THINGS TO KNOW BEFORE YOU GET TO THE PROBE LAB

The following document outlines information that is useful to know before you start using the microprobe, regarding subjects such as sample preparation, helpful hints, actual use of the microprobe and what to expect when you reach the lab.

Sample Preparation:

The quality of your analyses depends most heavily on the quality of the physical preparation of your samples, and there are several factors to consider when preparing and handling your samples before you get to the microprobe lab. Foremost among such factors is the quality of the polish of your samples – the smoother and more level your samples are, the better will be the overall quality of your analyses. Smooth and highly polished samples with the surface of the rock or mineral parallel to the glass surface of the slide (i.e., not slanted relative to the slide) permit the electron beam to hit the sample at about a 90° angle, which is the ideal sample-to-beam geometry, whereas the microprobe software assumes this angular relationship is present when analysing the sample. Samples that are not flat or that have significant local relief may cause anomalous scattering of electrons and resultant, emergent X-rays; such X-rays may not emerge from the sample at an ideal angle (called the 'take-off angle, or TOA) to enter the WDS spectrometers at their full intensity. When this happens, your analyses are most likely to have low totals. Additionally, a poorly polished sample is unlikely to coat evenly when placed in a carbon-coater; an uneven carbon-coat will result in local build-ups on the sample of static electronic charge from the electron beam. We call this phenomenon 'charging', and it will result in the partial or complete repulsion of the electron beam away from the point on your sample that you are trying to analyse – you may end up analysing a point you didn't intend to, but more than likely, if the beam does hit the spot at which you were aiming, the repulsion of some of the beam by the charged area will result in a lower net intensity of X-rays produced by the target region, and this necessarily leads to low totals of the analysis.

Charging may also result from a poorly cleaned sample, even if the polish is of high quality. In order to clean your slides, the recommended procedure is to wipe the sample clean with a kimwipe or other lint-free material, and then to place the slide in a small beaker of acetone. The beaker with the slide should be placed in an ultrasonicator-bath and allowed to be cleaned for about 2 or 3 minutes. This procedure will remove all adhered particulate matter (dirt) and organic residue from the sample, such as the most common contaminant, namely oils from your fingers. There are very few things more frustrating than finding carbon-coated fingerprints on your slide when it is placed in the microprobe. When using this procedure, be sure to wear nitrile gloves when handling the slide so that you don't contaminate the slide with finger oils, and to protect yourself properly (be sure to consult the appropriate MSDS for safety information when handling any chemical). Also, handle the slide by the edges whenever possible, especially after having cleaned them, otherwise you will undo all your hard work to

this point, and will likely end up with poor analyses with low totals. Finger oils and dirt will charge under the influence of the electron beam, and cause anomalous scattering of the beam. Dirt and other contaminants generally will cause an applied carbon coat to adhere poorly at best, and to not adhere at all in the worst case. When cleaning and drying the slides after having removed them from a solvent bath, or when trying to remove dirt or dust from the surface, do not use 'canned air' products, because they can contain materials like aerosolized waxes that may attach themselves to your slides, perhaps altering the efficacy of carbon from the coater to adhere to your slides. A better alternative is to use an 'air puffer bulb' used to remove dirt from camera lenses, that use ambient atmosphere to dislodge or remove macroscopic particulates from the surface of the slide.

On a related note, before you put your slide into an acetone-filled beaker, be sure to test that the epoxy that has been used to adhere your sample to the slide is compatible with acetone, because if the sample doesn't tolerate immersion in acetone and is chemically reactive to acetone, you will likely ruin your slide. Reaction of the epoxy with acetone may also occur if the epoxy has not been cured properly. To test for compatibility, put a small droplet of acetone on a small area of epoxy on the slide and see if the epoxy starts to look cloudy as opposed to clear, and press lightly on the exposed epoxy with a needle-probe. If the epoxy looks cloudy or feels soft when you push the needle probe into it, then don't immerse your slide in acetone to clean it. Instead, try another, less aggressive solvent such as ethanol, pre-testing the compatibility as described above. BE AWARE that all common organic solvents such as ethanol, acetone and methanol have low vapour pressures and relatively low ignition points. This is important to know when placing your solvent-filled beaker into an ultrasonic bath, because ultrasonic waves will cause the solvents to heat up and enhance the formation of vapour clouds above the surface of the solvent, so be careful not to ultrasonicate your samples for more than about 3 minutes, otherwise the solvent could spontaneously ignite. Methanol is particularly hazardous in this context, being of low molecular weight, and isn't recommended as an ultrasonication medium. Under no circumstances should you clean your samples with water, as your samples may be hygroscopic. Any water absorbed by your sample will boil off in the vacuum of the microprobe chamber, especially when heated by the electron beam. Be sure to adequately dry your sample before you get to the microprobe lab and before you coat the sample in carbon, as any absorbed fluid (water, polishing media, finger oils) will boil out of the sample when exposed to a focussed electron beam, and this will result in disruption of the carbon-coat, leading to anomalous analytical results.

