IDEOM for LCMS-based metabolomics data processing:

A practical tutorial

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http://mzmatch.sourceforge.net/ideom.php

IDEOM

Aim: To interpret LCMS metabolomics data in a biological context

1. **Untargeted** approach (hypothesis generating):
    - Are there differences between samples?
    - What are the metabolites that differ?

2. **Targeted** approach (hypothesis testing):
    - What happens to metabolites X, Y and Z

Ideom is designed for untargeted analysis, but can be used for both...

Getting started

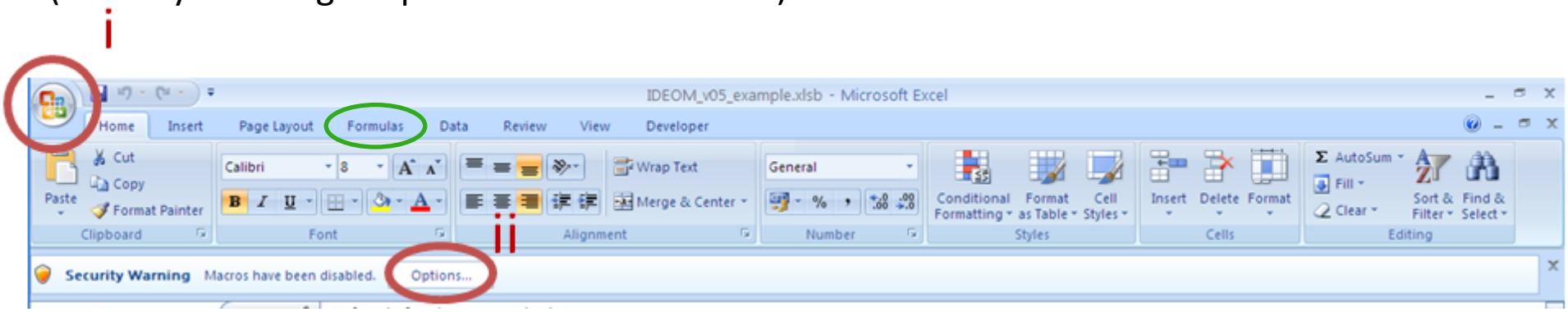
i. Open the Ideom_v19.xlsb file in Excel

To skip the data processing steps, open IDEOM_v19_Demo.xlsb file. This is an average sized dataset with ~6000 features (peaks) imported. For larger datasets (or slower computers) allow a few seconds for Excel to re-calculate formulas each time you do something. If it is particularly slow consider turning the 'Calculation' option to 'Manual'. Formulas >> Calculation Options >> Manual

If you do this, remember to hit the 'Calculate' button each time you change something.

ii. Allow macros to run

(Security Warning>>Options>>Enable Macros)



Note: if the security warning doesn't appear: click the Office button (i), go to 'Excel Options' >> Trust Centre >> Trust Centre Settings >> Select 'Disable all macros with notification'

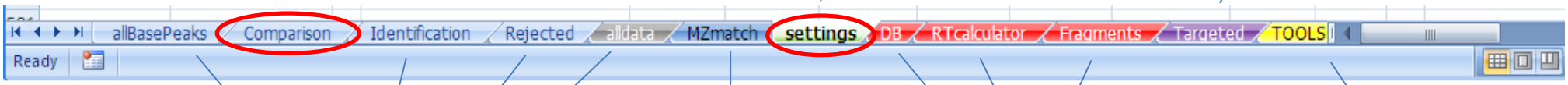
Getting started

IDEOM Navigation:

Comparison:
Main results sheet for data visualisation

Settings:
HOME PAGE for processing data and adjusting parameters

Targeted:
Separate sheet for targeted metabolite analysis



Results sheets:
Detailed data for each peak, organised according to:
- **All Base Peaks** (features)
- Putative **identifications**
- **Rejected** identifications
- **All features**

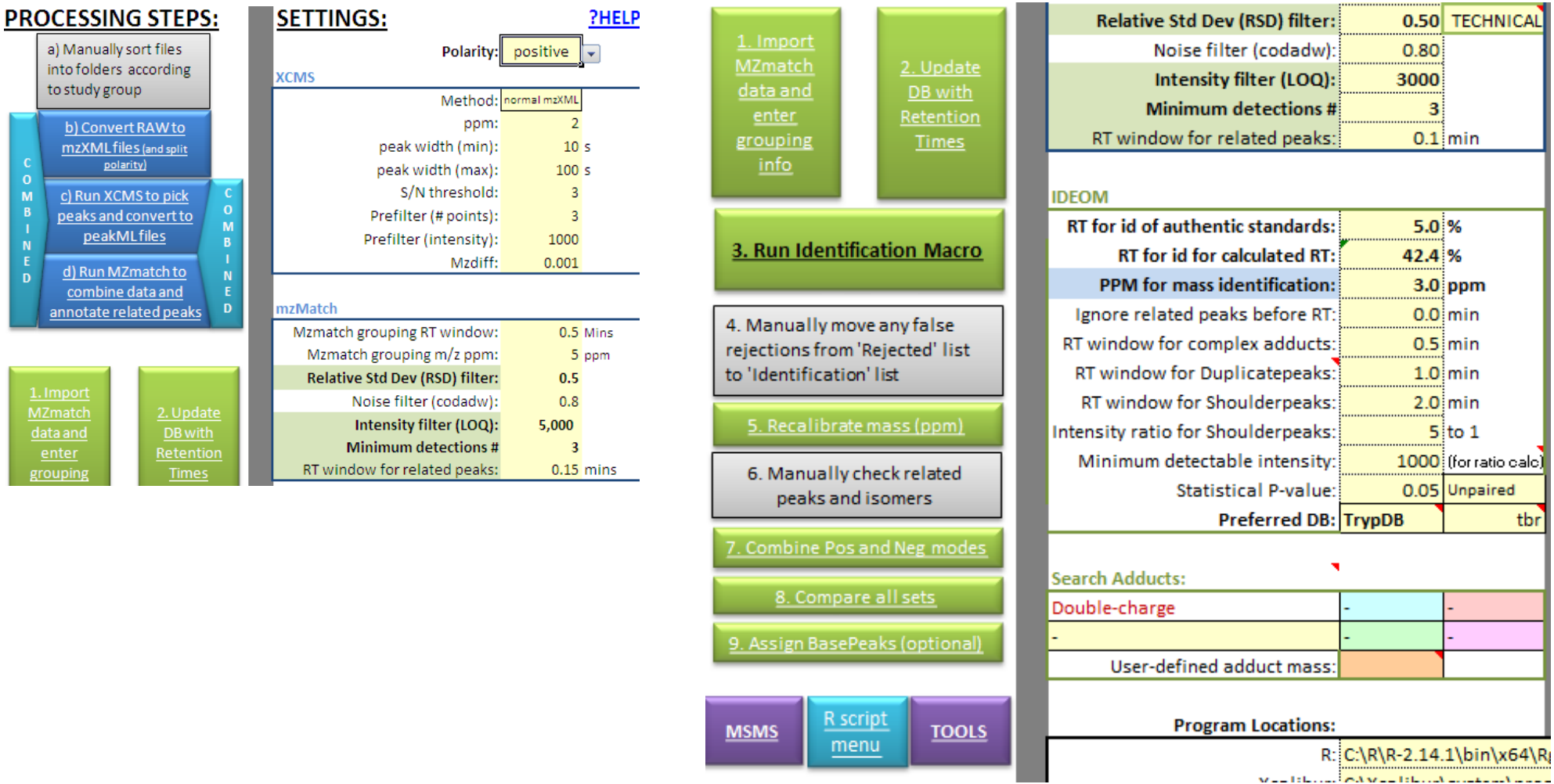
Raw data:
Imported data from mzMatch

Databases:
Databases and formulas for metabolite identification and noise filtering

Miscellaneous:
Examples of IDEOM formulas and sheets to store study-specific metadata (methods)

LCMS Pre-processing

Settings & automated data processing



Part 1

Using IDEOM (and XCMS/mzMatch) for pre-processing LCMS metabolomics data

LCMS Pre-processing

Installing required software/packages

Installation not required for the workshop

Pre-processing raw data requires

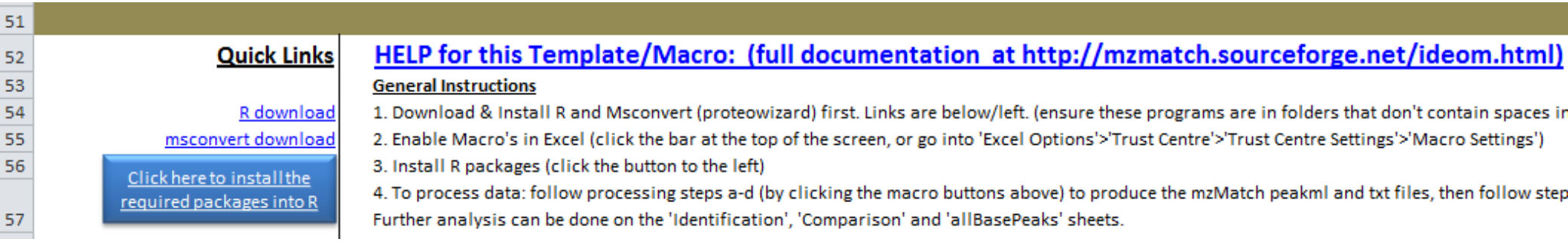
a) Msconvert (a proteowizard tool) to convert .raw files to .mzXML

b) R statistical package to run xcms and mzMatch

Both programs are free and easily installed from the websites (available in Ideom: go to the settings sheet and scroll down to the help section)

c) R packages (xcms and mzMatch): click the blue button in Ideom's help section (settings sheet) to install these packages. This function can also be accessed from the 'R scripts menu' on the Settings sheet.

Check that all install OK, you may need to select a download mirror and agree to installing all components. Re-install all packages if there is an error.

