CHM 317H1S
Winter 2018

Section C – FTIR & Raman Spectrophotometry

C: FTIR Spectrophotometry

1. List of Experiments

1. Attenuated Total Reflectance (ATR) FTIR Spectrophotometry
2. Diffuse Reflectance Infrared Fourier Transform (DRIFT) Spectrophotometry
3. Pigment Identification by Raman Microscopy

2. Locker Inventory

Equipment for this experiment may be found in a designated drawer in room LM6. This should include the following items; please check that you have everything below at the beginning of your first laboratory period for this set of experiments.

✔	Quantity	Items for Experiment C1 (2 drawers)
	5	25 mL volumetric flasks with stoppers
	2 each	10 mL volumetric flasks with stoppers
	2 each	1, 2, 5, 10, and 20 mL transfer pipettes
	2 each	5 and 10 mL graduated (Mohr) pipettes
	2 each	50 & 250 mL beakers
	1 each	100 & 500 mL beaker
	2	3-valve pipette fillers
	10	Pasteur pipettes and rubber bulbs
	1	Plastic wash bottle for distilled water
	1 box	Collection of known, labelled polymer samples
	2 each	Large and small spatulas
	1 box	KimWipes™

✔	Quantity	Items for Experiment C2 (2 drawers)
		Labelled vials containing ground tablet samples
	1	Pestle and mortar set
	~50	Small disposable plastic sample containers with lids
	2 each	Large and small spatulas
	1 box	KimWipes™

3. Instrumentation

During this set of experiments, you will be using one of the different FTIR spectrophotometers located in room LM9. These include:

- Thermo Scientific iS50 (with MTIR/ATR – experiment C1)
  - Built-in single-bounce diamond ATR sample accessory
  - Pike Technologies variable angle MTIR accessory kit
- Perkin-Elmer Spectrum One (with DRIFT – experiment C2)
  - DRIFT accessory kit

4. General Operating Instructions

The following pages provide general information on using the FTIR spectrophotometers in the Analest laboratory. Please take time to read through these instructions carefully **before coming to the laboratory.**

4.1 Starting the Instrument:

(a) The FTIR instruments are left on at all times. If the software is not already running on the attached computer, login to the computer using the appropriate username and password:

PE Spectrum One: username chm317ftir4 and password ftir4

Thermo iS50: username chm317is50ftir and password is50ftir

Once Windows has started, locate and double-click the desktop shortcut for the relevant FTIR software, either:

- **Spectrum for Windows** (PE Spectrum One)
- **OMNICS** (Thermo iS50)

When the software launches, it will present a dialog that should show the name of the instrument being used in a drop-down list. Simply click on the **OK** button to proceed.

(b) Some of the accessories used in these experiments mount directly into the sample compartment of the instrument, and require some adjustments be made to obtain the highest possible energy throughput. Laboratory technical staff will have already made these adjustments for you.

4.2 Running the Instrument via Software:

Collection of background and sample spectra is similar whether the instrument is being operated in conventional transmittance mode or using one of the reflectance accessories, although there are some slight differences in the software interface and sequence between the two instruments. There is also one additional step in the procedure when using the DRIFT accessory. Once a spectrum has been collected, the both software packages have similar features in terms of displaying, manipulating, and printing the data, although item names and locations on the screen will be different. See the relevant section for each experiment for further details.

4.3 Obtaining Excel-Compatible Versions of your Spectra:

Both software packages allow you to save a collected spectrum as a comma-separated variable (.csv) or tab-separated text (.asc or .txt) file which can then be imported into Excel or a similar application for additional processing and plotting. Make sure the spectrum you want is selected in the main software window, then use the standard **File** menu **Save As…**function and choose the appropriate option. Remember to save your data to a folder in the networked drive

Once you have all the files you need converted and stored on the network drive, go to one of the public terminals at the far (south) end of LM9; you will then be able to transfer the files to a USB key.

4.4 Finishing Up Your Experiment:

When you have completed *all* your measurements and transferred any data required for your calculations and report, make sure that the instrument and area around it are clean and all waste properly disposed of. Exit the software and sign out of Windows, but do **not** turn off the instrument.

5. Experiment C1: Attenuated Total Reflectance FTIR

This experiment explores the use of attenuated total reflectance FTIR (ATR-FTIR) for the simple analysis of liquids, solutions, powders, and film samples. The primary advantage of ATR-FTIR over more traditional methods such as liquid cells and the nujol mull technique is that samples can be measured *without* the need for a solvent or liquid medium: as a result, none of the sample peaks are obscured. Preparation time is also reduced, and solid samples can often be reclaimed after analysis.

In ATR, a special accessory is placed in the sample compartment of the instrument that diverts the IR beam through a high refractive index IR-transparent crystal in such a way that the beam is subject to total internal reflection. Variants include single-bounce (one internal reflection) and multiple-bounce (also known as multiple total internal reflectance or MTIR) configurations. Internal reflection results in a special standing electromagnetic wave (an *evanescent* wave) which propagates a short distance (~1 μm) away from the surface; attenuation of the evanescent wave by absorption results in a corresponding reduction in the intensity of the main beam. This allows the IR spectrum of material *in close contact* with the surface of the crystal to be obtained.

Further information can be found in chapter 17 of the course text, and from the manufacturer of one of the accessories used in this experiment, available at:

http://www.piketech.com/files/pdfs/ATRAN611.pdf

After performing this experiment, you should:

- Understand the principles and advantages of ATR-FTIR
- Be aware of which samples are best suited to analysis by ATR-FTIR
- Know how ATR-FTIR can be used for quantitative and qualitative analysis

Chemicals:

- Absolute ethanol (solvent cabinet)
- Distilled water

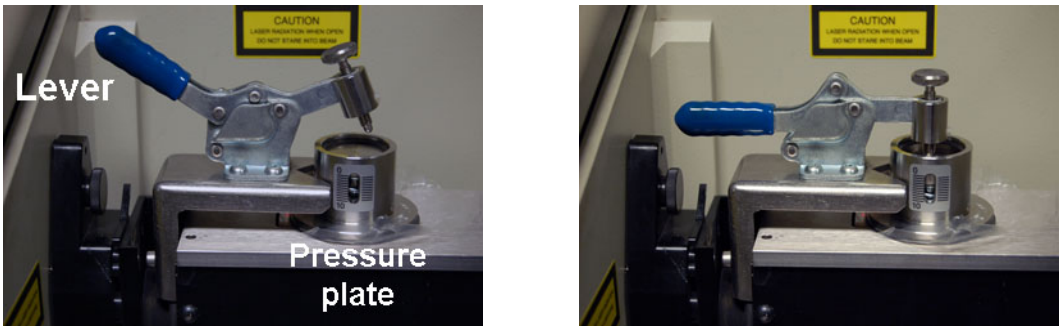
Sample(s):

- A selection of polymer films and organic liquids
- A vodka (or similar) sample
- Polymer samples: bring in your own samples of soft plastic (bags *etc*.); you will need at least *three different samples to identify*. Samples should be soft or flexible – hard plastics cannot be analysed, as it is difficult to obtain uniform contact with the ATR element surface.