It is strongly recommended that you clean your slides ultrasonically before you arrive at the lab to avoid any delays – your samples need to be clean and dry before you have them carbon-coated.

Related to the preparation of your sample and the quality of the analyses you hope to get is the *nature* of your sample (some of these types of concerns are covered in the *Considerations*

When Picking Points to Analyze section, below). The nature of your sample in the context of sample preparation mainly speaks to the issue of how well the carbon-coat will function. Thin sections that have a good polish, aren't plucked or have voids in them and where the rock/mineral slice covers more than about 85 % of the slide contiguously tend not to charge much even with just an average quality carbon-coat, because the carbon coat is contiguous and there is very little epoxy to have to coat on the slide margins. Epoxy, even if it is fairly well-cured, is susceptible to beam damage more so than an average silicate mineral, so ideally, we don't want to have any exposed epoxy on the slide – this is highly unrealistic, however. One of the worst-case scenarios occurs when you have grain mounts, with a lot of grains suspended in a sea of epoxy. Every time you zoom in on a given cluster of grains or on an individual grain using the SEM, you are concentrating the energy dosage experienced by the region upon which you are zooming – this is exactly the same situation as when you alter the focal distance of a magnifying glass on a piece of wood, focusing the sun's energy on the wood, causing it to burn. Whereas the epoxy is susceptible to beam damage, every time you concentrate the focussed electron beam on an individual grain, you will 'burn' the epoxy, and thereby disrupt the carbon-coat that lies on the surface of the epoxy surrounding your grain-of-interest. In turn, this physical disruption of the carbon-coat will cause an interruption of the ground-path of electrons trying to make it to the edge of the sample holder and away from your sample. If the excess electrons are unable to find a ground path, they will accumulate on or near your grain resulting in the phenomenon of charging, discussed above. Charging will result in poor quality analyses.

Carbon Coat

The quality of your analyses is also highly dependent on the quality of the carbon-coat, assuming for the moment that the sample is well-polished. Contaminants on the surface of your slide will cause the carbon-coat to adhere poorly to your sample. The reason why the carbon-coat and its quality are critical factors in obtaining good analyses is that most materials, particularly rocks and most minerals, are not good electrical conductors. If the slides were not coated in carbon, almost all the electrons shot at the sample would accumulate to form a static cloud on the surface of the sample, and this static cloud would repel incoming electrons, preventing them from hitting your sample and producing measurable X-rays. Coating the sample in carbon permits excess electrons to conduct away from the focussed electron beam during analysis, and reduces the effects of charging. Even the electrons that produce X-rays have to conduct away from the point being analysed – they don't magically disappear after having ionized your sample, resulting in the production of X-rays (conservation of matter). So, hopefully you now have a better appreciation of why the cleanliness and quality of polishing of your slides/samples are so important – they both effect the quality of the carbon-coat, which in turn effects the quality of your analyses. The thickness of the carbon-coat is also critical to getting good results, as will be explained, below.

So, the above discussion, consistent with the goal of this document to provide you with helpful advice *before* you get to the microprobe lab, begs the questions, "How do I know if the carbon-coat is good before I get to the lab?", and "What can I do if the coating is poor?". In answer to the first question, qualitatively, there are a couple of visual indicators that might indicate whether the quality of the coat is good. If the sample has an even and consistent, light-grey or even slightly metallic appearance, the coating is probably good. If the coating has a slightly gold or reddish-orange tinge to it, and/or is not even across the slide, the coating is probably not thick enough for the purposes of analysis. If the coating is very shiny and metallic or exhibits mirror-like reflectivity, the coating is probably too thick. The next and more definitive way to assess the quality of the coating is to use a multimeter to measure the resistance of electricity across the sample. To make this assessment, set your multimeter to the 'resistance' setting (designated by the symbol Ω) and place the measuring probes at opposite ends of the slide. If the resistance reads as an open circuit/open line, then the sample doesn't likely have an optimal thickness to effectively reduce charging. If the reading is around 600 kΩ or less, then the thickness of the carbon coat is likely good enough to produce good analyses. In general, the lower the resistance reading the better. Optimally, the resistance should be less than about 300 kΩ for the best results. In answer to the second question above, if the coating leads to a less-than-optimal resistance reading, simply recoat the sample – you may need to do this 2 or 3 times to achieve the optimal resistance reading in the multimeter. I have found that benchtop coaters tend to produce a carbon-coat that is less than ideal for the purposes of quantitative analysis, even when set to produce a 'thick' coat, particularly where carbon thread is used rather than a carbon rod.