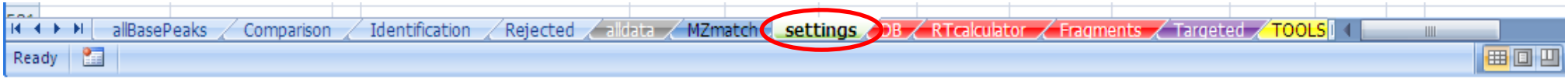


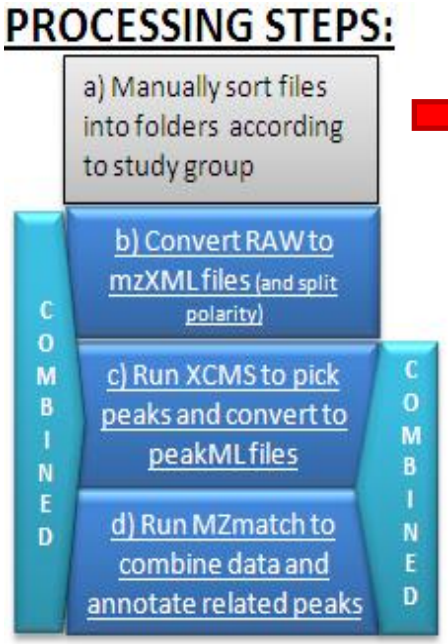
LCMS Pre-processing

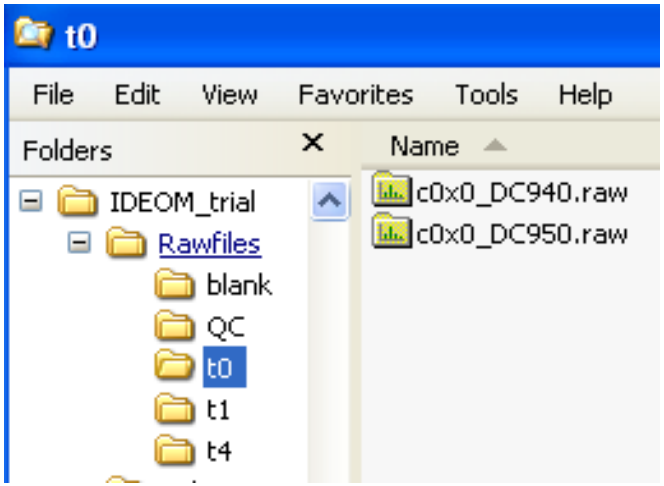
Raw Data Processing

Go to the ‘Settings’ page of Ideom

Not enough time in the workshop for these steps







LCMS Pre-processing

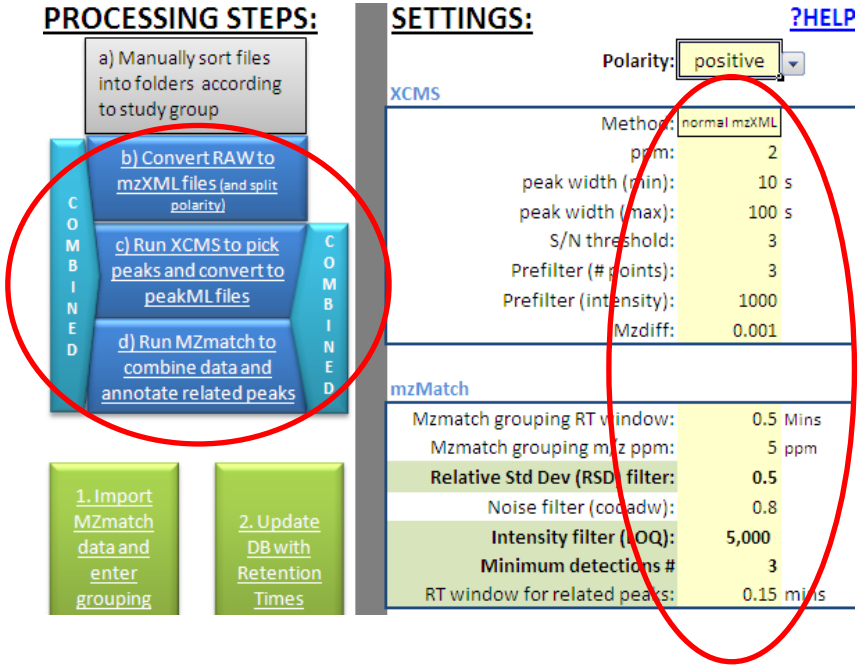
Automated peak picking, grouping and filtering

Not enough time in the workshop for these steps

Double-check the default parameters in column E (change if necessary)

Run steps b to d in R by clicking the appropriate blue buttons in Ideom

- Usually use the 'combined' button to automatically run all steps sequentially
- Separate buttons are available in case you need to run some functions on another computer with more RAM or processor speed
- These steps are time-consuming, usually set it going overnight
- On completion you should have an mzMatch_output.peakml file and a mzMATCHoutput.txt file to be further processed with Ideom



LCMS Pre-processing

Ideom processing (filtering and metabolite identification)

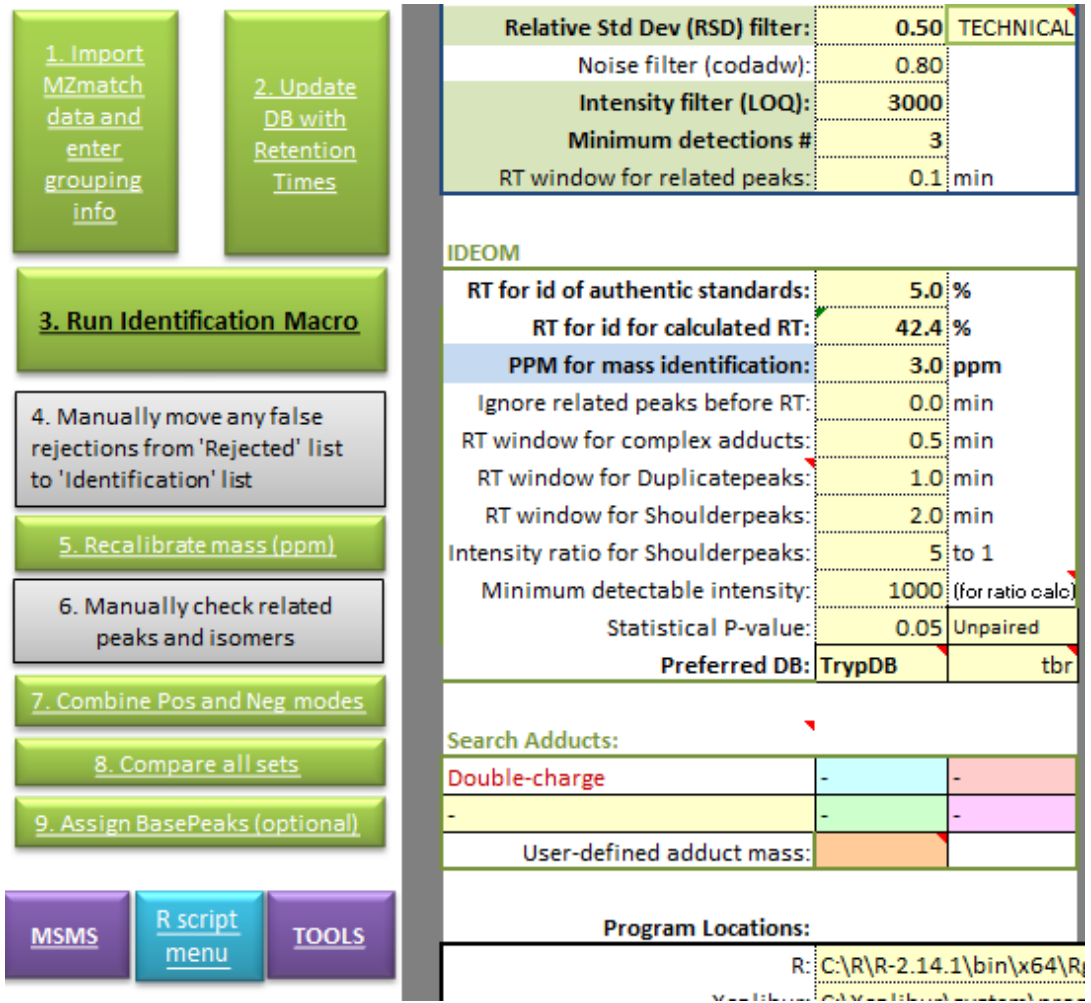
Whilst most Ideom processing is automated, some study-specific input is required from the user. Optimal results are achieved by clicking steps 1-9 (green buttons) and following the on-screen prompts.

Steps 1 and 2 can be run in any order, but must both be completed before running the main processing macro (step 3).

Steps 4 and 6 are optional.

Step 7 is only relevant for Exactive data (or when both modes are analysed in quick succession on the same column)

For Workshop only:
Open a fresh IDEOM_v19 file, SaveAs with a new name and load the Example data from the TOOLS menu



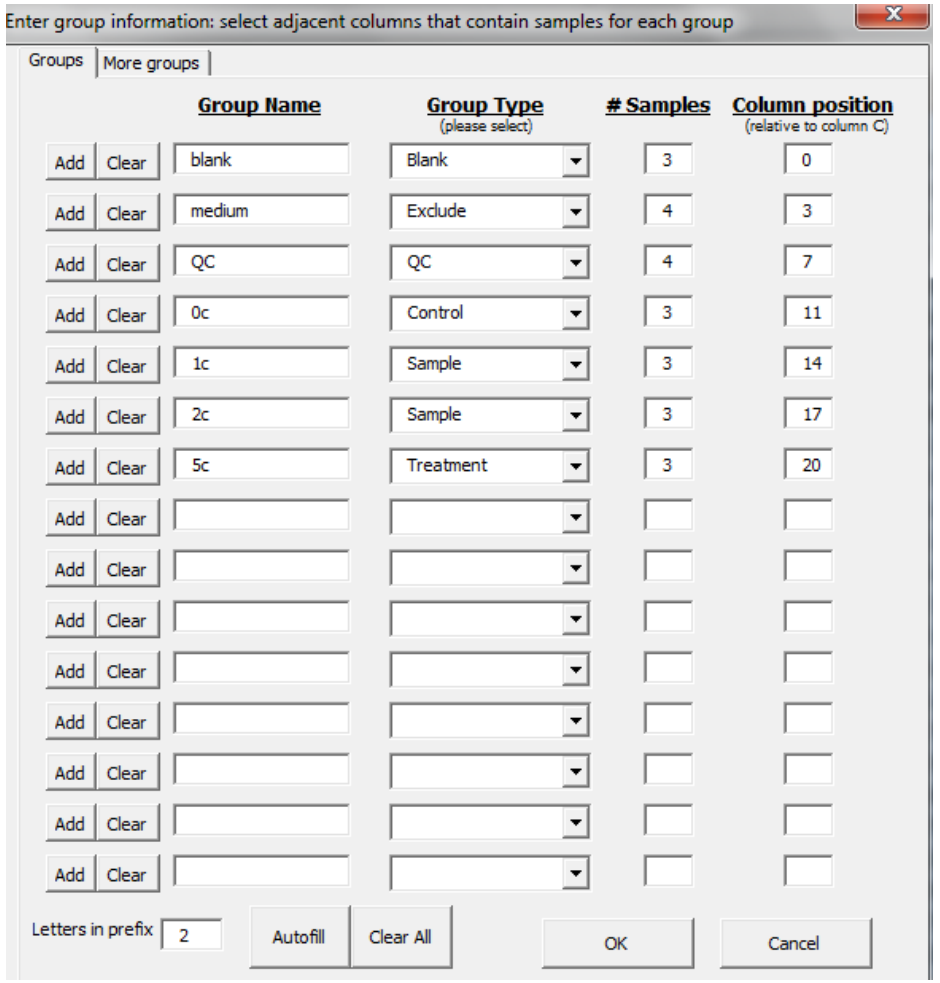
LCMS Pre-processing

Step 1: Import data and set grouping info

1. Import MZmatch data and enter grouping info

- Use this function to import data from the mzMatchoutput.txt file produced by mzMatch. If you have already entered data manually, or by the 'import example data' or 'import Mzmine data' buttons, you may press cancel at the import file screen to skip this step.
- The second part of this function asks the user to enter grouping information. 'autofill' can be used if the prefix of the sample names refers to the grouping, otherwise manually select groups using the 'add' buttons.
- Set-Type needs to be selected for each group using the drop-down lists: always set one group as 'Treatment' and another as 'Control' to allow comparisons
- ** mzMatch output data needs to have replicates in adjacent columns. Please adjust this if needed (by changing column order in the txt file) prior to import. This should automatically happen if your samples are labelled by group, and/or you used the mzMatch grouping function.