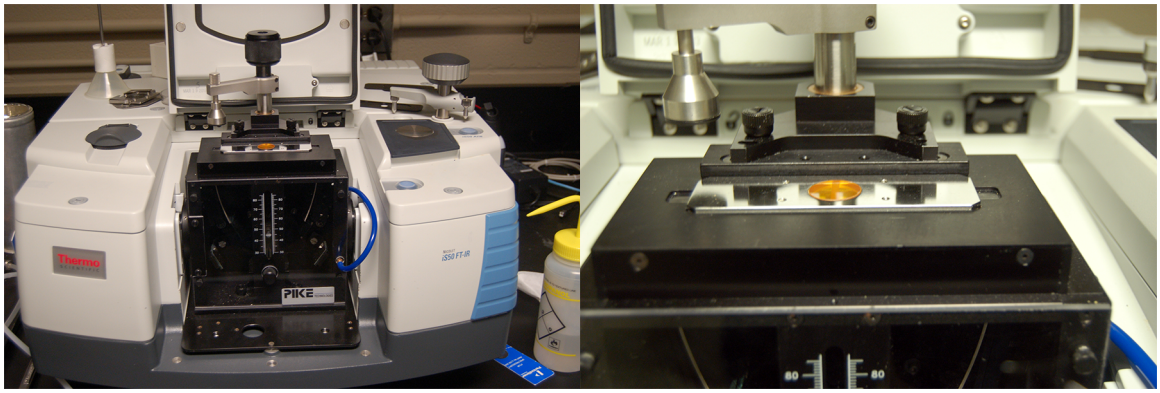
A. Instrument Setup and Polymer Identification by ATR-FTIR (is50 FTIR)

1. The instrument should already be setup with either the multiple bounce or variable angle ATR accessory (shown below). Log in to the computer associated with the instrument and

launch the FTIR software as set out in section 4.1. Note that, with the variable angle or multiple bounce accessory installed, you are not able to close the sample compartment lid.



Multiple bounce ATR accessory with pressure clamp released (left) and engaged (right), with a film sample between the ATR crystal and the pressure plate.



Variable-angle single bounce ATR accessory with pressure clamp released (left) and close up of the sample area (right). Avoid touching the yellow ATR element with your fingers.

2. Check that there is nothing currently placed on the ATR: the clamp should be raised and the exposed surface of the ATR element – a zinc selenide crystal – clearly visible. Make sure that the ATR element appears to be clean and dry. If the crystal has dust on it, use the small air can provided to blow the dust off. If it is dirty, spray a small amount of isopropanol on to a KimWipe™ to clean the surface and allow it to dry completely before placing any sample on it.

3. You will need to tell the software which accessory you wish to use: Click on the **Expt Slct** button in the top-left of the main window and then select **VeeMax ATR** as the Experiment from the relevant menu, and click **OK**. Note that the software will automatically apply the ATR correction to the collected spectra for you. You will need to collect a background spectrum before running any samples: make sure that there is nothing on the ATR crystal and that the clamp is **not** engaged, then click on the relevant button. Note that the system will purge the accessory unit with dry nitrogen gas for 15 seconds before collecting the data

4. You will be provided with a box containing a variety of different polymer films; choose one of these as your first sample, and record its identity in your lab notebook.

➔ Be **extremely careful** that you do **NOT** mix the samples up: remove one at a time, and replace it correctly before taking the next sample.

Place the first sample on the ATR crystal so that it covers the central area, swing the clamp into place, and then apply pressure by gently turning the large knob until you hear a "click" from the unit.

5. Obtain a sample scan for the instrument, being sure to use a use a unique filename for this scan. Once the spectrum has been obtained, make sure you use the button to add it to the current window. To label the peaks, click on the relevant button in the tool bar and then drag the horizontal line cursor so that only the main peaks in the spectrum are labelled. To print a copy, you will first have to either add the labelled spectrum to a new window or replace the existing one. Compile a list of the key peaks for each polymer in your lab notebook as you obtain each spectrum.

6. Repeat steps 4 and 5 until you have obtained and printed spectra for all the polymer films in the box. You can now obtain a spectrum from each of your polymer samples in turn; match the peaks in your sample spectra with those in the samples provided, and identify the composition of your plastic samples.

B. Quantitative Analysis by ATR-FTIR

7. Use the **Expt Slct** button in the top-left of the main window and then select **is50 ATR** as the Experiment from the relevant menu, and click **OK**. This will switch from the variable angle to the built-in single bounce ATR accessory for you. You will need to perform a new background scan, since you are now using a different accessory: make sure that the ATR element is clean and dry and that the correct wavenumber range is ebing used, then record a new background scan using the same procedure as previously.

8. Use the volumetric glassware provided to accurately prepare a set of standards containing between 0 and 40% by volume of absolute ethanol in distilled water.

➔ When diluting the contents of each flask to volume, add the distilled water in stages and swirl the flask in order to mix the contents *before* finally diluting to the mark – the volume of the solution will change as the water and ethanol mix!

9. Using a Pasteur pipette, place a drop of distilled water in the liquid sample holder so that it covers the ATR element *without* flowing everywhere. Record the ATR-FTIR spectrum of the distilled water following the same procedure as before.

10. Use a KimWipe™ to remove the water from the sample area; use the air can to blow the ATR element dry. Now place a drop of your first calibration standard on the element and record the spectrum as before. Continue this process until you have overlaid the spectrum of pure water with all of your ethanol–water standards, and the sample provided. Identify the main peaks in the spectra, determining which are due to water and which are due to ethanol; compile this information in your lab notebook.

11. In order to construct a calibration curve, you may need to convert the peak intensities from units of %T to absorbance (since we need to use Beer's law in order to obtain a linear

calibration). Make sure that all your spectra are selected (section 4.2(d)), and then choose **Absorbance** in the **Process** menu to have the software convert the data for you.

Zoom in on the relevant portion of the spectrum either by resetting the display axis or by dragging the mouse pointer across the plot and double-clicking within the resulting green rectangle. Position the vertical line cursor over the most intense peak, and tabulate the absorbance values together with the corresponding ethanol concentrations. Use this information to determine the ethanol content of the sample.

C. Investigation of the Signal-to-Noise Ratio

12. For this part of the experiment, you will investigate the effect of the number of scans on your signal-to-noise ratio. In general, increasing the number of scans should increase your signal-to-noise ratio, thus improving the quality of your absorption spectrum.
13. Make sure that both the sample well and the ATR element are clean and dry. Measure a background scan as previously. Using a Pasteur pipette, place a drop of distilled water onto the ATR element. Measure a sample scan by selecting **Scan Sample** from the **Instrument** menu and set the number of scans to 1. *It is essential that resolution and scan range are the same for the background and sample scans.*
14. Repeat the above procedure using 2, 4, 8, and 16 as the number of scans for your analysis. Make sure that both the sample well and the ATR element are clean and dry before the background scan for each set.
15. Apply background correction to your four absorption spectra by selecting the relevant item in the **Process** menu. *Do not apply smoothing to any of your spectra, as this will drown out the noise at the baseline regions.* Make sure the y-axes are set to transmittance, and export your spectra as ASCII (text) files (**File**: **Save As**). Obtain a copy of these text files by transferring them first to the network drive and then to a USB key from one of the data stations at the far end of LM9. See section **E(c)** for additional details regarding the data analysis.