The *thickness* of the carbon-coat also plays a part in getting good results, particularly when considering the aim of your particular type of analysis. So far, the carbon-coat and its role have been discussed in the context of common analytical beam conditions for quantitative analysis, namely using an accelerating voltage of 15 kV and a beam current of 20 nA, with average counting times on X-ray peaks. There are times when you will want a thicker than normal carbon-coat on your samples, such as during X-ray compositional mapping, where the beam current used is about 100 to 200 nA, or when analysing Th, U, Pb and Y in monazites for the purpose of geochronology, where counting times may exceed 7 – 12 minutes per point. In these cases, the carbon-coat will need to be thick in order to minimize beam-damage during data collection.

Practically speaking, a good way to assess the quality of the carbon-coat oncw your sample is inside the probe is to look for horizontal discharge bars in SEM images – absence of such bars is a good indicator that the coating thickness is sufficient for quantitative analyses at the voltage and beam-current settings you are using for the imaging. Another way to assess the quality and thickness of the coating is to compare the difference between the 'nominal' Beam Current with the Farraday cup inserted (i.e., beam not hitting the sample) *versus* the Absorbed Current with

the Farraday cup withdrawn (i.e., beam hitting the sample in imaging mode). The closer these 2 numbers are, the less is the likelihood that charging will occur.

Another reason why consistency is required in the thickness in carbon-coats is that, if there is a significant difference in the thicknesses of coating between the standard and the sample, the resulting analyses will be poor, with totals being either too low or too high. A disparity in coating thickness between the standard and sample will lead to a proportional difference in X-ray intensities produced by both for the same concentration of a given element. For this reason, it is best for standards and samples to be coated by the same apparatus using an identical procedure and operating conditions. Even using identical protocols in this way may still result in disparities in coating thicknesses between the standards and samples if the quality of the carbon sources differs, e.g. carbon rods or carbon threads from different production lots.

IMPORTANT !!! If you have your slides coated at our lab, the coating will be of the optimal quality, as the probe technologist always checks the coating thickness and conductivity. If you coat the slides at another lab, you will have to check the conductivity using a multimeter, as described above. The lab technologist will check the conductivity of your slides even if you have them coated at another lab – if the coating isn't sufficiently conductive for use in the microprobe, you will have to have them recoated, without exception, if you wish to analyse the slides. If you wish our lab technologist to clean and recoat your slides in our lab, there's no problem with that, except that it will require about 2 – 3 hours to complete the process, and this will delay your starting time. If the lab technologist determines that the coating on your slides is inadequate for microprobe analysis, you will not be allowed to analyse your samples, as the likelihood that you will get sub-standard analytical results is high, and no-one wants to have to pay to redo analyses, nor do we wish to waste microprobe time.

If you wish to have your samples coated at the microprobe lab, please be sure to give them to the microprobe technologist a few days in advance of your booked microprobe time to avoid any delays. If you show up at the lab the day you are slotted to use the microprobe and your samples are uncoated, there shall be a delay of 2 – 3 hours in your starting time such that the samples can be coated.

Marking of Slides

There has always been a difference of opinion between researchers as to whether they should mark regions of interest on their samples using ink pens or not (e.g. using vegetable pigment-, aniline-based or glycol-based pens such as Sharpies™). The rationale goes as follows. Those against marking slides *before* coating their samples argue that the ink doesn't allow for the carbon to coat the sample evenly, or that the ink will electrically isolate/insulate the minerals grains of interest, which could promote charging of the grain owing to an inadequate ground

path. The argument has a variation in that some users argue that above-described insulation effect is made worse when the grains of interest are completely circled by ink, therefore, they promote encircling regions of interest by dots of ink. If you wish to mark your samples before coating them in carbon, I would recommend simply drawing lines from the edge of your slide to the region of interest, and not encircling said regions at all.

Still other users promote marking on their samples *after* coating their samples in carbon. This practice isn't recommended, given that the friction of a felt-tipped pen against the carbon-coat and the glass slide might easily remove the carbon-coat.

If you really want to mark indicators on your slides, I recommend using a pen that has conductive ink, such as a Pilot ™ Supercolour Marker (no xylene), and to mark your samples before coating them with carbon. These pens used to be available at Staples, and are also available from Ted Pella Inc, an SEM supplies distributor.