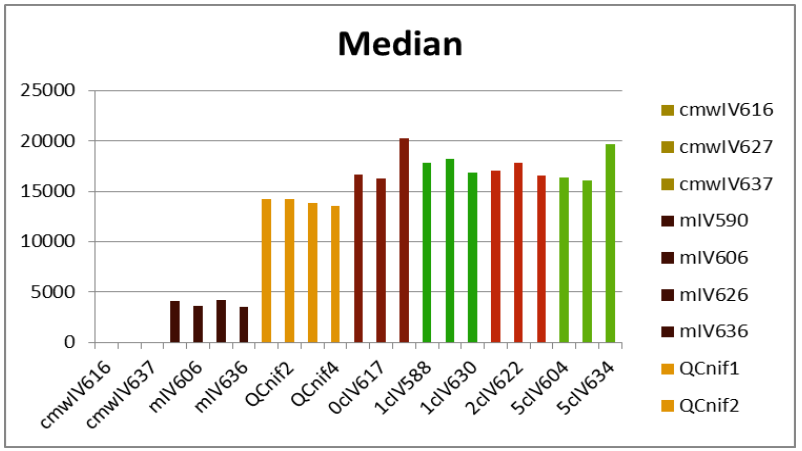
For the workshop (using the Example data), enter these groups:

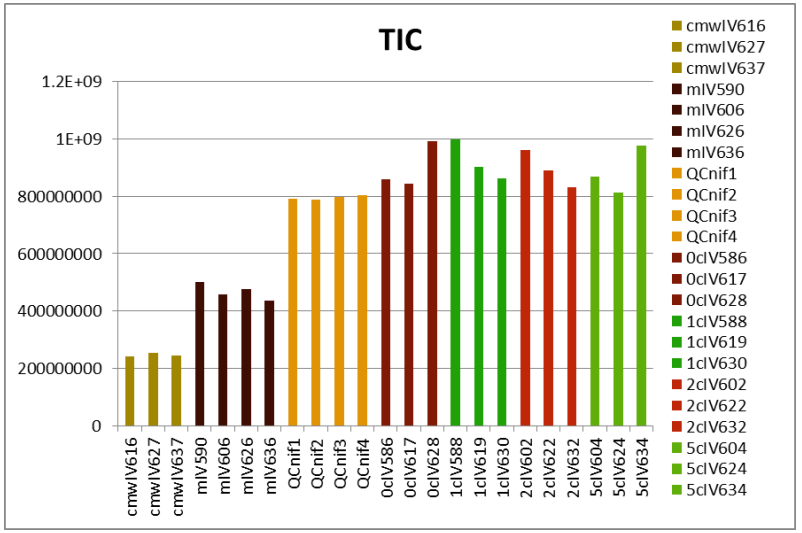


LCMS Pre-processing

Step 1: Import data and set grouping info

- The third part of this function plots average sample intensities to allow a quick check of whether the data is consistent. Internal (external) standards will also be plotted if you have entered them in V2-AD2 of the settings sheet
- The fourth part gives the option of normalising the data either by TIC, median, or user-defined values (column R on settings sheet). Normalisation is not routinely recommended for LC-MS data due to non-linear responses and the unpredictability of ion-suppression.
- If you subsequently decide to normalise the data, you may re-run the whole step 1 any time before running step 3 (Identification Macro).





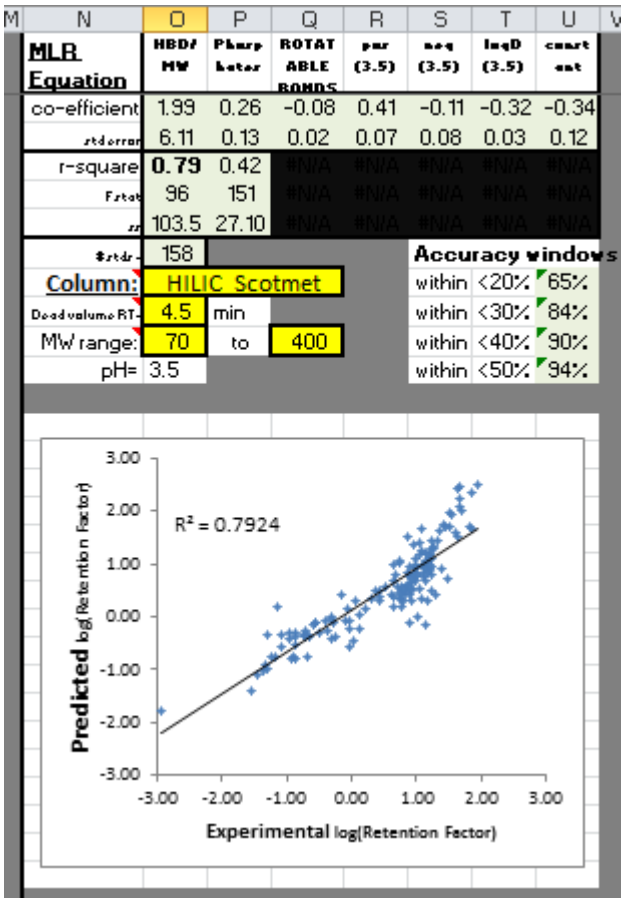
2. Update DB with Retention Times

LCMS Pre-processing

Step 2: Update DB with retention times

- This function enters standard retention times into the database (DB sheet), and (optional) enters predicted retention times for other metabolites.
- A list of retention times from authentic standards is required (create this list either using the Targeted Sheet, or externally using ToxID, Xcalibur or similar)
- The list of standard RTs may be either imported from any excel readable file, or entered directly into columns A and B on the 'RTcalculator' sheet
- If importing .csv files of retention times: "_" in metabolite names will be replaced with ","
- All authentic standards (column A on the 'RTcalculator' sheet) must have names that exactly match those in the DB sheet.
- RT calculator uses physico-chemical properties in the DB sheet to predict retention times based on a multiple linear regression model with the authentic standards. (QSPR approach)
- The column (cell O8) and dead volume time (cell O9) should be entered before running this macro. Other data in columns N-U show the accuracy of the current RT prediction model.
- The mass range for application of the prediction model is defined in cells O10 and Q10. The default QSPR model is accurate for the Formic Acid:ZIC-HILIC method from MW 70-400

For the workshop use the default settings and standards (don't import standard RTs from another file):



LCMS Pre-processing

Step 2: Update DB with retention times (cont)

- Columns W:X allow standard retention times to be uploaded to the database without being included in the prediction model. (e.g. for large metabolites outside the validated mass range)
- Columns Z:AI allow predicted retention times to be uploaded to the database based on class properties, according to specific annotations in the DB sheet (if no RT calculated by the prediction model)
- Prediction model variables (Headers E1:J1) can be adjusted to other phys-chem properties (from drop-down menus) if you wish to attempt to apply RT prediction to different chromatography.

- You have the opportunity to check the model fit before annotating all metabolites in the database.
- If there is no good prediction model you can still use this function to upload standard retention times for those metabolites where you have authentic standards.
- If you don't run this function then metabolite identification will only be based on exact mass (not retention time) - hence you will get a lot more false-identifications.

Compound Name (if not in RTcalculator)	standard RT		Pathway	Group RT		Map	Group RT		Property	limit	Group RT
Folate	9.33		Glycerophosphocholines	7.5		Lipids: Fatty Acyls	5		logP >	0	5.5
S-glutathionyl-L-cysteine	19.52		Glycerophosphoethanolamines	6		Lipids: Glycerolipids	5		correction:		+
Glutathione disulfide	18.99		Glycerophosphoserines	6		Lipids: Glycerophospholipids	5.5		pos (3.5) >	0.5	0.5
Bis-gamma-glutamylcystine	19.4		CDP-Glycerols	6		Lipids: Sphingolipids	5.2		neg (3.5) >	0.5	0.5
NAD+	19.74		Glycerophosphonocholines	7.5		Lipids: Sterol lipids	5		cation >	0	1
Thiamin diphosphate	36.43		Glycerophosphonoethanolamines	6		Lipids: Prenols	5				

LCMS Pre-processing

Step 3: Run Identification Macro

3. Run Identification Macro

- Double-check the parameters on the settings sheet
  Common changes might be:
  Polarity: This determines which adducts/fragments are filtered (manual changes to adduct/fragment filtering can be made on the 'Fragments' sheet)
  RSD: depending on your data quality
  RSD level: Generous, Strict or Technical (or Off)
  Intensity filter (LOQ)
  # detections: depending on how many replicates
  RT for ID of authentic standards: depending on the chromatographic reproducibility
  RT for ID for calculated RT: depending on the prediction model
  ppm: depending on the mass calibration of the instrument
  Preferred DB and map: depending on the organism

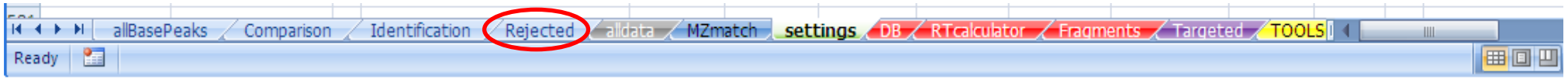
- Click step 3 to run the main data processing macro

This step is where most of the automated filtering and identification takes place