D. Finishing Up

16. Make sure you have printed copies of your spectra, then exit the software and log out from the computer.

    Dispose of all your solutions in the aqueous organic waste container. Used Pasteur pipettes should be rinsed thoroughly with distilled water before being disposed in the teal-coloured **decontaminated glass bin**. Clean all your glassware, using some alcohol on a KimWipe™ to remove any labels, and return it to the correct bench drawers.

➔ Please keep all the transfer and graduated pipettes in a different drawer to the rest of the glassware, in order to minimize accidental breakage

Check all areas where you have been working – balance, bench, and instrument – to make sure that they are clean and tidy, and that all chemicals have been returned to the correct shelves. When done, have your TA validate your lab notebooks before leaving.

E. Data Analysis

(a) Review your polymer spectra; identify both the bond type and vibrational mode for each of the key peaks in each spectrum. If there are any peaks in your own samples that are *not* present in the specimens provided, try and identify what these might be. Make sure you summarise this information in your lab notebook.

(b) Construct a calibration curve by plotting the absorbance for ethanol against concentration (as %v/v); remember to subtract the absorbance due to water from that of each water–ethanol standard! Use linear regression analysis to determine r or r^2, $s_{y/x}$, limit-of-detection, limit-of-quantitation, and linear range of your method. Also calculate the concentration of ethanol in the sample provided, along with its uncertainty. Glue a copy of your calibration curve and calculations into your lab notebook.

(c) To determine the signal-to-noise ratio for any one spectrum, choose representative regions of the spectrum for both your baseline (noise) region and your signal. As your baseline region, choose a relatively flat (horizontal) region on the absorption spectrum near 100% transmittance, and one that exhibits considerable noise at a low number of scans. The standard deviation in the % T reading over this interval is a quantitative measure of your *noise* for that scan.

As your signal, select a representative peak in the absorption spectrum, and use its distance from the baseline region as your *signal*. A quantitative measure of your signal-to-noise ratio is thus simply the ratio of the two above-defined quantities. Do this for all four spectra measured (i.e. for 1, 2, 3, and 4 scans), *using the same assignment for both the baseline region and the peak you choose as your signal for all spectra*. Assess the effect of the number of averaged measurements on your signal-to-noise ratio. For your lab report, plot the signal-to-noise ratio as a function of the square root of the number of scans, and explain the trend you observe.

6. Experiment C2: Diffuse Reflectance

This experiment explores the use of diffuse reflectance FTIR (DRIFT) for the simple analysis of powders and solid mixtures. The primary advantage of DRIFT the nujol mull technique is that samples can be measured *without* the need for the liquid medium: as a result, none of the sample peaks are obscured. Preparation time is also reduced, and solid samples can often be reclaimed after analysis. One significant difference between DRIFT and attenuated total internal reflectance (ATR) is that sample materials can be coarser and harder: hard crystalline substances are generally unsuitable for ATR measurements as only very limited contact can be established with the ATR crystal, which can be damaged by hard substances like silicates.

In DRIFT, a special accessory is placed in the sample compartment of the instrument that directs the light beam onto the surface of the sample at an angle of incidence other than 90°. Some of the light is reflected between faces of particles before escaping from the surface at a range angles *other* than that corresponding to regular reflection; this is termed *diffuse* reflectance and, since this light has interacted more with the sample than light emerging at the regular angle of reflection (termed *specular* reflectance), it is attenuated more by sample absorption. The accessory is designed to capture as much diffusely reflected light as possible and direct it towards the detector, while blocking specular reflectance.

Further information can be found in chapter 17 of the course text, and from the manufacturer of one of the accessories used in this experiment, available at:

http://www.piketech.com/files/pdfs/DiffuseAN611.pdf

After performing this experiment, you should:

- Understand the principles and advantages of DRIFT
- Be aware of which samples are best suited to analysis by DRIFT
- Know how DRIFT can be used for quantitative and qualitative analysis

Chemicals:

- Anhydrous potassium bromide (desiccator in LM6 office)
- Caffeine and β–D-glucose (experiment storage drawers)

Sample(s):

- Pre-ground samples of common pharmaceutical tablets (drawer); see supplementary list on public course web site.
- Any tablets of your own you wish to examine (please check with the lab instructor first!)

A. Basic Setup and Investigation of Anomalous Dispersion

Anomalous dispersion – which can also be observed with solid samples using the nujol mull technique – occurs when both reflection of light takes place at frequencies close to strong absorption bands. One consequence of this is that solid samples for analysis by DRIFT must be

diluted with a non-absorbing solid such as dry KBr or KCl. The same material is also used to obtain the background scan, as this has to be obtained by diffuse reflectance also.

1. Dispense about 500 mg of KBr into one of the small plastic containers – this is sufficient material to reach the lowest filling mark on the container. Press a lid onto the container, and take it through to room LM9 to record the background scan while your lab partner(s) prepare samples for the first part of your experiment.

   Background Scan: open the scan dialog, enter your settings, and then press the icon in the top-right to obtain the background. For DRIFT experiments, use a range of 4000 to 550 cm^{-1}, 16 scans, and a scan resolution of 8 cm^{-1}.

2. Using the small plastic sample containers provided, prepare a set of samples containing various ratios of caffeine and dry potassium bromide. Note that the actual masses dispensed do not have to be exactly those specified, but you *do* need to know those masses as accurately as possible. Using one of the electronic analytical balances in LM6:

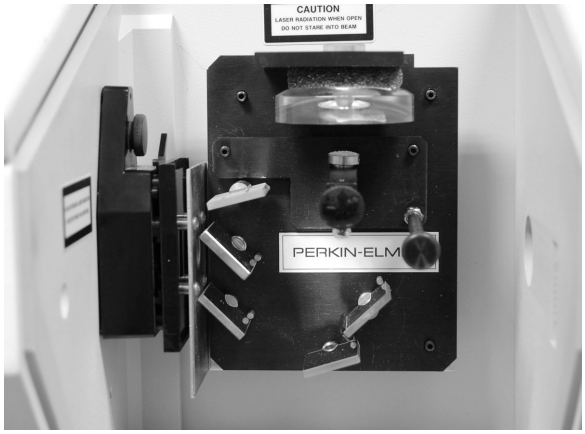
   - Dispense accurately by difference about 500 mg of KBr into a second container. Add about 50 mg of caffeine, and determine the actual amount added by re-weighing the container. Press a lid onto the container, and label this as 'A'. Note the *actual* masses of KBr and sample in your lab notebook. Shake the container for about 10 seconds to thoroughly mix the contents.
   - In the same way, additional samples labelled 'B' and 'C' consisting of about 500 mg of KBr with 125 mg and 350 mg of caffeine, respectively. Cap the containers, label them, and note the actual masses of KBr and sample in each in your lab notebook. Shake each container for about 10 seconds to thoroughly mix the contents.
   - Finally, dispense about 500 mg of caffeine into a fifth container, cap it, and label it as 'D'.

