Developing MIR Calibrations to estimate soil properties

# Setting up for calibrations

Start OPUS

Click <Setup Quant2 Method>

* Components Tab
Enter all the soil properties (“components”) in the table with laboratory data.
IMPORTANT: Components must be in same order as in the table of laboratory data
	+ <Add Component>
		- Enter Name, Unit, Digits after the decimal point for each soil property
* Spectra Tab
	+ <Add Spectra>
		- Select Spectra for calibration set
	+ <Set Sample Numbers>
		- Enter “Number of Spectra per Sample”
		(This is the number of replicate scans per sample. The recommended number is 4)
		- <Set>
		- <Exit>
	+ Switch to .CSV file with laboratory data
		- Highlight all data values (NOT header row or columns with sample ID)
		- Copy (<Ctrl>c)
	+ Switch back to OPUS
		- Highlight top left cell
		- Paste (<Ctrl>v) data just copied from .CSV file
	+ CHECK DATA LINES UP CORRECTLY
	+ CHECK AGAIN THAT DATA LINES UP CORRECTLY
* Parameters Tab
	+ “Preprocessing in calibration regions”: No spectral data preprocessing
	+ “Mean Centering”: Check
	+ “Calibration Regions”: from 4000 to 600
* Validate Tab
	+ Select “Cross Validation”
	+ “No. of samples leaving out”: 1
* Settings Tab
	+ “Select preprocessing options for Optimize” by highlighting:
		- No spectral data preprocessing
		- Vector normalisation (SNV)
		- First derivative
		- First derivative + Vector normalisation (SNV)
	+ “Maximum test range:” 4000 to 600
	+ Uncheck “Run optimization in background”
* Store Method Tab
	+ Select “Store only Spectra List + Parameters”
	+ <Store Method>
		- Filename: …\MIR\_Calibration\Sample & Component List.q2
* Click <X> to exit Quant 2
* You should now have a basic template to use for calibrating each soil property. Take care NOT to overwrite this file

# Calibrating a soil property e.g. Clay

Click <Setup Quant2 Method>

* Load Method Tab
	+ <Load Method>
		- Open Sample & Component List.q2 (created in previous section)
* Spectra Tab
	+ <Set Data Set>
		- <Special Settings>
			* Select <Blank>
			* Select soil property currently being calibrated e.g. Clay
			* <Set> (This excludes any samples with no data)
			* <Exit>
		- <Exit>
* Validate Tab
	+ Uncheck all Validation parameters except soil property of interest
	+ <Validate>
		- Give validation a name e.g. “Clay\_O” (O for ‘original’)
	+ Once validation has run QUANT 2 will switch to the Graph Tab
* Graph Tab
	+ Use graphs to evaluate the calibration. R2 should be as high as possible
	+ Rank should be as low as possible (“Rec:” shows OPUS’ recommendation)
* Store Method Tab
	+ Select “Clay\_O”
	+ Check “Store validation results”
	+ <Store Method>
		- Name “Clay.q2”

## Optimizing the calibration

* Settings Tab
	+ “Maximum test range:” 4000 to 600

### Optimize using General A

* Optimize Tab
	+ Select <General A>
	+ <Optimize>
	+ Wait for optimization to complete – may take several hours
	+ When complete the optimization runs will ordered from best (i.e. lowest RMSECV) to worst (highest RMSECV)
	+ Select run at top (i.e. the best) by clicking on its “Number”
	+ <Use Parameters”
* Validate Tab
	+ <Validate>
		- Give validation a name e.g. “Clay\_A1” (A for General A and 1 for first run)
		- When finished QUANT 2 will switch to Graph Tab
* Graph Tab
	+ Use graphs to evaluate the calibration. R2 should be as high as possible
* Optimize Tab
	+ Select next best run from top which has a lower rank by clicking on its “Number”
	+ <Use Parameters”
* Validate Tab
	+ <Validate>
		- Give validation a name e.g. “Clay\_A3” (A for General A and 3 for third best run)
		- When finished QUANT 2 will switch to Graph Tab
* Graph Tab
	+ Use graphs to evaluate the calibrations. Choose the calibration that has the lowest rank that has an R2 > the greatest R2 of all the runs minus 1.
	e.g. If Clay\_A1 has R2 of 80.00 and rank 10 and Clay\_A3 has R2 of 79.1 and rank of 8, then choose Clay\_A3

### Optimize using General B

* Optimize Tab
	+ Select <General B>
	+ <Optimize>
	+ Wait for optimization to complete – may take several hours
	+ When complete the optimization runs will ordered from best (i.e. lowest RMSECV) to worst (highest RMSECV)
	+ Select run at top (i.e. the best) by clicking on its “Number”
	+ <Use Parameters”
* Validate Tab
	+ <Validate>
		- Give validation a name e.g. “Clay\_B1” (B for General B and 1 for first run)
		- When finished QUANT 2 will switch to Graph Tab
* Graph Tab
	+ Use graphs to evaluate the calibration. R2 should be as high as possible
* Optimize Tab
	+ Select next best run from top which has a lower rank by clicking on its “Number”
	+ <Use Parameters”
* Validate Tab
	+ <Validate>
		- Give validation a name e.g. “Clay\_B3” (B for General B and 3 for third best run)
		- When finished QUANT 2 will switch to Graph Tab
* Graph Tab
	+ Use graphs to evaluate the calibrations.
	+ Choose the best one using the same criteria as above, e.g. Clay\_B1
* Store Method Tab
	+ Select “Clay\_B1”
	+ Check “Store validation results”
	+ <Store Method>
		- <Select All>
		- Name “Clay.q2”