Relative Std Dev (RSD) filter:	**0.50**	TECHNICAL
Noise filter (codadw):	0.80	
Intensity filter (LOQ):	**3000**	
Minimum detections #	**3**	
RT window for related peaks:	0.1	min
IDEOM		
RT for id of authentic standards:	**5.0**	%
RT for id for calculated RT:	**42.4**	%
PPM for mass identification:	**3.0**	ppm
Ignore related peaks before RT:	0.0	min
RT window for complex adducts:	0.5	min
RT window for Duplicatepeaks:	1.0	min
RT window for Shoulderpeaks:	2.0	min
Intensity ratio for Shoulderpeaks:	5	to 1
Minimum detectable intensity:	1000	(for ratio calc)
Statistical P-value:	0.05	Unpaired
Preferred DB:	**TrypDB**	tbr
Search Adducts:		
Double-charge	-	-
-	-	-
User-defined adduct mass:		

LCMS Pre-processing

Step 4: Manually retrieve False Rejections



- Check the rejected sheet for metabolites that you believe to be wrongly rejected

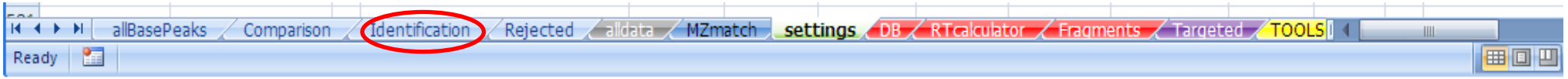
If you think a metabolite was wrongly rejected:
Check if the metabolite has already been identified (as another feature) by double-clicking the confidence level (in column F)

If you can justify why a feature was incorrectly rejected: retrieve the metabolite by clicking the red ‘Retrieve Row’ button at the top

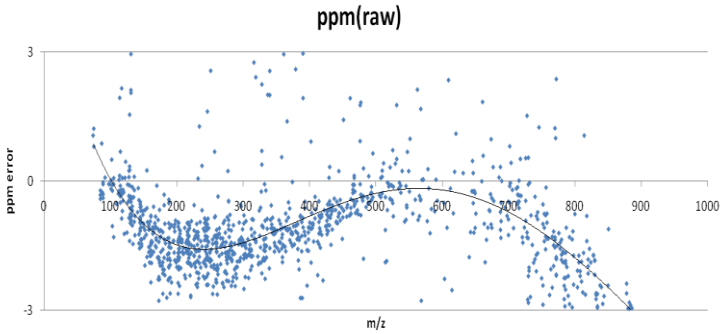
E.g. In example data find Folate (which was rejected because it appears to be a dimer of another peak).

LCMS Pre-processing

Step 5: Recalibrate mass (ppm)

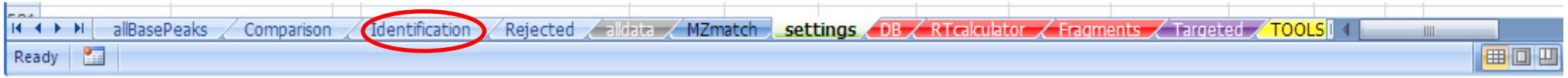


- Go to the Identification sheet or the settings page
- Run step 5 by clicking the green ‘re-calibrate mass (ppm check)’ button
- This will plot the relationship between mass and mass accuracy (ppm error) for all 'identified' metabolites, with standards in red, and fit a 5th order polynomial function. (this should allow for the calibration errors observed on Thermo Orbitrap)
- If the polynomial function appears to fit your data, agree to re-calibrate masses. If the curve is not a good fit, but you see a trend, consider manual re-calibration efforts.
- After calibration, check the new plot of mass errors, and set a new ppm window to remove outliers (false-identifications). In some cases it is worth checking the rejected peaks (bottom of 'rejected' list) for alternative identifications by clicking the 'altppm' column



LCMS Pre-processing

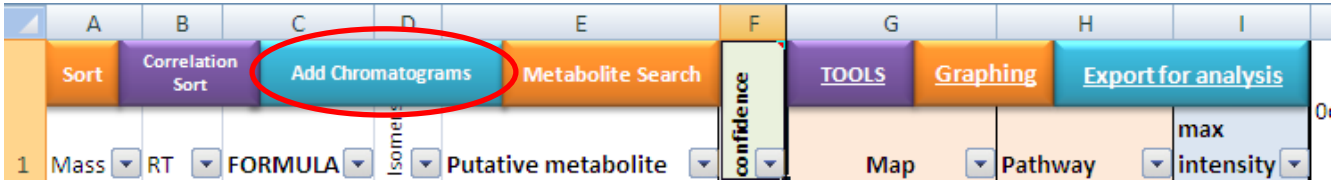
Step 6: Manually check for False Identifications [optional]

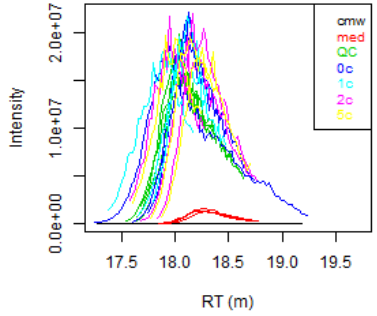


- For thorough analysis, check all the information supplied for each peak in the 'identification' sheet.
- You may wish to skip this step initially, and return later to double-check specific metabolites of interest.
- Whilst it is always a good idea to return to raw data for confirmation of specific metabolites, the identification sheet allows rapid access to a large amount of meta-information to simplify the process of manual data curation and metabolite identification.
- Click the ‘colour’ button in the TOOLS menu to make viewing of data easier
- Click the 'Hyperlinks' button in the TOOLS menu to allow links to metabolite websites. Click again to turn off hyperlinks if it slows your computer too much. Hyperlink websites can be edited in the hyperlinks table in columns AF:AI on the settings sheet
- Check the information in each column, and sort by each column to see if metabolite metadata suggests false identification (e.g. RT is before dead volume, maxRSD is too large, ^{13}C isotope doesn’t confirm the number of carbons in the formula, isomeric peaks appear to be chromatographic artefacts, related peak information suggests a fragment or adduct)
- False identifications can be removed (or merged) with the red ‘Remove row’ button

LCMS Pre-processing

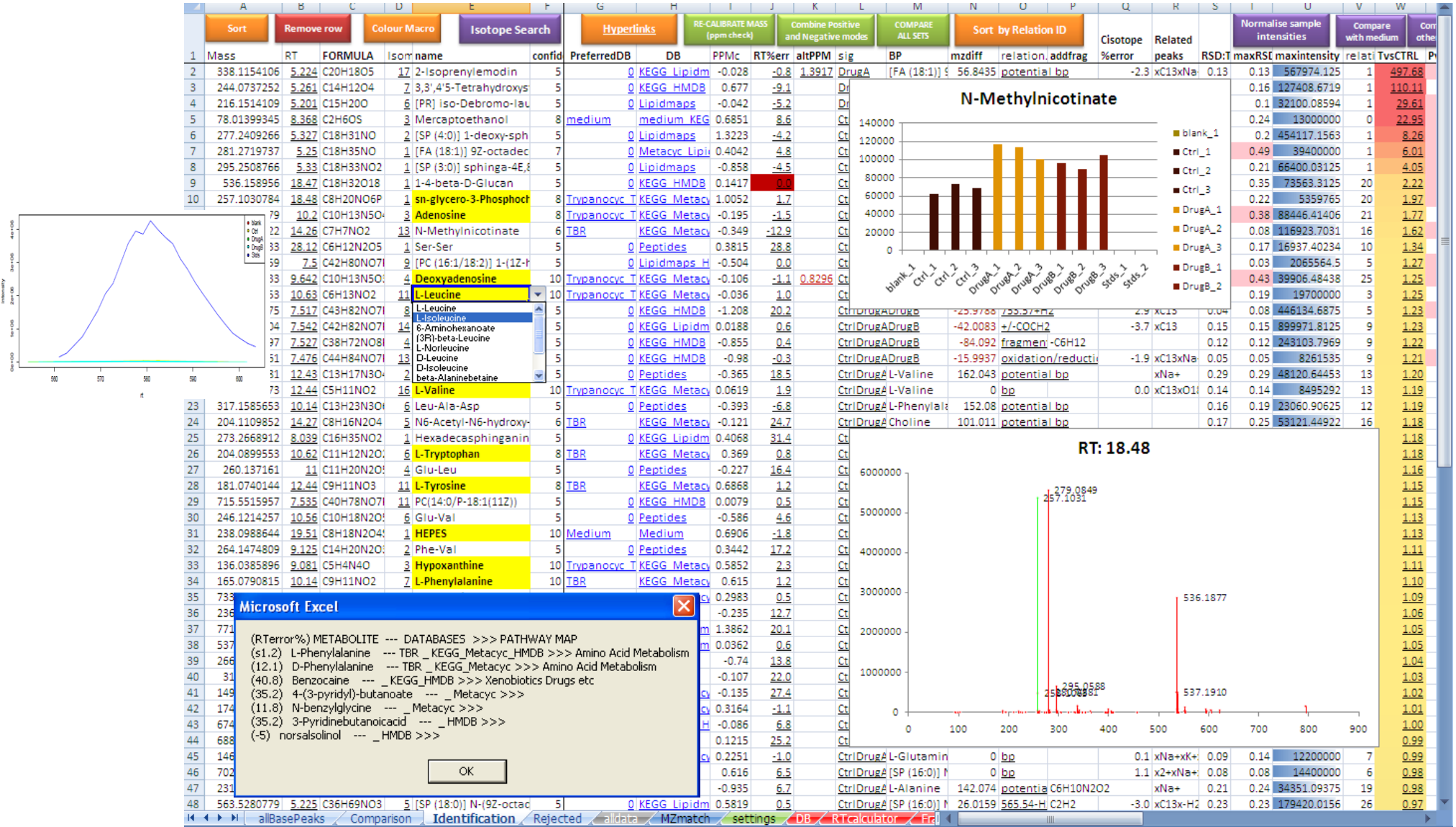
Add Chromatograms





- Add EICs to column A of the 'Identification' sheet by the 'Add Chromatograms' button
- This function requires access to the corresponding peakml file. NB: chromatograms are uploaded based on the peakID number. Therefore, take care to use only the peakml file that corresponds to the data matrix (mzMatchoutput.txt) uploaded to IDEOM.
- Alternatively (if your computer cannot load the peakml file) the chromatogram images can be generated from a peakml file on any machine using the R script available on the 'settings' sheet (this folder of chromatogram images is generated by the default script in step d)
- You may run this step on any results sheet at any time
  (e.g. Identification, Comparison, allBasepeaks)

LCMS Pre-processing: step 6



LCMS Pre-processing

Step 7: Combine Positive and Negative data [optional]

Skip this step for the workshop (using the Example data)

- This step should be performed on positive and negative datasets that have already been pre-processed up to step 6.

- Simply click step 7 and then select the pre-processed Ideom file for the opposite polarity (note combination of files may take a few minutes)

- Duplicate peaks (with the same corrected mass and retention time within the 'duplicate peaks' window) will be removed for metabolites detected in both Pos and Neg mode. The peak with the higher maximum intensity is retained.