   Take these labelled sample containers through to room LM9 to the FTIR equipped with the DRIFT accessory to begin your experiment, while your lab partner(s) prepare the next set of samples.

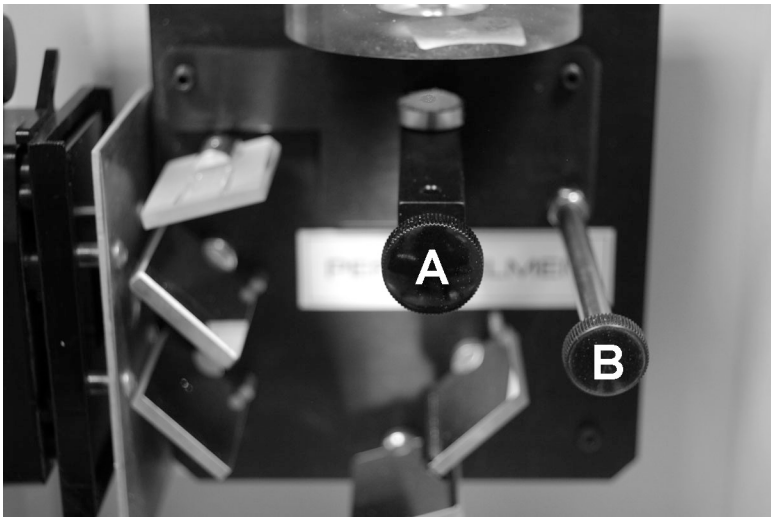
3. *Obtaining the DRIFT Background*: The DRIFT accessory is mounted in the sample compartment of the instrument as shown below. The main controls you need to be concerned with are the sample mount slide (A) and the height adjustment control (B).

CAUTION!

The controls are mechanical and should be handled GENTLY at all times. This is NOT a game console controller!



DRIFT Accessory mounted in the FTIR spectrometer



Close-up showing the sample mount slide (A) and the height adjustment control (B)

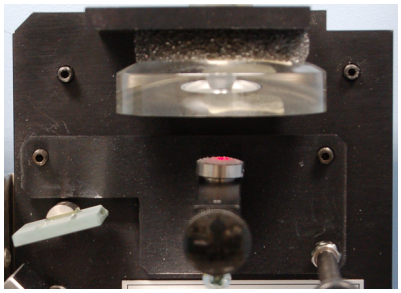
Gently pull the sample mount slide (A) towards you. You will now be able to lift the metal sample cup from the slide mount; place this on a KimWipe™ on the bench beside the instrument. Fill the sample cup with dry KBr by **slowly** pouring it from the plastic container. Lightly tap the sample cup on the bench to level the sample in the cup. Replace the sample cup into the slide mount – if the cup does not drop smoothly into the well on the slide, rotate it slightly until it does so. Once it is in place, **gently** push the slide mount towards the instrument until it stops.

➔ It is important to be as consistent as possible when filling the sample cup. If you have a different level of material each time, the diffusely reflected light will come off at a different angle, and may fail to re-enter the optical path of the instrument. This will generate a misleading error message in the next step.

4. Once the sample cup is in position, select the **Monitor** item from the **Instrument** menu in the software. In the resulting dialog box, make sure that the **Energy** mode is selected, and click on the **OK** button. The beam energy will be displayed as a bar on the screen;

adjust the height control (B) to obtain the highest possible beam energy to the detector then close the monitoring window. You can now gently close the sample compartment lid and obtain the background scan following the procedure set out in section 4.2(a).

➔ If you get an error message about laser power, it is likely that the sample cup is filled differently. Visually check the location of the red laser 'dot' on the sample: this should be slightly off centre (towards the right). If it is hitting the very edge of the sample, adjust the height control until the dot is closer to the centre and repeat step 4.



Close-up of the DRIFT sample cup in position, showing the red laser spot on the sample surface.

5. *Measuring the Caffeine/KBr Mixtures*: Once you have obtained the background scan, open the sample compartment and **gently** pull the sample mount slide towards you. Remove the sample cup, dispose of the contents into the KBr waste container provided, and wipe out the sample cup. You can now fill the sample cup with your first caffeine/KBr mixture, replace the sample cup on the slide mount, and maximise the beam energy reaching the detector in exactly the same way as you did for the background.

   Once the sample is in place and you have adjusted the height to obtain the maximum beam energy to the detector, gently close the sample compartment lid. You can now obtain the DRIFT spectrum for your sample. Make sure you use a unique file name for the spectrum, and enter a suitable description for it.

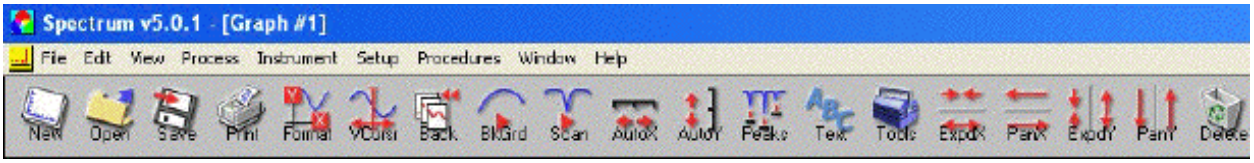
   Once the spectrum has been acquired, open the sample compartment, **gently** pull the sample mount slide towards you, remove and empty the sample cup, wipe it out, and repeat the measurement procedure for the next sample. Make sure you use a different file name for each sample spectrum.

   Once you have obtained the spectra of all the caffeine/KBr mixtures, label any significant peaks with their corresponding wavenumber, and print copies for each student. Note that the auto-peak criteria may need to be reset before the software will label anything for you: select **Options…** from the **Setup** menu. Switch to the **View** tab to change the number of decimal places used to display intensity values; switch to the **Peaks** tab to change the height threshold for detecting peaks within the spectrum.

B. Determination of Caffeine by DRIFT

6. Extend the range of samples used in part A by preparing additional samples containing different amounts of caffeine powder in KBr, ranging from 1 – 5 % by mass. Obtain the DRIFT spectrum for each standard using the same procedure as previously. Remember to

use the K-M peak values (which are analogous to absorbance values). For best results, calculate the peak height relative to a baseline running across the bottom of the peak. The software includes a baseline subtraction option to make this possible. Alternatively, you can export the data as a text file, transfer it to a USB key, and process it in Excel later (see step 15 of experiment C1).



