

1P1b: Introduction to Microscopy

Safety

| <i>Hazards</i> | <i>Control measures</i> |
|------------------------------------|--|
| Hot surfaces – risk of fire | <ul style="list-style-type: none">• turn microscope off after use• do not replace dust cover until microscope has cooled down |

Central to the study and characterisation of metals and many other materials is the microscope, ranging from the magnification of, say, $\times 1$ to $\times 35$ in a simple stereo binocular microscope, to resolutions of less than 0.1 nanometre in the most powerful transmission electron microscope. For many purposes the most basic and versatile tool is the optical microscope. During the practical course in both the first and second years several experiments make use of the optical microscope coupled to a digital camera and simple image analysis software. The purpose of this introduction is to give you an insight into the variety of structures and information that can be observed in this way, and to familiarise you with the micro-scale, measuring features down to about $0.5 \mu\text{m}$ across.

The metallographic microscope

All the samples you will see this afternoon are metals; they are opaque and therefore must be viewed in reflected light. To achieve this light from a source at the rear of the microscope is reflected by a half-silvered mirror down through the objective to the sample and then back through the objective to the eyepiece. To do this the sample must be flat with its surface normal to the incident light beam. Typically samples are examined as polished and also after etching to reveal different aspects of their structure. The samples you will look at this afternoon are pre-prepared and the first will be set up on the microscope for you. The demonstrators will show you how to level subsequent samples.

The microscopes are equipped with digital cameras and a suite of image processing and analysis software. This system is used to display the

images which you can record on a CD and take away with you to examine and/or print out using your own or other computers.

Resolution

The resolution of an optical microscope, the smallest distance between two points that can be separated in the image depends on the wavelength of light and the properties of the objective lens. Beyond this increasing the magnification will not increase the resolution although it can undoubtedly make the image easier to study. A full treatment of the resolution of a microscope can be found in standard microscopy textbooks (a simple description is given in B. Boussfield, 1992: Surface preparation and microscopy of materials, (Chichester: Wiley), 252-58). The resolution is given by:

$$R = 0.61\lambda/NA$$

where λ is the wavelength of the light and NA is the numerical aperture of the lens, which is defined by:

$$NA = n \sin \alpha$$

where n is the refractive index of the medium between the objective and the sample and α is the angle between the axis of the objective and the most oblique ray passing through it, and is a function of the dimensions of the lens. With the system you are using the lenses are always in air so that $n = 1$.

For a typical wavelength of 500 nm the maximum resolution of the microscope will be of the order of 0.3 μm . Therefore, to achieve the best results the sample should be prepared so that the maximum width of

scratches and other defects in the surface is of this order of magnitude. The samples you will examine are described below and the first three are designed to demonstrate this aspect of sample preparation.

Operating the microscope

The microscope will be calibrated and ready to use on arrival. The demonstrators will give a basic introduction to the microscope controls and will be standing by to answer questions. Please select Sample 1 and follow the general instructions below to obtain an image.

1. Starting Up the Equipment

- a. Turn on the computer if it is not already on. Please sign in using the generic username and password printed on the top of the computer console.
- b. Turn on the microscope.
- c. Check that camera unit on top of the microscope is on (the green indicator light on the right hand-side of the unit should be lit). Otherwise turn the camera on using the switch at the back.
- d. Place your sample underneath the microscope spotlight.

2. Operating the Software

- a. Open the LAS (Leica Application Suite) software from the desktop.
 - i. A live view of your sample will appear.
- b. Before taking an image, you must define a file path (location) to save the images.
 - i. Select the navigator tab at the top of the toolbar on the right of the screen **OR**

- ii. Select the “Browse” tab from the top workflow menu and the navigator tab will appear in a panel on the left-hand side.
 - iii. Create a new folder for your group in an easily accessible location (i.e. desktop) using the “create new” tab in the navigator panel.
 - iv. Select your folder and click the “assign capture location” button.
- c. Select “acquire” from the top workflow menu to return to the live view.