LCMS Pre-processing

Step 8: Compare all sets

8. Compare all sets

- Run this function to summarise data into the Comparison sheet
- You have the option to only include the identified peaks, or to also include all significant base peaks (including unidentified and those with low identity confidence)
- This step can be repeated as required for different ‘control’ groups or with/without basepeaks

Step 9: Assign Basepeaks [optional]

9. Assign BasePeaks (optional)

- Sometimes base peaks are actually a related peak (adduct/fragment) of a smaller peak. This function takes all unidentified base peaks and annotates them if any related peaks have been (putatively) identified.
- This step could be run before step 8 if you want this information in the Comparison sheet

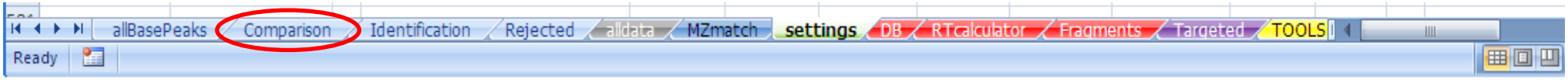
Ideom Tutorial: Part 2

Data Interpretation/Visualization Tutorial plan

- Getting Started
- Finding differences
  - sort
  - filter
  - graph
- Checking data integrity “Is it a real difference”
- Metabolite Identification
- Exporting to external programs or websites
- Changing groups for comparison

Getting started

Select the ‘Comparison’ sheet

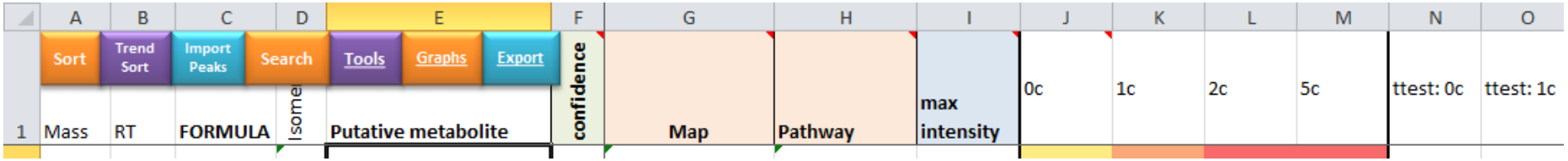


Scroll up and down to see the list of metabolites

- Metabolites (column E) highlighted yellow have been identified with authentic standards, all other metabolites are putatively identified from the database
- Red names (column E) indicate more than one peak has been identified as that metabolite
- Formula’s (column C) in red text indicate more than one peak is present with that formula (i.e. isomers... or shoulder peaks)
- Masses (column A) are highlighted according to the polarity mode of detection:
  - Red = positive ionisation
  - Blue = negative ionisation
  - White = detected in both positive and negative modes

Getting started

Scroll across to see the information and results available



Column:

- A: Neutral exact mass (re-calibrated, from mzMatch)
- B: Retention time (from mzMatch) in minutes
- C: Formula from DB with closest match to mass (if within ppm window)
- D: Number of isomers in DB with this exact formula
- E: Putative Metabolite name: best match from DB for this mass and RT
- F: Confidence level (out of 10) according to parameters on 'settings' sheet (10 = highest)
- G: Map: the general area of metabolism for this metabolite (usually from KEGG)
- H: Pathway: list of biochemical pathways for this metabolite (usually from KEGG)
- I: Maxintensity: The maximum intensity for this peak in any included sample

NOTE: columns G,H & I can be changed by choosing a different header in cell G1, H1 or I1

E.g. Click cell I1, then click the new down-arrow that appears to the right of the cell, and select 'groups'. The column now shows which sample groups have significant peaks detected for each metabolite.

Getting started

Scroll across to see the information and results available (2)

The number of additional columns is dependant on how many groups in your study:

- J onwards: Mean intensity of each included group relative to the 'control' group (as set when the 'Comparison' macro was run). Significant values (t-test) are in **bold**.
- NEXT columns: P-values for unpaired t-test between each included group and the control
- NEXT columns : Mean Intensity for each included group
- NEXT columns : Standard deviation for each included group
- NEXT columns : Relative Standard deviation for each included group
- NEXT columns : Fisher ratio for each included group, relative to the control group
- LAST column : PeakID: identifier essential for cross-referencing within the software and peakml files.
- ADDITIONAL: columns are added when you run some IDEOM functions. You may add your own additional columns to the right of existing data. Please do not insert columns between existing data.

$$\text{Fisher's ratio} = \frac{(m_1 - m_2)^2}{v_1 + v_2}$$

m = mean, v = variance
1 & 2 are study groups

Ideom Tutorial: Part 2

Data Interpretation/Visualization Tutorial plan

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  - sort
  - filter
  - graph
- Checking data integrity “Is it a real difference”
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Finding differences

Sort by specific columns to find the most changing metabolites

Fold-change

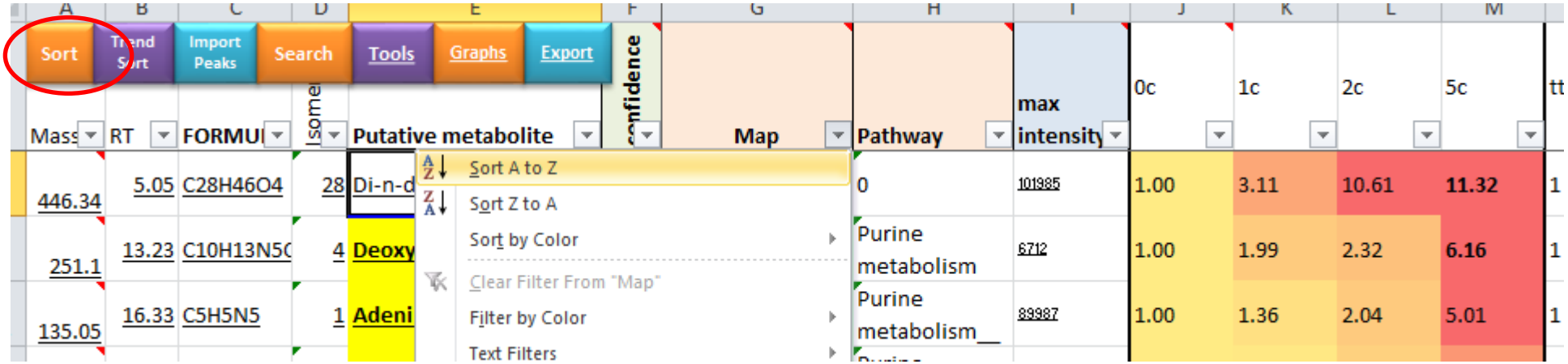
0c is currently set as the ‘control’.

To find the differences between 1c and 0c, sort by ‘1c’ (column K)

1. Sort using the orange ‘Sort’ button at the top-left and follow the instructions

(If using the inbuilt Excel sort or autofilter function please double-check that all data is selected)

1



Finding differences

Sort by specific columns to find the most changing metabolites

T-test

To find the significant differences between 1c and 0c, irrespective of whether they are increases or decreases, sort by 'ttest:1c' (column O) to find the metabolites with the lowest p-value

Caution: When metabolites are completely absent in a study group a p-value cannot be calculated. There are probably the most significant differences, but will appear as "NA" or "Div/0". NB: p-values are not corrected for multiple testing, please consider this before publishing significance (available in the R export menu).

Fisher ratio

To find the biggest significant differences between 1c and 0c (taking into account the extent of change and the variance), irrespective of whether they are increases or decreases, sort by 'Fisher:1c' (column AE) to find the metabolites with the highest fisher ratio

Caution: This cannot be calculated when the metabolite is completely absent from either group. In this case it will be marked as:

- NA = not in either group
- Low = not in treatment group
- High = not in control group

Finding differences

Sort by specific columns to find the most changing metabolites

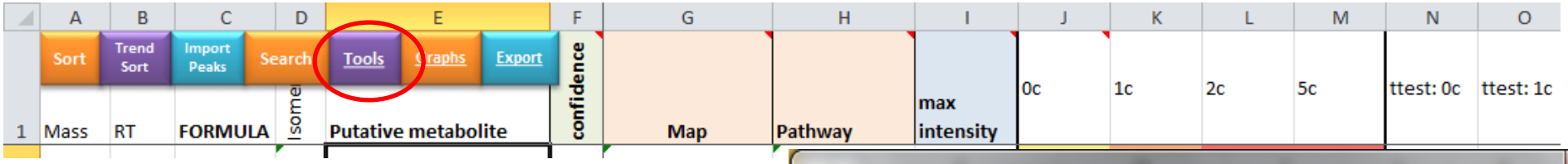
Other columns

It is less meaningful to sort by the “mean” columns, because LCMS response is different for every metabolite. For example, two metabolites with the same concentration could give 1000-fold different LCMS peak intensities due to their differing ionisation properties.

You may wish to combine sorting by differences with sorting by pathway (or other metabolite properties) to assist with interpretation.

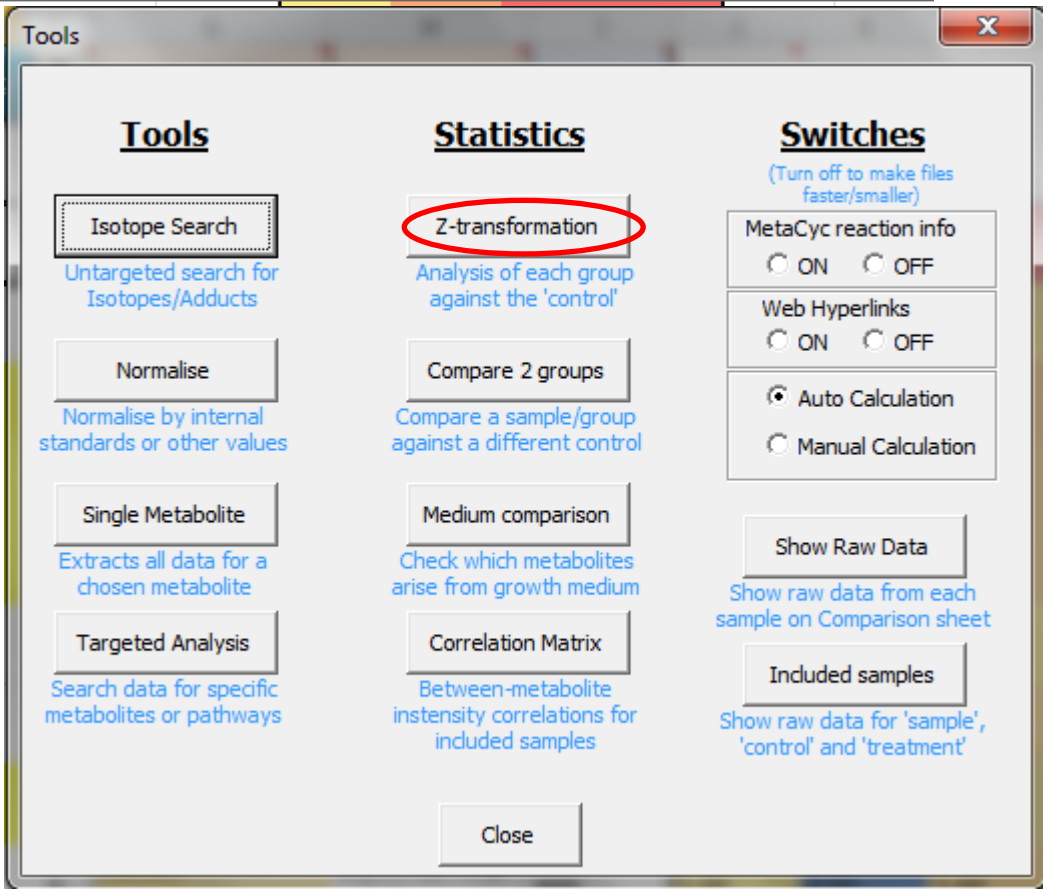
Finding differences

Z-transformation



Z-transformation is performed on all samples to express variance relative to the standard deviations from the mean of the control group