Main Window Toolbar for Spectrum for Windows

(a) *Sample Scan:* First check that successive sample scans will be overlaid within the same window – select **View → Overlay/Split Display** and check that the **Overlay** sub-menu item is checked. Now select **Scan Sample** from the **Instrument** menu. In the resulting dialog box (similar to that for the background scan), enter a suitable unique **Filename** (e.g. aspirin), avoiding spaces, hyphens, and punctuation marks.

Make sure that the **Ratio** radio button is selected, and that the scan range and resolution are the *same* as those used for the background. For DRIFT measurements, you should select K-M (Kubelka-Munk) units. Note that, whichever units you select here, you can change the way the spectra are displayed at any time once acquisition is complete.

Once you have finished checking the sample scan settings, click on the **OK** button to start the sample scan. A progress window will display the data as it is being collected. Once the sample scan has been completed, the full spectrum will be displayed in the graphics window.

(b) *Selecting your Spectrum:* Successive sample spectra should be displayed on the same plot in the same window; the corresponding file names will then appear as a list in a panel directly below the plots. You can select an individual spectrum by clicking on the file name; the text should become bold while all other file names appear in plain text. You can remove a selected spectrum by clicking the **Delete** button in the toolbar; this will *not* delete the corresponding file, only remove the plot from the window. You can recall the spectrum at any time by clicking on the **Open** button.

(c) *Processing a Spectrum:* The Process menu contains numerous options for manipulating your spectra once collected. First, make sure that the spectrum you wish to process has been selected (see (b) above). If you did not choose the Kubelka-Munk (K-M) option for units in the **Scan Sample** dialog, choose **Process → Kubelka-Munk**. The software will correct the reflectance spectrum so that the relative peak intensities are similar to what would have been observed in a traditional transmission measurement displayed in absorbance (rather than %T) units

(d) *Labelling Peaks:* There are several options for identifying individual peaks and determining their wavenumber within a selected spectrum.

- Clicking on the **Peaks** button in the toolbar will label all peaks over a certain threshold transmittance value in the currently selected spectrum. You may find that this either labels too many peaks, or that it fails to label some of the less intense peaks.
- Another method is to use the cursor: click the **VCursr** button in the toolbar to show the vertical cursor; clicking a second time on the button hides it again. Position the mouse pointer over the vertical cursor so that the pointer icon changes to a double-headed arrow. You can now drag the vertical cursor to any point in the spectrum; double-clicking on the vertical cursor will label the spectrum at the point where the cursor crosses the spectrum.
- All labels can be dragged to avoid overlap with other labels and peaks. You can remove individual labels by selecting then with the mouse pointer and pressing the **Delete** key. You can remove all labels by choosing **View Remove → Peak Labels**.

(e) *Printing Spectra:* When you have configured the plot window (including any text labels) to your satisfaction, simply press on the **Print** button in the main toolbar.

(f) *Exporting Data:* You may export your spectra as comma-separated variable text files to a USB key.

Since Analest is a shared facility, it is strongly recommended that you reserve a single USB key for your data, and scan it for viruses regularly. All files should be transferred to the network drive, as you will only be able to transfer then to a USB key from there on one of the data stations.

Make sure that the spectra are visible in the plot window, and that the corresponding file names appear in **bold** text. Select **File → Save As**, switch the **file type** to **ASCII (*.ASC)**, and save the files to your USB key. When you open the files in Excel, you will see various lines of information (including the sample information if you entered it originally), followed by `#DATA`, followed by the actual wavenumber and transmittance values. Save the file as a standard Excel file (*.xls or *.xlsx) before attempting to plot the data for yourself! Note that Excel has an option under **Format Axis** to reverse the direction for the wavenumber axis.

7. Obtain a spectrum of the caffeine tablet sample: accurately dispense about 25 mg of the ground solid and 500 mg of dry potassium bromide into a clean dry plastic sample container, cap and shake to mix the contents thoroughly, before obtaining the DRIFT spectrum.

8. Compare your standards with the caffeine tablet sample, and identify one or two peaks to use in order to construct a calibration curve for the determination of caffeine. Remember to subtract the baseline from the caffeine peak. You may need to dilute the sample more to bring it within your calibration range; for best results, you should also bracket the sample with additional standards.

C. Identification of Solid Samples by DRIFT

9. Prepare a set of samples consisting of about 25 mg of ground solid in about 500 mg of dry potassium bromide, following the same procedure as for the caffeine/KBr mixtures in part A (step (b)). Make sure you clearly label the containers as you prepare each sample. The samples you should prepare are listed in the supplementary information for this experiment posted on the public course web site. For each sample, determine the wavenumber values for the key peaks and tabulate these in your lab notebook. Many of the tablets contain a mixture of ingredients, including cellulose (a polymeric form of glucose), stearic acid, *etc*. Print one copy of each spectrum making sure that it is clearly labelled.

D. Finishing Up

10. Make sure you have printed copies of your spectra, then exit the software and log out from the computer.

    Dispose of all your solid waste in the container provided, including used sample containers. KimWipes should be disposed of in the regular garbage bins. Make sure you return the tablet samples to the drawer for this experiment, and return all other chemicals to the appropriate location.

    Check all areas where you have been working – balance, bench, and instrument – to make sure that they are clean and tidy, and that all chemicals have been returned to the correct shelves. When done, have your TA validate your lab notebooks before leaving.

E. Data Analysis

(a) Calculate the actual composition of every mixture studied in this set of experiments as the percentage by mass in KBr. Study the spectra obtained with the caffeine/KBr mixtures, and estimate the maximum composition that can be used without the resulting spectrum being overly distorted by anomalous dispersion.

(b) Similarly, use the actual compositions of your caffeine standards to construct a calibration curve and determine the caffeine content of the tablet sample you were provided with. Remember to properly characterise your calibration curve using linear regression analysis, and to account for the fact that caffeine represents only a fraction of the tablet contents that were mixed with the KBr in your experiment.

(c) Summarise the spectral information on the tablet samples, indicating the extent to which specific sample components could be identified unambiguously. Lookup spectra of the pure active ingredients for comparison purposes; remember that these will typically have been recorded in conventional transmission mode (as liquid film, *etc*.), whereas the Kubelka-Munk correction is analogous to converting from %T to absorbance units.

5. Experiment C3: Pigment Identification by Raman Microscopy

While FTIR can be used to identify many substances based on bond vibrations, not all of these vibrational modes are found in the resulting spectrum – they are said to be IR inactive. This can be circumvented using Raman spectroscopy, a technique based on light scattering rather than simple absorption. In short, some small fraction of the molecules in a sample can be excited into a non-quantized "virtual state" by a photon having an energy that does not correspond to any electronic energy state transition; when the molecule relaxes, a photon is released. If this process results in excitation from and relaxation to the same vibrational energy state, the absorbed and emitted photons will have the same energy (Rayleigh scattering.) Some small proportion of molecules will, however, relax to a higher vibrational energy state, while an even smaller proportion will be excited from a higher vibrational energy level and relax to a lower one. This inelastic scattering gives rise to photons of lower and higher energy, respectively.