3. Taking an image

- a. With the sample in view, select the auto exposure button from the top of the “acquire” panel at the left of the screen.
 - i. This will set the brightness and contrast sliders to moderate values.
- b. Replace your sample with a white piece of paper and select the auto white-balance button (to the right of the auto exposure button)
 - i. This will remove any tint in the image to reproduce the color of the sample.
- c. With the sample back in view, select an objective lens by turning the objective lens carousel on the microscope.
 - i. When changing objectives, take care to ensure that the objective lens does not hit the stage or the sample.
 - ii. The default objective lens is x5
- d. Select the corresponding objective setting in the software

- i. Under the “acquire” workflow, select the “mic 1” tab and click the button that matches the objective in use.
 - ii. This will reset the scale bar.
 - e. Focus the image by turning the knobs on either side of the base of the microscope. Large knob = Coarse, Small knob = Fine.
 - i. This will move the stage up/down until the sample is at the focal length of the objective lens.
 - ii. Take care not to drive the sample into the lens!
 - iii. The sample can be positioned finely by turning the knobs underneath the stage.
 - f. Once the live view looks as you want it, click the “acquire image” button in the bottom left of the screen.
 - i. You will be asked to confirm the magnification of the objective lens.
 - g. This will take you to the “browse” tab, where images you’ve taken will appear in a scroll at the bottom of the screen.
 - i. A scale bar will be automatically overlaid on the image.
4. Annotating an image
 - a. To add additional annotations (lines, arrows, comments, etc...), select the annotations button from the toolbar at the right of the screen **OR**
 - b. Select the process tab from the top workflow menu and the annotations panel will appear on the left-hand side of the screen.
 - c. Annotations (show) > Line (show) > select (line, arrow, etc...) from the dropdown menu > use the mouse to overlay the annotation on the image.

- i. Overlays can be edited after being generated, but only one overlay can be generated at a time.
 - d. To add multiple overlays of the same type (line, text, etc...) to an image:
 - i. First make an annotation while in the “annotations” or “process” workflow using the “basic annotations” toolbox.
 - ii. Then click the “merge all” button to flatten the current annotation and the image below it and save it as a new image.
 - iii. A dialogue box will appear asking if you want to “replace” or “duplicated” the image.
 - 1. Select replace to create a single image with all annotations **OR**
 - 2. Select “duplicate” to create multiple copies of an image with difference annotations.

5. Shutting Down

- a. Close the LAS software.
- b. Turn off the microscope.
- c. Turn off the camera (switch at the back of the camera).
- d. Remove your samples, and return them to the demonstrators.
- e. Saved images can be transferred via email, file sharing services, or USB.
- f. Do not put dust cover over the microscope until it has cooled down.**

Examining the samples

The samples have been chosen to give an introduction to sample preparation, to the resolution of the different objectives, and to some of the basic types of metal microstructure. You will see more of these later in the course. Save the images to your own workspace or a datastick. Always remember, use “Live” images to scan samples, select magnifications etc., and then snap the image to measure or annotate.

Sample 1: Pure copper ground with 600 grit SiC paper.

When a sample is cut and mounted for examination in a metallographic microscope the first step in preparation is to grind the cut surface flat and remove the mechanical damage in the sample caused by the cut. Then finer and finer grinding papers are used, each step erasing both the scratches made by the previous one and the area of deformed metal the grit has made below the surface. The grade 600 grit corresponds to a maximum particle size of 25 μm but, of course, on the sample the scratches from many grit particles will overlap. Using “Annotations” mark a 100 μm scale bar and estimate how many scratches it covers.

Sample 2: Pure copper polished with 6 μ m diamond suspension.

The series of SiC grinding papers stops at 4000 grit which has a particle size of about 6 μ m; further preparation is then done with a suspension of diamond in water (or oil if water attacks the sample). The first step in polishing is with 6 μ m diamond; the sample shows you the difference from a ground sample, but of course the polishing scratches could still be resolved with most microscopes.

Sample 3: Puddled wrought iron polished with 1 μ m diamond suspension.