Something to be aware of when marking slides, is that the electron beam will blast away components in the ink, and these components will contaminate the column. This contamination will lead to poor image quality and interfere with quantitative analysis. As the microprobe technologist, I advocate not marking your slides at all, so as to minimize contamination of the microprobe. With the advent of digital photography, it is much easier to have a detailed image of your slide in order to navigate than it is to mark them with ink.

The bottom line for marking slides is that the decision depends on the types of analyses you are doing; if you are looking at a texturally and compositionally undifferentiated sample, it may not matter exactly which grains you analyse, so long as you pick good grains. That type of decision should be decided in consultation with your academic advisor before you get to the microprobe lab, in order to save time and worry, and to meet the analytical needs of your particular project – the best approach is to have a plan before you arrive. Prior planning prevents poor performance.

Photographing of Slides

An alternative to marking slides with ink is to photograph the samples and mark points for analysis on the images, digitally. Having enlarged, digital scans of your samples makes navigation easy. You might think it is easy to remember certain features on a given slide whereas the slide is only about 2 cm by 3 cm, but at magnifications of 40 X or above, it may seem that you are looking at a map made to a scale of 1:100,000! Also, keep in mind that whereas images you have seen of your samples in the optical microscope are in full colour and have a certain depth of field, images rendered by the SEM are in greyscale only, with almost no depth of field – this can be rather confusing, if you have never used an SEM before. Also, recall

that the maximum magnification of a typical petrographic microscope is about 1500 X, whereas the maximum practical magnification factor of an SEM is about 100 times greater than the petrographic microscope, making it very easy indeed to 'lose your way' when using an SEM.

The Probe for EPMA™ software available for use with our microprobe makes it possible to import digital images from a camera, cellphone or tablet and have them digitally auto-scaled to the coordinate system of the microprobe stage, making navigation of your slides in the microprobe as easy as clicking a mouse button. It is, however, always advisable to keep a sketch or hard-copy of any images you intend to use for navigation purposes, whereas digital storage devices are known to malfunction from time to time.

Storage and Transportation of Slides

When storing slides, you may wish to have a small amount of dessicant in your slide box or holder/storage vessel if your samples are hygroscopic or prone to oxidation. This method is particularly useful to preserve slides from oxidation by water vapour, especially if there is a significant gap between the time your slide was prepared and the time that you are scheduled to use the microprobe. More useful still for such samples, is the practice of storing your slides in a vacuum dessicator or in a dessicator chamber.

Once your slides have been carbon-coated, it is important to handle them as little as possible, and to handle them by the edges only, and to wear gloves to minimize or eliminate the transfer of finger oils from your hands onto the slide. Avoid using fabric-based gloves when handling slides, because they reduce your grip on the slide – bad things can happen when the slide shifts in your grip, ranging from dropping the slide onto a hard surface, to having the slide twist in your fingers such that you end up holding the slide by the front and back surfaces and removing a large portion of the carbon-coat. Also, fabric gloves enhance the possibility of transferring fabric fibres onto the surface of the slide, and fibres charge and move around the surface of your slide inside the microprobe in an unpredictable manner.

Once your samples are coated, it is not recommended to wrap them in kimwipes to 'preserve' them during transport, because kimwipes can potentially remove some of the carbon coating from the slide. Wrapping samples in Kleenex is even worse, as the Kleenex can both remove some of the carbon coating and will deposit small paper particles on the slide surface that are difficult to remove owing to 'static cling'.

Regarding the storage of samples after your analyses have been completed, the lab and its personnel accept no responsibility whatsoever for your samples. You must arrange for their storage and/or transport, as the lab is not a repository for neglected samples. Periodically, samples that are left in the lab are purged.

Considerations When Picking Points to Analyze

Even though you aren't sitting at the microprobe and setting points right now, it is useful to have some helpful pointers before you do start setting points. An important factor when selecting points to analyze is to select a flat region of the sample, for reasons discussed in the 'Sample Preparation' section of this document. Points selected for analysis should be well away from pits, cracks, plucked areas, pieces of dirt, fibres and 'puddles' of fluids, because al of these types of features are highly prone to charging, and will also cause anomalous scattering of primary electrons and emergent X-rays. Whenever possible, points should be picked at least 5 microns away from grain boundaries, because the direction in which these boundaries dip is unknown; analyzing too close to a grain boundary may result in a composite analysis of adjacent grains owing to a shallow dip-angle of impingement of said grains. Picking points too close to the edge of a slide is not advisable, as the minerals near the edge tend to have sloping edges, and analysing near edges will most likely cause anomalous scattering of emergent X-rays. This phenomenon also occurs when analyzing adjacent grains that have significantly different hardness, where the harder of the two grains tends to exhibit a higher physical relief than the softer grain does, leading to rounded edges on the boundary regions of the harder grain.