The difference in the energy of the exciting and scattered photons corresponds to the energy of vibrational excitation; where a specific vibrational mode is IR active, this corresponds to the energy – and therefore wavenumber – of the corresponding absorption band. Since the probability of Raman scattering is low, the technique requires a high intensity monochromatic source: lasers, and particularly modern diode lasers, have made the technique feasible and contributed to its increasing adoption as an analytical tool in many areas. Of particular interest is the use of Raman microscopy to examine historical objects and rare samples (such as meteorite fragments). The technique can be used not only to identify chemical substances, but also to create chemical "maps" of an area to provide additional insight on complex materials.

This experiment will consist of two main parts:

- An examination of standard materials to familiarise you with the instrumentation
- An examination of unidentified pigment samples from the Royal Ontario Museum

Chemicals:

- Isopropanol wash bottle (between Raman microscope and Spectrum One FTIR)

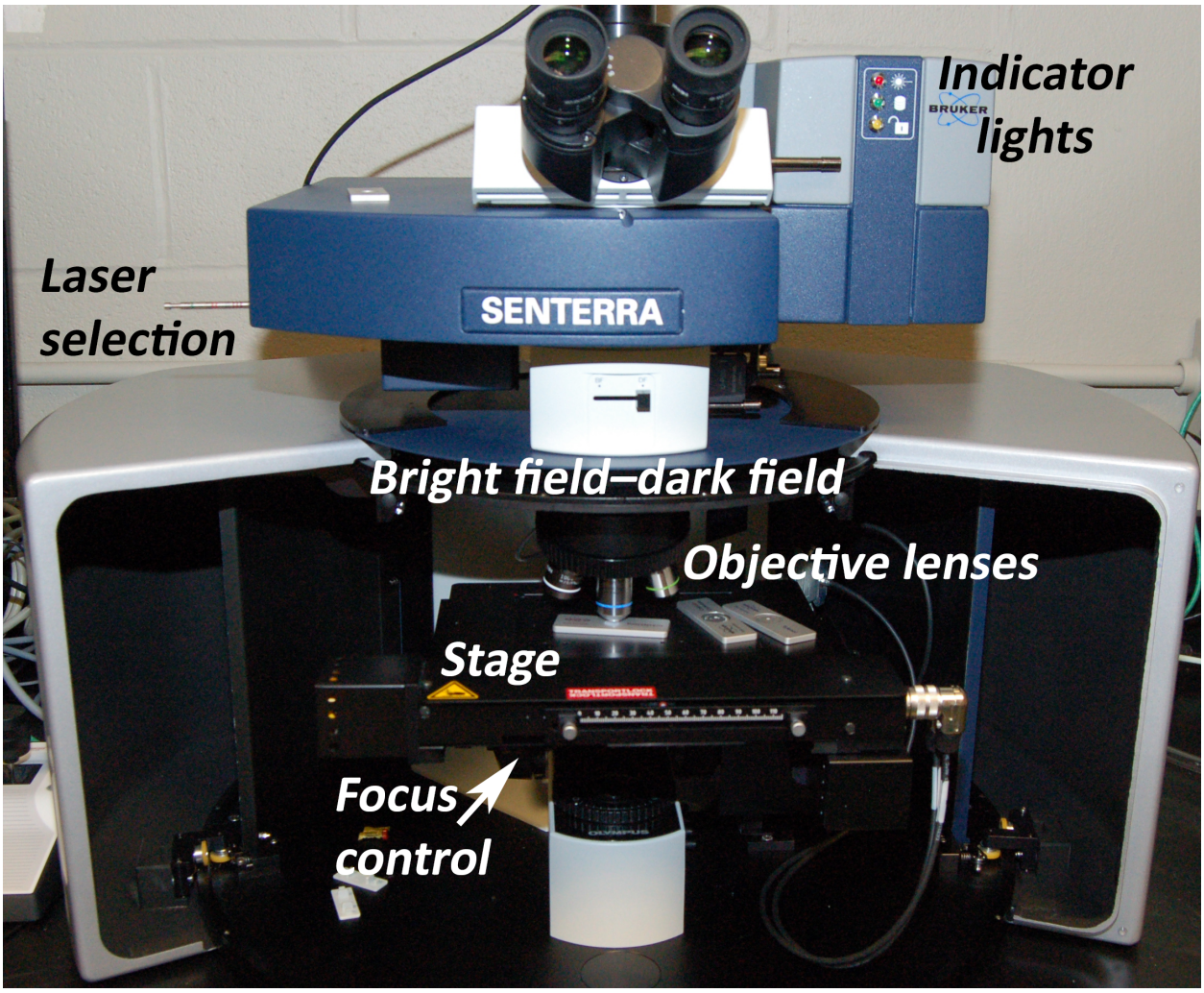
Sample(s):

- Polystyrene, calcium carbonate, and silicon test samples (Bruker, pre-mounted)
- Up to five of the pigment samples from the Royal Ontario Museum

A. Examination of the Standard Test Samples

The instrument should already be turned on and the software running when you arrive in the lab. If not, please ask laboratory staff for assistance. If the test samples are not out, please ask your TA or the lab instructor for them.

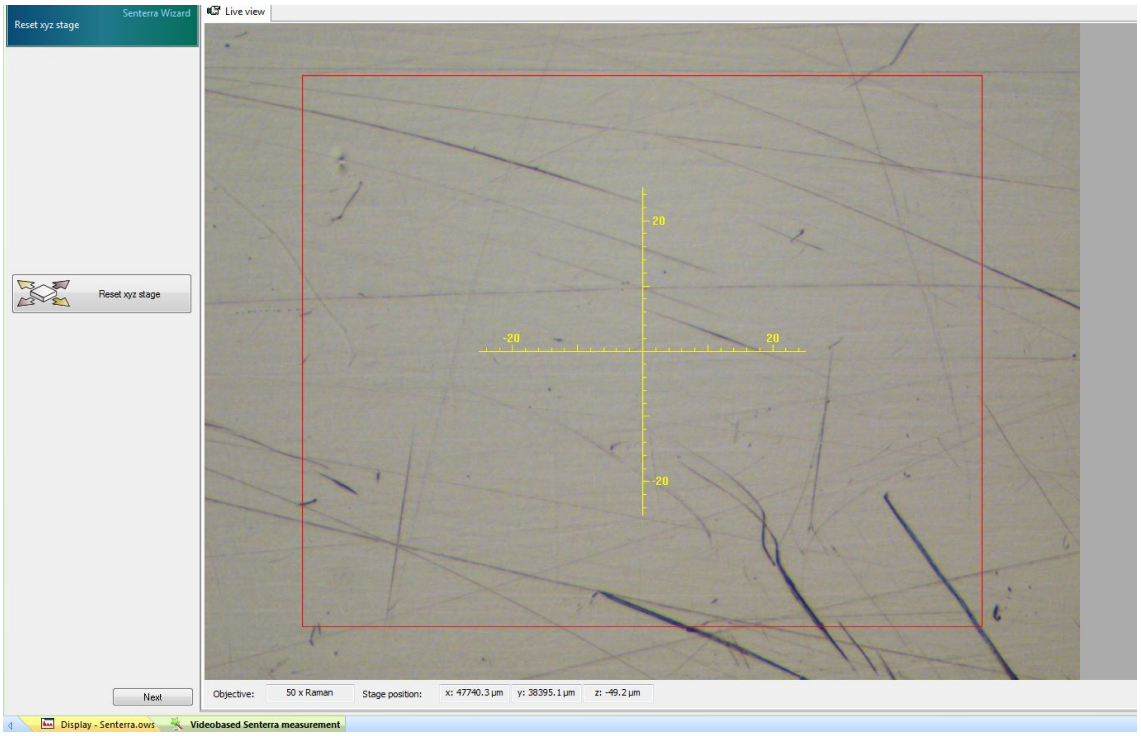
1. Open the sliding doors on the sample compartment of the microscope to reveal the stage, and place one of the sample slides in position directly below the objective lens. Make sure that the X4 objective (the smallest one with the lowest magnification) is selected.