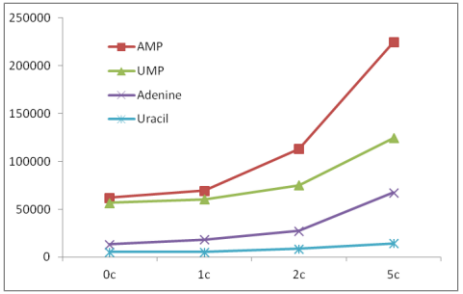
Data will be added to columns to the right of existing data



Finding differences

Correlation (Trend) Sort

Click the 'Correlation Sort' button to find metabolites that have a similar intensity trend across all study groups relative to:



1. A specific metabolite:

   Select a metabolite from column E

2. All other metabolites:

   Select the an empty row to do an iterative (non-specific) correlation sort

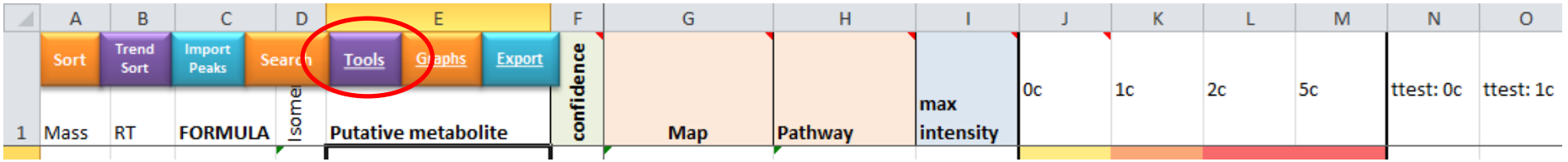
3. A specified intensity trend

   Enter a dummy metabolite (at the bottom of the page) with the desired intensity trend entered in columns J onwards (remember to delete this row before running other functions)

NB: On the Comparison sheet this correlation is based on group means. To calculate correlation of individual samples go to identification or alldata sheet.

Finding differences

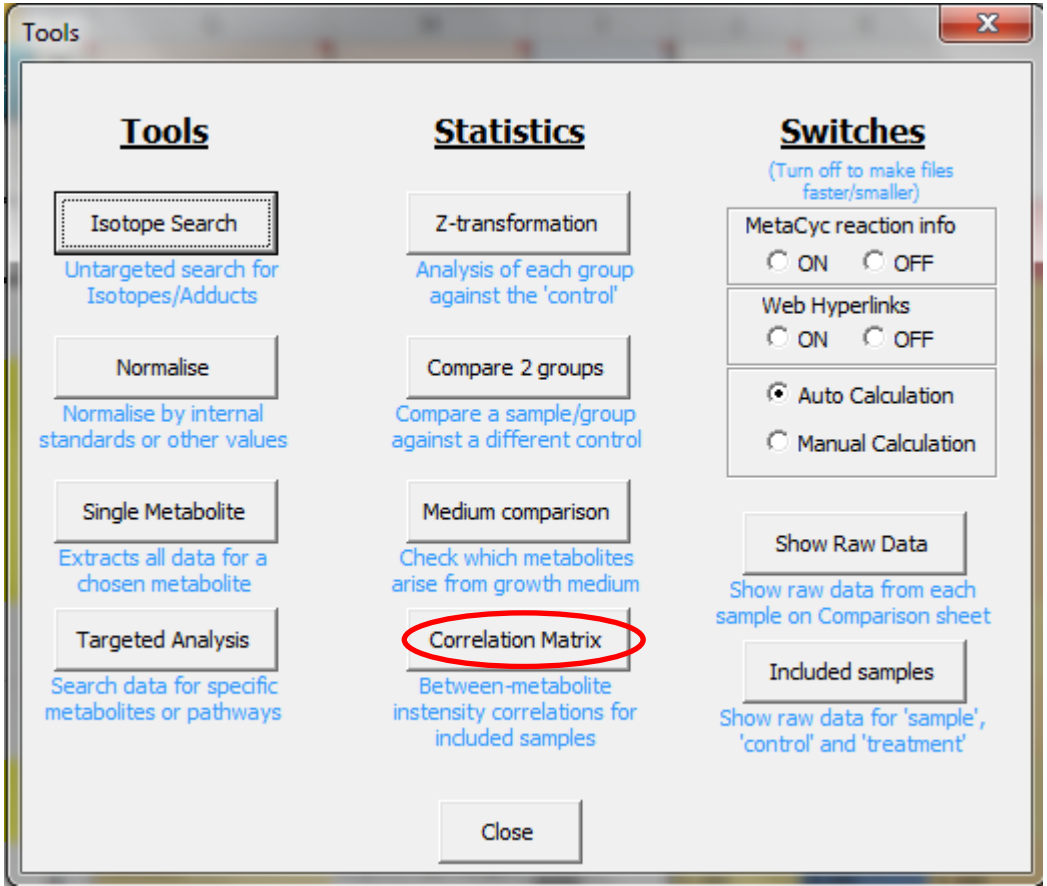
Correlation Matrix



Click the 'Correlation Matrix' button in the TOOLS menu to find the cross-correlation of intensities between metabolites.

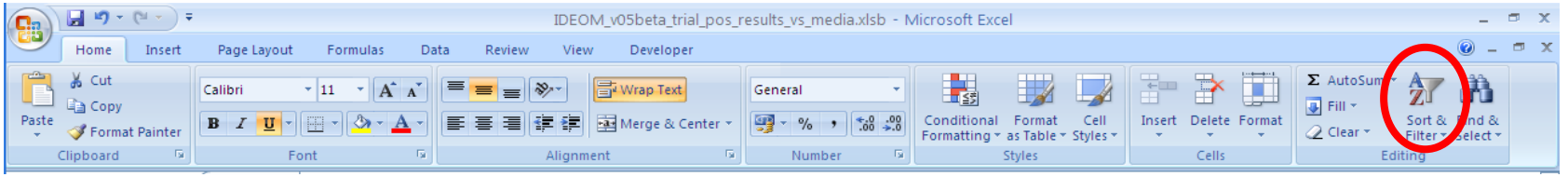
Pearson correlation coefficients will be written to a new worksheet and coloured in a heatmap format.

CAUTION: Metabolites with very high correlation may actually be LCMS artefacts that escaped the filtering steps. Double-check these by analysis of retention times.

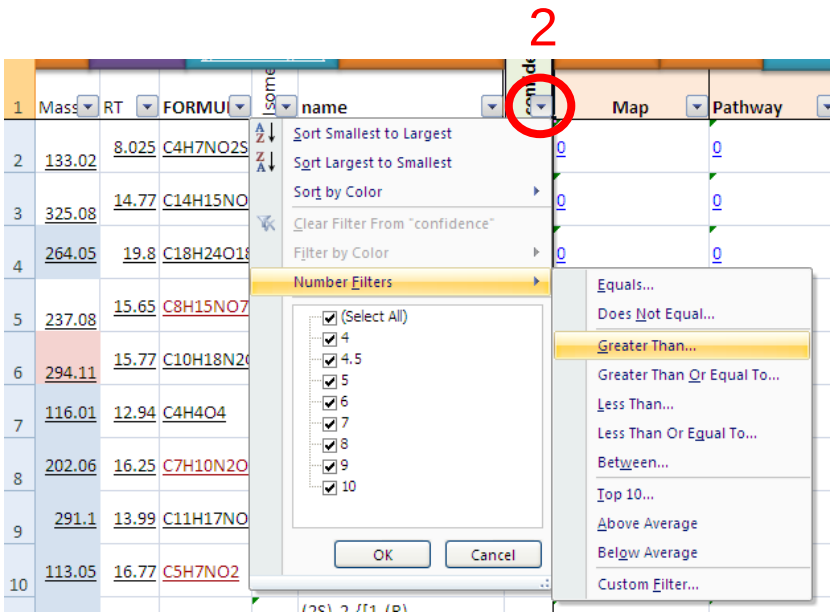


Filtering your list

Excel's Autofilter function is a very useful way to tidy your dataset to optimise visualisation, graphing or export functions for your metabolites of interest



1. Activate the filter (if it is not already activated)
2. Click on the down-arrow for the column you wish to filter by
3. Filter by selecting/deselecting the checkboxes, or set a number, color or custom filter



<u>Examples:</u>

Filter by Confidence > 6 (to get more confident ID's)

Filter by specific maps/pathways, or by "Text that contains": Lipid

Filter by P-value < 0.01 for a specific study group

Filter by maxintensity > 10,000, or in Groups "text that contains": 5c

Filter name by color to see only metabolites identified with authentic standards

Finding specific metabolites

The two best ways to find a specific metabolite are:

1. Metabolite Search

Click the orange Metabolite Search button

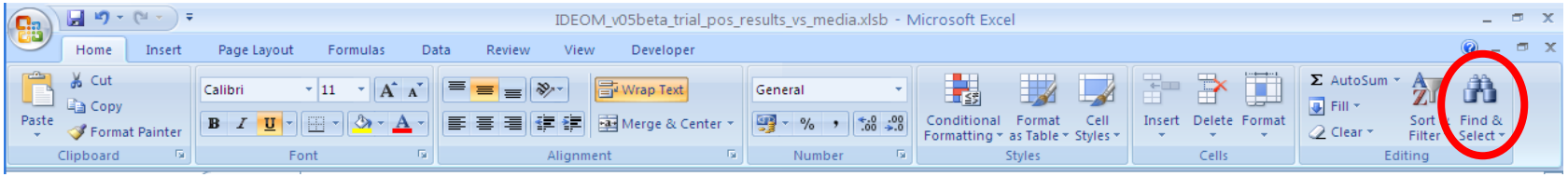
Enter the metabolite name

If the name doesn't exactly match a metabolite in the database you will be shown a list of similar metabolites to select the metabolite you are looking for

