FTIR User Instructions

ATR Mode

Data Collection:

Hardware:
- Attach ATR accessory to chamber of device – tighten two screws
- Be sure that metal plate underneath cup and rubber cap is clean (100% EtOH)

Software:
- Open software on HHL’s laptop (Merlin)
- SCAN -> SCAN -> BACKGROUND TAB -> SETUP (bottom) -> ALIGN, CALIBRATE, AUTOGAIN, O.K.
- Scan background first (i.e. PBS)
  - SCAN -> BACKGROUND TAB -> SCANS TO CO-ADD (# OF SCANS, i.e. 200) -> NAME SPECTRUM -> BACKGROUND BUTTON
- Scan samples
  - SCAN -> SAMPLE TAB -> ENTER # OF SCANS (i.e. 200), ENTER RESOLUTION (lower value, higher resolution, i.e. 2), TYPE: ABSORBANCE, COMMENTS: NAME SAMPLE -> SCAN (on bottom)

NOTES:
- Gently clean off surface near crystal (metal plate under cup and rubber cap) after sample scan – use 100% EtOH and kimwipe

Data Analysis:
- Correction:
  - TRANSFORMS -> ATR CORRECT -> Default setting (1.00) -> ADD (instead of replace spectrum)*keep consistent* - rename orig files NAME_ORIG
- Smoothing (select only new files):
  - TRANSFORMS -> ADVANCED -> SMOOTH (Boxcar, 7 or 5– higher value, more smoothing)
- Setting Range for Spectrum (select only new files):
  - VIEW -> SET DISPLAY LIMITS FOR -> WHOLE DOCUMENT OR CURRENT DISPLAY (lower limit around 550 for atr mode)
- Exporting to word/powerpoint:
  - EDIT -> COPY TO WORD/POWERPOINT

Measuring Area Under the Curve:
PEAKS -> NEW PEAK -> Create a set from file -> BROWSE -> Peaks3 (FTIR folder) -> OK
- Click on peak and adjust the boundary markers to indicate area under peak of interest
- PEAKS -> DISPLAY PEAK TABLE: Will provide info about peaks
- Peaks3 file for Amide I, Phosphate, and Carbonate peaks
Materials: KBr
- black box
- mortar and pestle

Diffuse-Reflectance (D-R) Mode

Data Collection:

Hardware:
- Attach D-R accessory (BE CAREFUL not to touch laser on side of device)
- Locate small kit box and mortar and pestle in FTIR drawer (contains: mirror accessory, cups, brush, spatula, dishes, etc.)
- Place mirror accessory into holder and place onto track (if in front hole, holder should be back, if in back hole, holder should be slid forward)

Software:
- SCAN -> SCAN -> BACKGROUND TAB -> SETUP (bottom)
  - Maximize signal with mirror accessory by rotating knob (remove top of chamber)
  - ALIGN, CALIBRATE, AUTOAGAIN, O.K.

Sample Preparation
- Samples must be dehydrated prior to scanning (place in desiccator)
- Select cup size (determine if samples will fit within cup diameter)
- Place cup between 2 black dishes
- Grind KBr with mortar and pestle so that there is enough to fill cup
  - Use razor edge to flatten top
- Place sample onto surface and push in with flat spatula (or grind sample in with KBr)
- Place cup into black holder and place onto track on device in chamber
- Check to see that laser is on KBr surface

Scan Background
- Maximize signal again by turning knob
  - AUTOAGAIN -> O.K.
  - Scan background first (i.e. KBr)
    - SCAN -> BACKGROUND TAB -> SCANS TO CO-ADD (# OF SCANS, i.e. 200) -> NAME SPECTRUM -> BACKGROUND BUTTON
  - Scan samples
    - SCAN -> SAMPLE TAB -> ENTER # OF SCANS (i.e 200), ENTER RESOLUTION (lower value, higher resolution, i.e. 2), TYPE: ABSORBANCE, COMMENTS: NAME SAMPLE -> SCAN (on bottom)

NOTES:
- Be sure to change the KBr powder for every sample
- Have 2 similar sized cups, can prepare one sample while other is being scanned
- only need to scan background once at beginning
Data Analysis:
- Smoothing (select only new files):
  - TRANSFORMS -> ADVANCED -> SMOOTH (Boxcar, 7 – higher value, more smoothing)
- Setting Range for Spectrum (select only new files):
  - VIEW -> SET DISPLAY LIMITS FOR -> WHOLE DOCUMENT OR CURRENT DISPLAY (lower limit around 550 for atr mode)
- Exporting to word/powerpoint:
  - EDIT -> COPY TO WORD/POWERPOINT

error: signal overload analog
- FTIR on B-TCP samples (neg control)
  - should always be on (warm 30 min)
  - log onto Helen (Startie)
  - program = Digilab Merlin
  - use large cup holder
  - brush off mirror ⇒ sample holder (2 positions)
  - scan - scan - setup (Signal strength 4)
  - take off windshield adjust screw until # maximized
  - align
  - calibrate OK
  - take out mirror and put in cup
  - mix sample w/ kar (grip up) - use funnel
    - fill cup w/ kar + seed
    - flatten w/ razor
  - scan - scan - setup
  - calibrate OK
  - autogain, max signal
  - adjust knob against max signal OK
  - scan - background (200 scan) - (Resolution 4) - (Attenuance)
  - put sample on top ⇒ flatten
  - scan - scan
  - copy to word edit

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