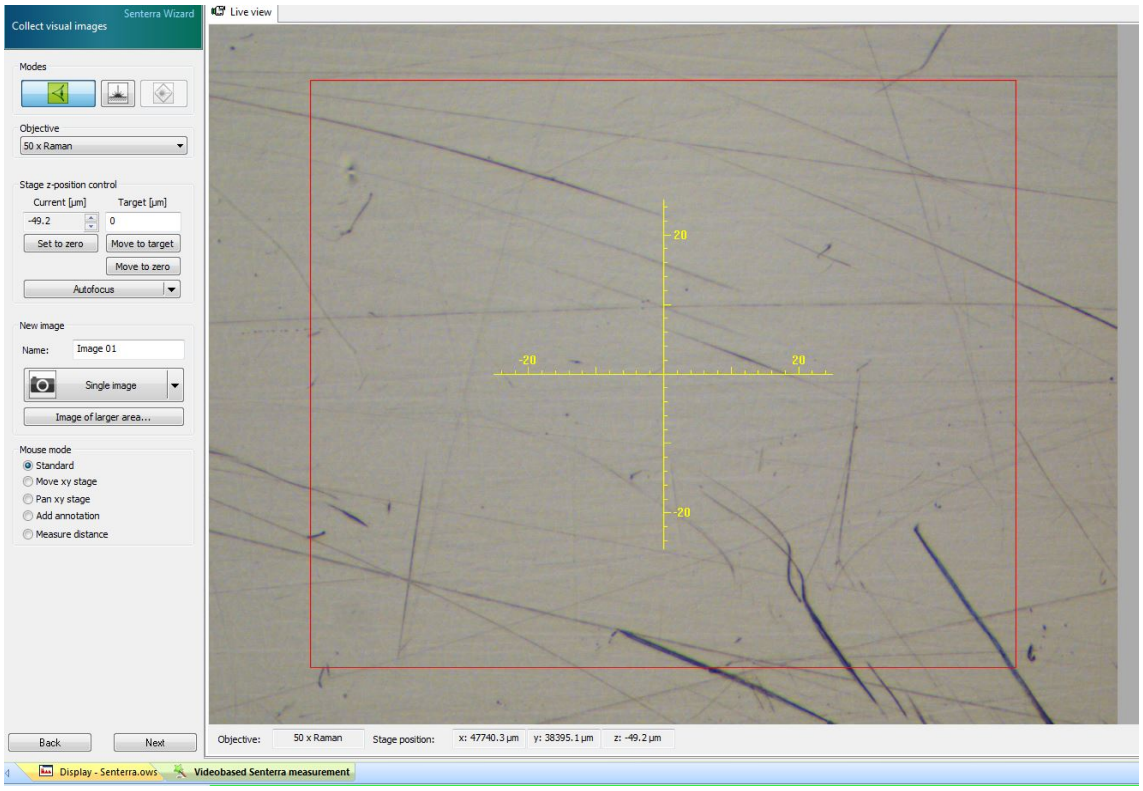
The Bruker Senterra Raman Micrscope with the sample doors open

2. Make sure the microscope is set to "bright field" (BF) – the lever should be fully to the *left*.
3. In the software, click the "Video Wizard" button in the main toolbar. This should produce a live video display from the microscope on the screen.
4. Use the focus control at the bottom-rear-left of the microscope stage to focus the image on the screen. Make sure you don't over-adjust the focus so that the objective lens contacts the sample.
5. Once the sample is in focus, fully close both doors. The yellow "Lock" light on the top-right of the microscope should go out.
6. Make sure that the selector rod on the top left is in the correct position to select the 532 nm laser (the last click stop when the selector is pushed in to the instrument).
7. You can adjust both the sample location and the focus using the joystick beside the microscope; the laser will be aimed at the spot indicated by the intersection of the

crosshairs on the screen. Click the "Next" button in the Video Wizard to proceed to the next step.



The initial "Live view" provided by the video wizard, with the polystyrene sample in place.



The initial settings window of the Video wizard, showing the Objective drop-down menu.

8. Set the drop-down menu in the side bar to match the selected objective lens (there is no communication between computer and microscope apart from the video and spectral data, so this must be done manually). Click the "Single image" button in the side bar to record an image of the sample area, then click "Next".

9. Set up the initial laser settings: make sure that the "Laser" drop down matches the one selected on the microscope (532 nm initially), and that a low laser power is selected (5 mW is recommended; high power settings can burn samples, especially organics.) Use the default settings for the remaining options initially.

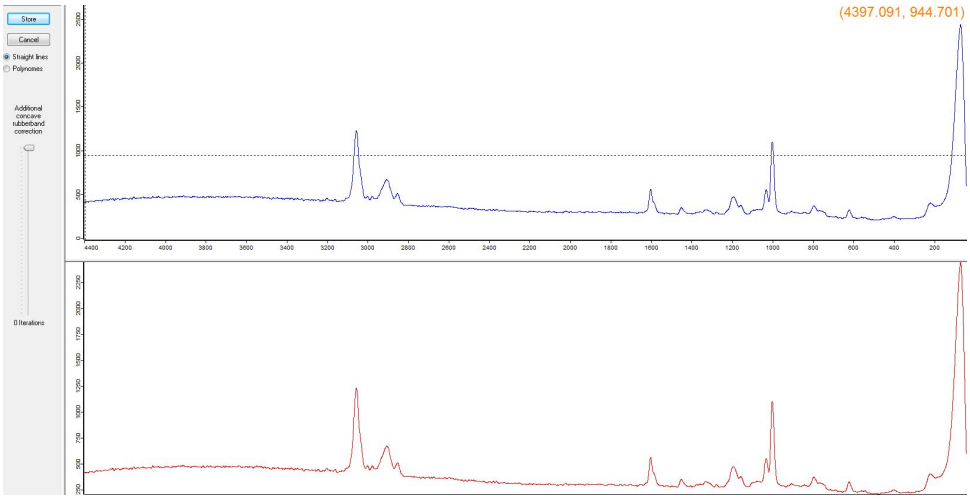
10. Switch the microscope from bright field to dark field (DF); this will cause the live video feed to go dark, but the sample snap shot should still be visible.

