy and conventional nondestructive testing.

# Chapter XIII

## Future Microscopies

The grains in most materials range from microns to millimeters in diameter and may contain billions of atoms. Newly created versions of well known materials called nano phase materials or nano materials are less than 100 nm in diameter and contain only tens of thousand of atoms. A three-nanometer diameter cluster contains about 900 atoms and is about a million times smaller than the period at the end of this sentence. These tiny grains respond to light, electricity and stress very differently than micron or millimeter size grains, displaying an array of novel attributes. Nanophase materials because of many unique properties such as an increase in strength of a metal such as copper or an increase in breaking strength of a nanophase ceramic are now of great technologic interest. The control of the structure of these materials is dependent on observations by the most up to date kinds of microscopy using electrons and scanning probe techniques.

The ultimate in this technology is found in a recent report of a transistor made of just one atom. Paul McCuen and his colleagues at Cornell University and the University of California at Berkeley constructed the device out of a single cobalt atom held in an organic compound. They put a gold wire 10 nm in diameter on a silicon substrate and covered it with the cobalt compound. Making a tiny gap in the wire allowed molecules of the coating to slip into the space and to carry a cobalt atom along. The two ends of the wire then act as contacts with the third and final connection being made to silicon dioxide which insulates the gap from the substrate. The manufacture of such device will certainly require advances in the technology of visualization of such tiny objects.

In the words of John Maddox “What remains to be discovered is not necessarily what will be discovered”. The advances in microscopy stem both from the curiosity of individuals such as Leeuwenhoek and Fox Talbot and by the incremental advances dictated by the necessities of modern engineering. Two unique fields come to mind. The first is a new concept in microscopy called magnetic resonance microscopy (MRM). This technique is similar to the magnetic resonance imaging techniques (MRI) used in many hospitals to visualize soft tissue. Both of these techniques use radio frequency energy to excite and detect protons in the water within tissues. In MRI the image is created in a gradient magnetic field using radio frequency currents to excite the protons. The microscope gets a resolution a million times greater by using more powerful super conducting magnets, stronger gradients to perturb the magnetic field and smaller imaging coils to accommodate tiny specimens. MRM can create three dimensional data sets which can be computerized to display the results.

Computer visualization techniques can render selected parts of a specimen translucent while leaving others opaque. Using this technique internal structures can be viewed at varying depths and in their natural arrangement without destroying the specimen by cutting it up. In a recent publication Bradley R. Smith at Duke University reports on a remarkable study of a large collection of human embryos in various stages of development. The potential of such technology is enormous both for the training of clinicians and the bringing of valuable image data into the laboratories of researchers. This particular study is valuable in another way in that it is available as digital images and can be transmitted world wide as an on-line resource.

The second topic in future microscopies has to do with the drive of engineering to incrementally improve the technologies of modern microscopy. In the world of semi-conducting devices the driving force is the production of faster, denser, lower power consuming and more reliable products. These drives are known to accelerate with time.

Many of today's devices are already beyond the view of ordinary optical microscopes. Materials used in manufacturing semiconductor devices including starting materials, insulators, conductors and packaging are already under the control of various kinds of electron microscopes as well as probe microscopes. The instruments are central to design, development, manufacture and testing. Failure analysis is also part of this picture.

As the technology of semi conductor devices has developed the microscopic analysis has evolved from visible light microscopy to scanning electron microscopy and then to more sophisticated high resolution and low voltage SEM. It has further progressed to newer techniques in transmission electron microscopy and scanning probe microscopies and now back to the optical microscope using the newest techniques as confocal microscopy. The best confocal microscopes can visualize feature down to  $0.25\text{ }\mu\text{m}$ . The future chip families will have features on the order of  $0.25\text{ }\mu\text{m}$ . This brings the techniques to their limit. A further increase in resolution will make use of short wave length ultra violet light bringing the useful resolution down to  $0.09\text{ }\mu\text{m}$ .

An important concern in the microscopic analysis of semi-conductors is the requirement for high spatial resolution in the specimen preparation technique. For SEM and SPM specimen preparation methods must produce the location of specific areas with a precision of 100 nm or better. For TEM, preparation is more difficult as the specimen must be thinned to electron transparency. The object of these examinations is to characterize failures. The preparation of multi megabit chips today and the future preparation of multi gigabit chips focuses on the problem of locating points of failure. All of which leads to the reduction of turn around time in the microscope laboratory. In turn this requires engineering improvements in microscopy but the real major step is when microscopy moves from its off-line function to on-line as part of the manufacturing process. Off-line methods of microscopy control generally use destructive sample preparation techniques. In today's

micro electronic industry individual wafers is so enormously expensive that to sacrifice one or more for production control has become a major problem. The future lies in having the microscopy techniques as part of the production line. The first step in this direction has to be the use of the SEM in electron beam lithography. The future, hopefully, will see the introduction of some of the microscopes as part of the in-line manufacturing process.