Chief among the points that you want to avoid while using the microprobe are altered grains, as they will almost always yield analyses that have low totals. This is particularly true when analysing hydrous alteration products (e.g., clays, serpentine, Fe-oxyhydroxides). If your goal is to analyse alteration products, be aware that low totals are in your future.

You should be aware that the microprobe can't analyse for water (structural or waters of hydration), OH-groups, H, He, Li or Be in WDS mode. Therefore, all mineral species (and glasses) that contain OH-groups or water will yield low totals for their quantitative analyses, e.g., clays, micas, amphiboles, zeolites. As it turns out, many hydrous species are also susceptible to beam damage during the analytical process, meaning that the structures of said species break down as you are trying to measure them; therefore, the very act of measurement is effectively changing the quantity of the elements you are trying to measure. You should also know that, the microprobe reports all elements at one specific valence state, e.g., Fe as either FeO OR Fe_2O_3, not both. Therefore, if you are analysing minerals such as chromite or other spinels that can have multi-valent cations, your reported totals may be low.

Speaking of beam damage, the electron beam also tends to damage glasses, both natural and synthetic, depending on the composition of the glass in question. If the glass is hydrous, then you experience the problem described in the previous paragraph. In glasses more so than in crystalline substances, Na, and to a lesser extent K, will physically migrate via solid-state

diffusion away from the centre of the electron beam as they are ionized by high-energy electrons and attempt to re-capture low-energy electrons to achieve electrical neutrality (the mechanism is a little more complicated than that, but all you need to understand is that migration of alkalis in the solid state will lower the totals of your analyses).

What to Expect to See in Microprobe Images

Most geologists are used to seeing thin sections in transmitted and reflected light. In both these cases, various colours are evident upon gazing through the optical microscope. Unlike optical images, SEM images are generated using an electron beam that is scanned across your sample. The resultant image is in grey-scale for SEI, BEI (COMP), TOPO and CL modes of imaging, and the image is largely representative of what is on the surface of the specimen, only – no subsurface features are seen. As a result, you won't be able to see items such as buried/hidden fractures or inclusions or 3-D grain boundary interactions through the depth of the sample. SEI and BEI are the two most common imaging modes upon which most users concentrate, as they reveal structural and compositional information that may not have shown up in optical imaging. Keep in mind that, optical microscopes have a magnification limit of about 1500 X – the SEM/microprobe has a theoretical limit of about 300,000 X magnification, although for most materials, ,ost users don't tend to go much above about 5,000 to 10,000 X. Even so, you may discover that features that looked homogeneous in the optical microscope may turn out to be quite heterogenous at the much higher magnification factors offered by the SEM.

SEI (secondary electron imaging) mode is very useful for discerning good points to analyse, as it reveals surface features such as smoothness/roughness, cracks, pits, pieces of dirt, plucked regions, grain boundaries and whether or not your sample is charging. Some of these features are not revealed by BEI (back-scattered electron imaging, also called BSE or COMP imaging). In fact, some physically undesirable features such as pits may show up as dark spots on a sample, and you could be fooled into thinking those pits are compositionally different regions surrounded by other minerals of interest.

BEI (back-scattered electron imaging) mode is used mostly to distinguish between phases of differing composition, and is useful for getting a rudimentary idea of the relative abundances of different phases within a given field of view. This mode allows you to tease out compositional details within individual grains, such as compositional zonation from the rim to the core that you won't be able to see in SEI mode. The key to mastering this mode is careful adjustment of the contrast and brightness settings of your image, which can both be adjusted 'on the fly'. BEI images tend to have a wider range of grey-scale values than do SEI images – this is important when deciding exactly which features of your sample you wish to accentuate and record as a

digital image, and this is the sort of thing you will want to discuss with your advisor, ahead of time.

Philosophical Considerations

Please keep in mind that, although the probe technologist can supply you with expert advice when it comes to sample preparation, EPMA theory and operation of the microprobe, he is *not* your academic advisor, and shall not presume to tell you what minerals/grains you should measure or how many points to measure on each grain. Those types of decisions regarding analytical strategy should be discussed with your advisor long before you step foot in the lab. Presumably, you have examined any thin sections prior to coming to the probe lab, using optical techniques and you will know your slide better than the technologist will. That being said, the technologist will do his best to answer any questions you might have – there is no such thing as a stupid question, except the one you *don't* ask when you don't know the answer.