11. Click "Live Spectrum"; the status bar at bottom will reflect progress. As each spectrum is collected, it will appear on the screen and be added to the list of temporary files in the "OPUS Browser" side bar. To stop collecting data, click the "Live Spectrum" button a second time.

12. At this point, you can adjust the settings to improve the quality of the collected spectra (*e.g.* increase the laser power or number of co-additions, change location, or switch to the alternate laser) or you can.

13. If satisfied, make sure that the "Live Spectrum" button is not active (greyed out), then click on the "New Spectrum Window" button in the toolbar; drag and drop one of the spectra from the OPUS Browser list into this window, then click on the "Rescale" button in the toolbar.

14. ***Baseline Correction***: A common issue with in Raman spectroscopy is the presence of sample fluorescence. When present, this is observed as a large change in the baseline over an extended wavenumber range, with the Raman peaks (often much weaker in intensity) superposed. To deal with this, click the "Baseline Correction" button, and click "Start interactive mode" in the resulting dialog box. The software tiles the original spectrum and the resulting corrected spectrum in the window, with a slider in the side bar. Adjust the slider until the lower spectrum has an acceptable baseline, and then click on "Store".



Baseline correction; note the slider in the grey side bar.

15. ***Peak Labelling and Printing***: Click on the "Peak Picking" button in the toolbar, and click "Start interactive mode" in the resulting dialog box. The software will place a horizontal dashed line across the spectrum with a box in the centre: ***right-click*** in this box to activate the threshold slider in the grey side bar so that you can set the threshold for labelling peaks; you can also drag the horizontal line to set the threshold for labelling the peaks. Once you are satisfied with your settings, select "Quick Print" from the "Print" menu.
16. Switch back to the main window, and click the "Video Wizard" button to set up for your next sample. Make sure you switch the microscope back to bright field, open the doors, and switch to the next test sample.

B. Examination of Unknown Pigment Samples

The lab instructor will assign you a set of four or five pigment samples. These are suspected to mostly be inorganic salts and, as such, may very well be salts of toxic metals such as lead, cadmium, mercury, and chromium. You should assume that all pigments are harmful be ingestion, inhalation, and contact with skin or eyes; take appropriate precautions at all times.

1. Note the sample numbers and appearance (i.e. colour!) of you samples. Using the spatula provided, dispense a small quantity of each pigment into different wells on the stainless steel sample plate provided. Press the powder down to ensure a reasonably even surface. Make a careful note in your lab notebook of which pigments are in which well.
2. Place the well plate on the microscope stage, and use the translation control to place the sample under the objective, and then close the sample compartment doors.
3. Following the same procedure as for the test samples (steps 3 through 9 from part A), focus the microscope on the sample and set up both the microscope and the software to obtain a sample spectrum using the 532 nm laser. Start with a low power setting and small number of co-additions.
4. Following the same procedure as before (steps 11 through 15 of part A), collect the Raman spectrum of the pigment using first the 532 and then the 785 nm source lasers. Identify the wavenumbers of the Raman peaks, and print the resulting labelled spectra. Note that some of the peaks may be quite weak, requiring either a higher power or a greater number of co-additions. Note also that the highest power settings for the 785 nm laser may affect the sample!
5. Once you have collected spectra for all your samples, make sure that the "Live Spectrum" is no longer active, and remove the well plate from the microscope. Dispose of the samples in the well plate into the solid waste container beside the microscope, and then clean the plate with isopropanol and wipes. Dirty wipes should be disposed of in the bucket near the microscope and FTIR.
6. ***Make sure you have cleaned any spilled chemicals***.

C. Finishing Up:

1. Once you have put everything away and finished cleaning up, return the samples to the lab instructor.
2. When done, have your TA validate your lab notebooks before leaving.

D. Data Analysis:

1. Look up the IR spectra of the standard test materials, and compare these to your Raman spectra. Identify which absorption bands are (a) Raman active only, (b) IR active only, and (c) both Raman and IR active. Summarise your findings.
2. For the pigment samples, use the information in the references below to determine the most likely identity of the unknown pigments. Take into account both the actual colour of the sample *and* the peaks identified in the spectra obtained using both excitation wavelengths.
3. For each pigment, suggest simple tests and additional analyses that could be performed to confirm or refute the chemical identity of the pigment. Clearly explain your reasoning.
4. Submit the results of your analysis, along with copies of your spectra, to your lab TA at the start of your next lab period.

References:

- Ian M. Bell, Robin J. H. Clark, and Peter J. Gibbs, "Raman spectroscopic library of natural and synthetic pigments (pre ~1850 AD)", *Spectrochimica Acta A*, 53 (1997) 2159-2179.
- P. Vandenabeele, L. Moens, H. G. M. Edwards and R. Dams, "Raman spectroscopic database of azo pigments and application to modern art studies", *Journal of Raman Spectroscopy*, 31 (2000) 509-517.
- P. J. Hendra *et al*, "Routine analytical fourier transform raman spectroscopy Part 2. An updated review", *Analyst*, 120 (1995) 985-991.
- T. D. Chaplin, R. J. H. Clark, D. Jacobs, K. Jensen, and G. D. Smith, "The Gutenberg Bibles: Analysis of the illuminations and inks using Raman spectroscopy", *Analytical Chemistry*, 77 (2005) 3611-3622.
- https://www.bruker.com/products/infrared-near-infrared-and-raman-spectroscopy/raman/senterra/overview.html
- http://www.chem.ucl.ac.uk/resources/raman/
- http://www.irug.org/resources/spectral-databases-for-raman

7. Elements for Report Discussion:

Your formal report for this technique should describe the experiments performed and present the results obtained, commenting on any special features observed in the spectra obtained. From these results, you should go on to discuss application of reflectance techniques to FTIR spectrophotometry, identifying any advantages and disadvantages compared to conventional transmission measurements using NaCl or KBr plates. Some (but *not* all) of the topics you should address include:

1. Although there is a long history of using these techniques in the UV and visible regions of the spectrum, ATR and DRIFT only became practical for IR measurements with the introduction of Fourier Transform, as opposed to dispersive, IR spectrophotometers. What specific feature(s) of FTIR spectrometers have made this possible?
2. What is anomalous dispersion, and why do we need a non-absorbing medium such as KBr to dilute the samples? To what extent could you use DRIFT to identify pharmaceutical ingredients in tablets *if you did not know in advance* what those ingredients were?
3. What are the advantages and disadvantages of reflectance techniques in FTIR compared to conventional measurements using nujol mulls or KBr disks? What criteria might you establish for deciding which technique(s) to use for different types of sample?