The ability to spot individual atoms becomes very important as electrical engineers continue to jam more transistors into computer chips. The "Holy Grail" of electron microscopists has been the imaging of atoms to a resolution of less than one Ångstrom. In the early 1900's a Japanese built, one and a quarter million volt machine constructed for a German laboratory achieved 0.95 Ångstrom resolution. This was at a cost of 10 million deutschmarks. At about this time the U.S. National Center for Electron Microscopy had constructed a three story million volt "Atomic Resolution" microscope that was able to resolve 1.6 Ångstroms. Also around this time Michael O'Keefe of the Lawrence Berkeley National Laboratory suggested a way to computer process through-focus

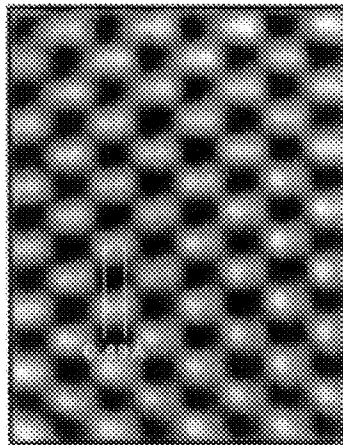


Figure XIII-1. Lattice image of diamond in [110] orientation shows columns of carbon atoms at 0.89 Ångstrom separation.

images to achieve higher resolution from a medium-voltage microscope. He suggested combining information from many images to achieve a single image with resolution approaching the information limit of the instrument. By 1999 the Berkeley group produced images of columns of carbon atoms in a diamond lattice 0.89 Ångstroms apart.

This becomes very evident as the latest advances in microscopy get reported in the newspapers. At the IBM Research center and the Nion Corporation an enabling technology has been developed that can resolve subatomic images with an electron beam that is only three-billionths of an inch wide. This technology makes possible a microscope with a resolving power less than the radius of a single hydrogen atom.

Applying this to a modern transistor where some components are no more than five atoms thick it becomes possible to create three-dimensional images up to 10 nm in depth. This is the equivalent of as many as 50 layers of atoms.

Since the lenses in an electron microscope suffer imperfection as all lenses do the images produced will be blurred. The strongest of these flaws is spherical aberration. Since the electron microscope was developed in the 1930's the aberrations have limited the resolution to about 50 times the wavelength of the imaging electrons. The IBM and Nion scientists have now reported a computer controlled aberration correction system in a dedicated scanning transmission electron microscope. This type of instrument is less sensitive to chromatic aberration than a TEM. The geometry of the STEM requires an extremely small electron probe scanning in a raster over the area of interest. The aberration corrector is very complex, consisting of seven major electron lens elements with many dipoles and quadruples at each stage to control parasitic aberrations. In all there are 35 separate windings with computer controlled power supplies. The axial aberrations are determined by software which analyses the electron shadow images and then adjusts the corrector. This work demonstrates the first attaining of sub-Ångstrom resolution

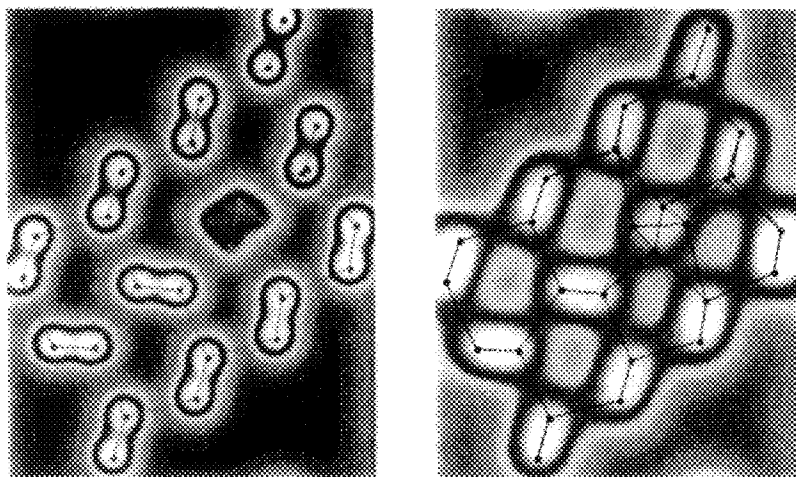


Figure XIII-2. Scanning transmission electron microscope images of Silicon. Aberration correction on left image produces a sharper image than on the right.

by an electron microscope in a single image. An example is shown in Figure XIII-2.

This aberration correction technology will open the way for lower voltage, smaller, more easily managed instruments that will be capable of routinely imaging and analyzing materials at sub Ångstrom resolution.

This shift in precise image control will allow routine atomic level characterization of the structure, defects and interfaces in bulk materials.

We are now seeing the future of microscopy.



## Further Reading

The intention of this book is to reach the scientifically interested general reader so there are no specific journal references. This list will guide the reader to more detailed work on specific subjects. In some cases I have specified material from references in the body of the text. The World Wide Web has been a source of much valuable information. In particular, such search services as Encyclopedia Britannica Online have been a valuable starting point for me as well as a place to check data on people whose names appear in the older literature.

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