The final stage in metallographic polishing for most general purposes is with 1 μ m diamond; at this level there should be few visible scratches. Any polished sample should be examined first in the as-polished state to look for corrosion, inclusions etc. The sample here was cut from a piece of early wrought iron railway track made in about 1820. Puddling was the first process by which wrought (carbon-free) iron could be made from pig iron, after which wrought iron became the main structural metal until superseded by steel in the late 19th century. The process left a great deal of slag in the metal which you can see, and the slag inclusions have their own internal microstructure. If time permits it may be possible to show you how the volume of slag can be measured.

All subsequent samples will be polished to a 1 μ m diamond finish and then etched to reveal features of their microstructure.

Sample 4: Cast aluminium-copper alloy

This sample is taken from an aluminium-copper alloy ingot made during one of the second-year experiments. Unlike pure metals, alloys in general do not freeze at a single temperature or with a homogeneous microstructure. Typically they will freeze into a mass of large crystals called grains and within each grain the solute element in the alloy will be segregated in such a way as to produce the tree-like patterns called dendrites. A feature you can measure is the spacing between the dendrite arms, a parameter controlled by the rate at which the casting cools from the melt.

Sample 5: Worked and annealed bronze

When a piece of metal is cold worked the process by which it is deformed causes it to harden and become brittle. To make the metal workable again it has to be heat treated – annealed – and a new, strain-free grain structure nucleates and grows. This structure is very different from that in the casting and the differences show how you can use the microstructure to understand how a piece of metal has been treated. This sample has been cut from a large bracelet made in about 1200 BC from a bronze containing 5% tin in copper; this shows something of how stable metal structures can be. You will see polygonal grains crossed by parallel-sided bands which are growth defects in the grains. Select a magnification that displays, say between 10 and 20 grains across the width of the image. Use the “Measure” menu to draw a line across, say, 10 grains and estimate an average grain diameter. You might also use tools from the same menu to outline a single grain and measure its area. (Note: the blue-grey inclusions in the metal are copper sulphide, the sulphur surviving because of inefficient smelting and refining of copper from a sulphide ore).

Sample 6: High zinc brass

When two metals are mixed to make an alloy it is often the case that, while the melt is a single liquid, there is not complete solid solution of one in the other. As one element is added it distorts the crystal lattice of the other to such an extent that it becomes unstable and a different structure, or phase, becomes stable. In brasses, which are alloys of copper and zinc, this happens at something over 30% zinc. Metallographic etches very readily highlight the differences between phases and the two different phases here are clearly visible.

Sample 7: Carbon-steel, air cooled

This steel is an alloy of iron with 0.8% carbon. When cooled slowly from very high temperature (say 950°C) a series of solid-state reactions occurs which results in the mixed structure you see. The white areas are effectively pure iron (called ferrite) while the dark areas (pearlite) are a finely divided mixture of ferrite and an iron carbide called cementite. If you use the highest magnification you should be able to resolve the structure of the pearlite into thin bands called lamellae. To do this you will almost certainly need the $\times 100$ objective. Again using "Measure" draw a short line across a bundle of, say, 5 or 10 lamellae and calculate the average width. You will very probably find a value of the order of 0.5 μm , showing you are getting very close to the resolution limit of the microscope. Using zoom on the screen might make the lamellae a little easier to see but does not increase the resolution.

Sample 8: Carbon-steel, water quenched

As a final example of the variety of structure that can be studied with the optical metallographic microscope this sample shows how the structure of an alloy can be radically modified by different thermal treatments. Here a steel similar to that in Sample 8 has been cooled many times more rapidly and a totally different structure called a martensite has been produced. This is much harder than the ferrite/pearlite structure and offers a good introduction to the versatility of steel.

Both this and the last steel you will encounter again in a 2nd year experiment on the properties of steels.

These samples have been chosen to give you a rapid overview of some of the principles and practice of examining metal structures. Save the images and when you meet them again during the course you will recognise them and be able to relate the theory you are learning to real pieces of metal.