If your metabolite is not identified, but the formula is present, it will be found

If there is more than 1 matching formula you will get the option to find all these isomeric peaks

2. Excel's native Find function



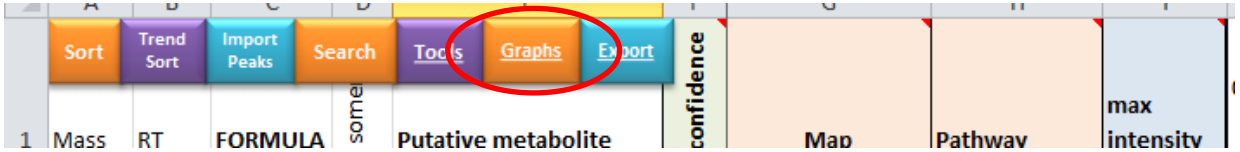
This will not find isomeric formulas if your metabolite wasn't 'identifed'

This may be quicker than the Metabolite Search function if you know approximately what you're looking for

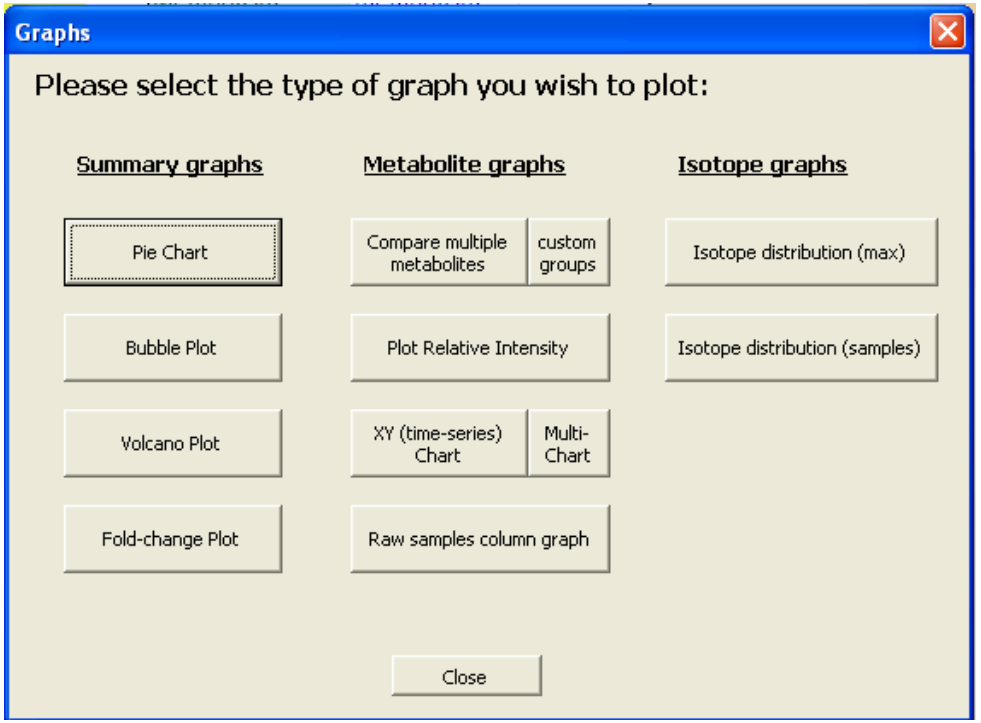
Graphing

Use graphs to find the most changing metabolites

Click on the “Graphs” button to access graphs and plots



Use Summary Graphs →

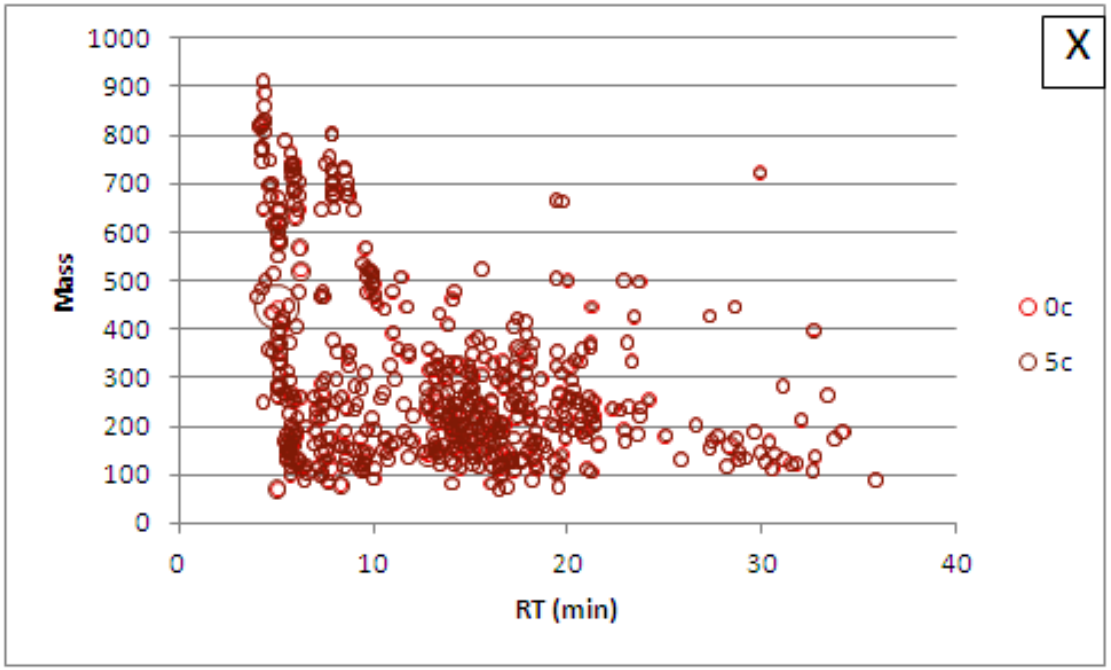


Graphing

Summary graphs: Bubble plot

Click the bubble plot to see the metabolites according to their detected masses and retention times. Larger bubbles represent larger relative intensities (compared to the control group)

- Edit graph as you would for any graph in Excel.
- If only interested in 0c and 5c, delete the extra series (click on the bubbles and press delete, or right click and 'Select Data')
- Make it bigger/smaller to suit
- Hover mouse over any point to get details of the retention time, mass and relative intensity
- Click the X in the corner to close (or select the graph and press delete)

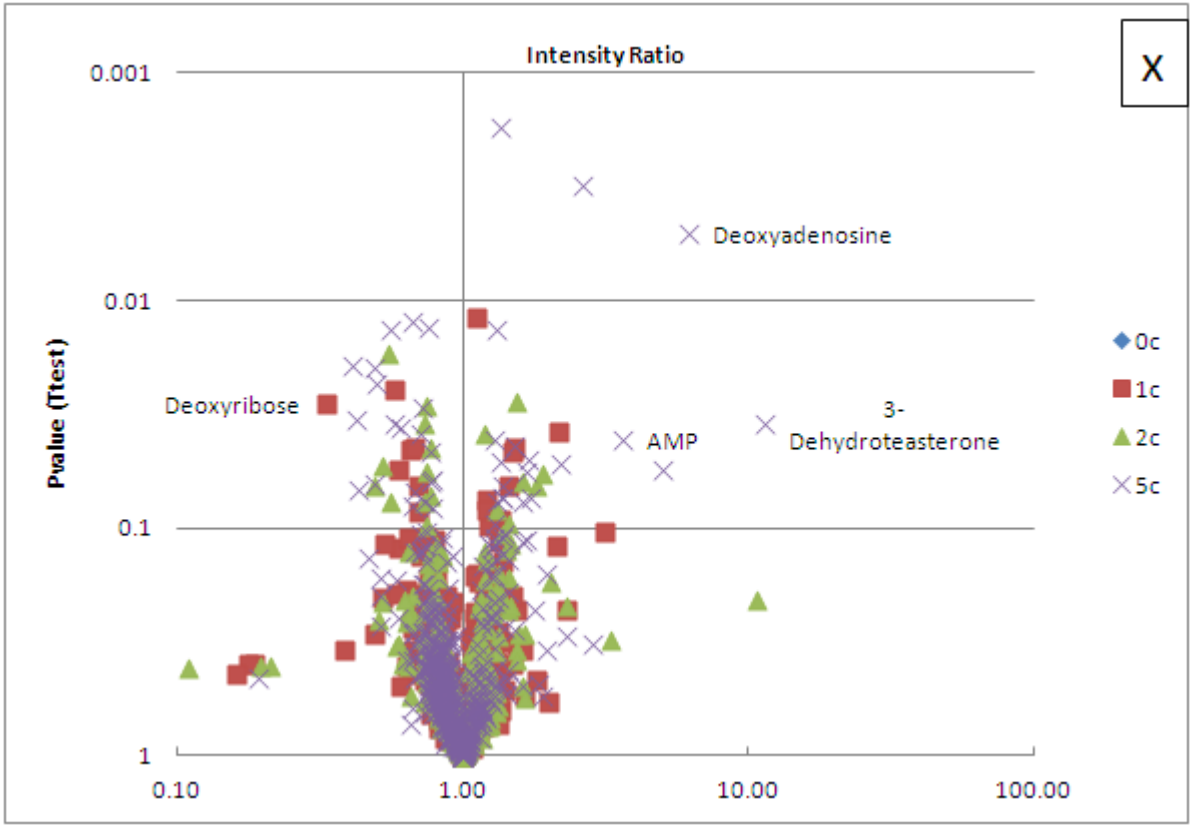


Graphing

Summary graphs: Volcano plot

Click the volcano plot to see the metabolites that differ most by p-value (from t-test) and relative intensity. Points furthest from the origin are most changed.

- NB: This plot uses log axes. Please agree to the Excel message about plotting 0.
- Edit graph as you would for any graph in Excel.
- Change size and included series as you did for the bubble plot
- If too many names are overlapping, consider starting again with stricter labelling thresholds

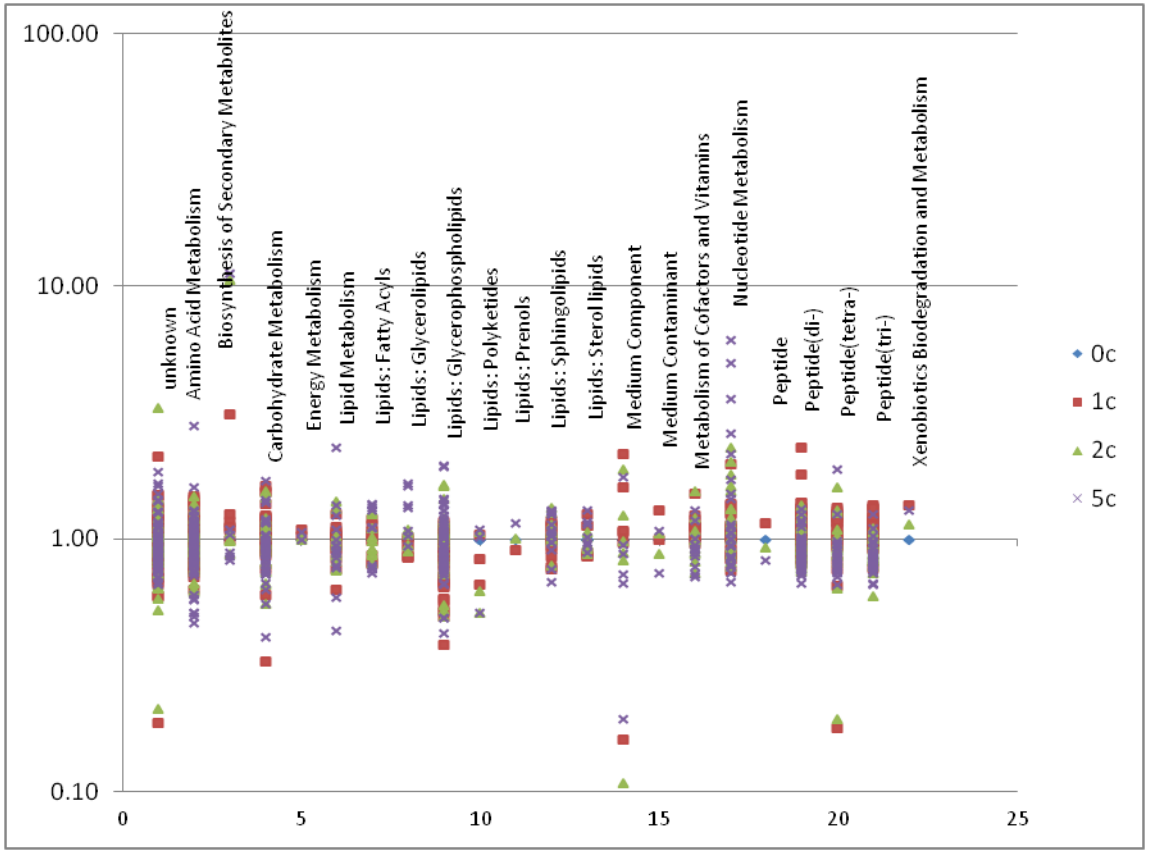


Graphing

Summary graphs: Fold-change plot

Click the Fold-change plot to see the relative intensity of all metabolites separately, or grouped by pathway. This looks better if you have fewer metabolites.

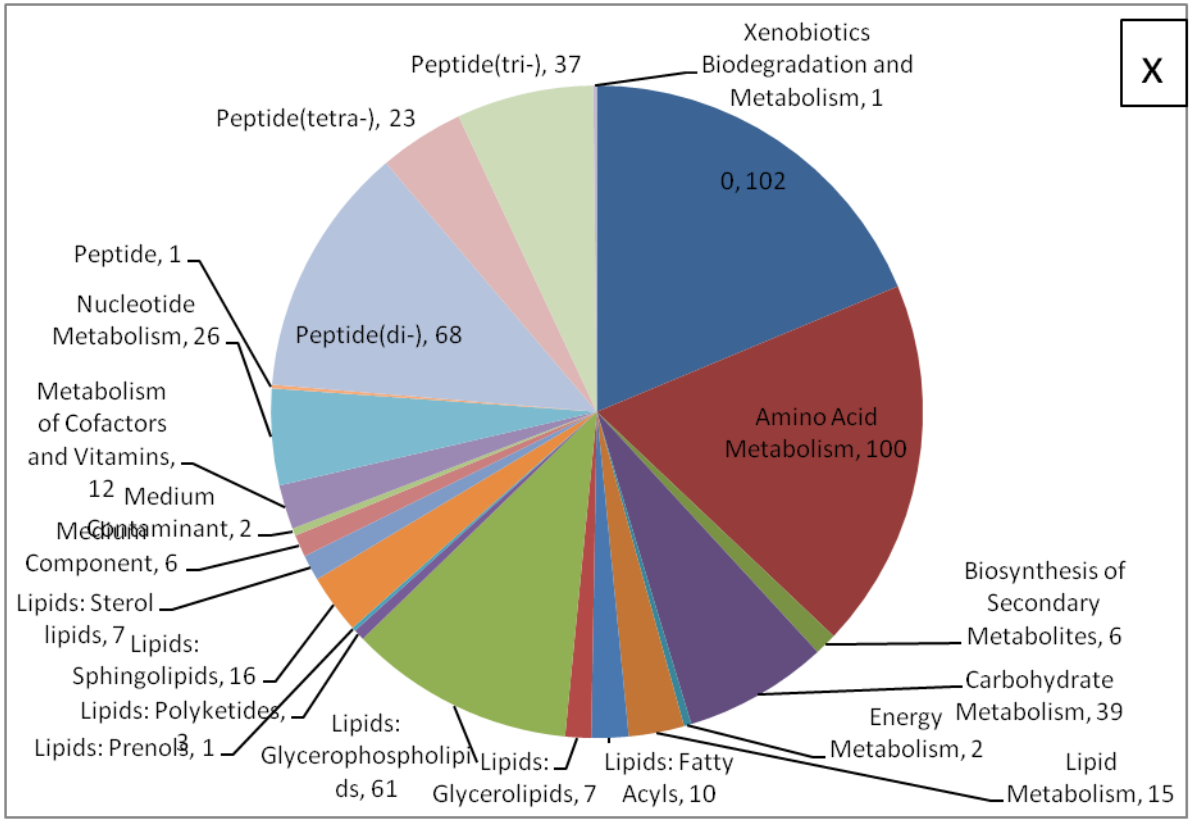
- NB: This plot uses log axes. Please agree to the Excel message about plotting 0.
- Edit graph as you would for any graph in Excel.
- Change size and included series as you did for the bubble plot
- If you grouped by pathway, try again by plotting all metabolites
- If too many names are overlapping, consider filtering the list beforehand



Graphing

Summary graphs

Click the Pie chart button to see a distribution of your identified metabolites (this doesn't give any information about changing metabolite levels). (NOTE: the pie chart is the only graph in Ideom that cannot handle filtered data)



Ideom Tutorial: Part 2

Data Interpretation/Visualization Tutorial plan

- Getting Started
- Finding differences
  - sort
  - filter
  - graph
- Checking data integrity “Is it a real difference”
- Metabolite Identification
- Exporting to external programs or websites
- Changing groups for comparison

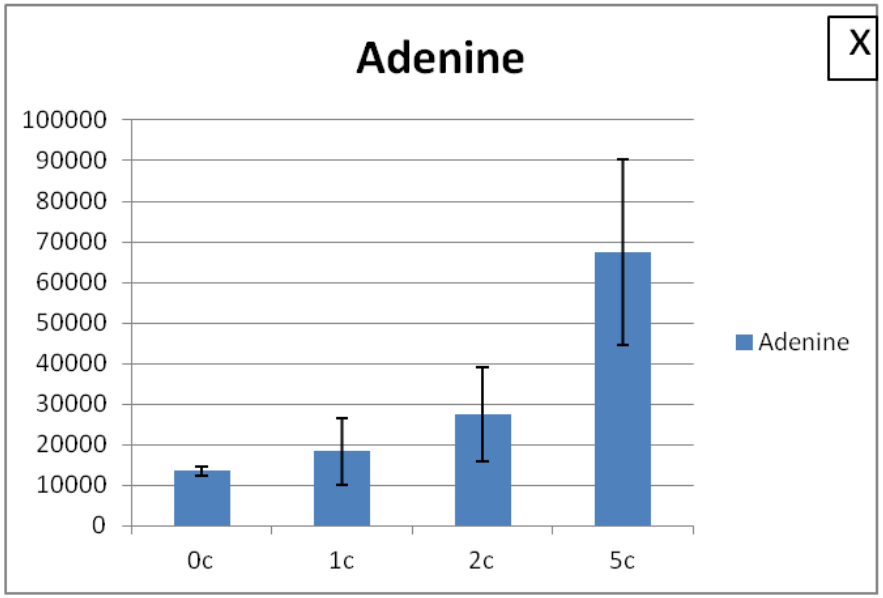
Checking differences

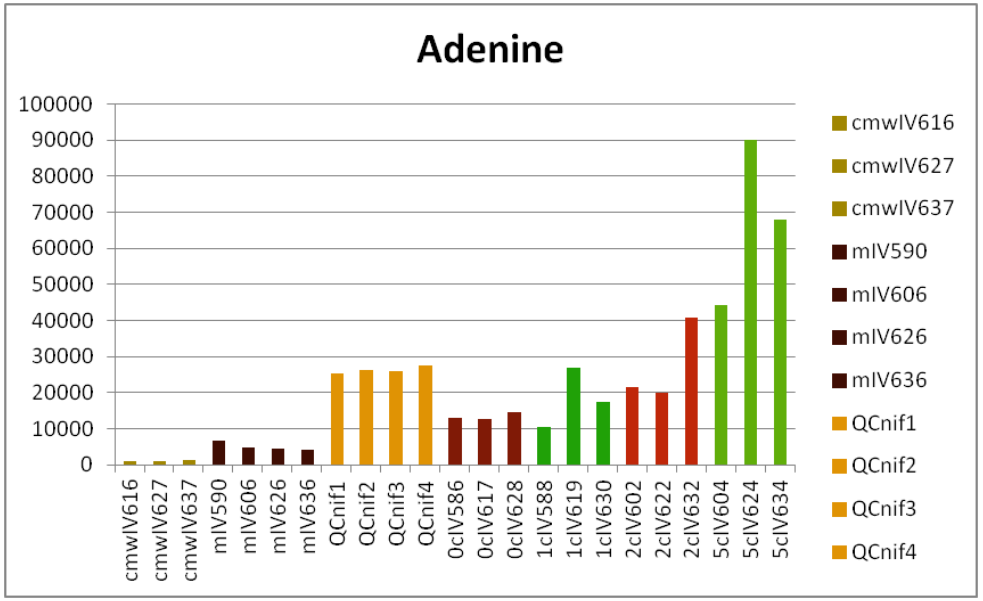
Is the difference real?

Means, standard deviations, t-tests and fisher ratios for each metabolite are available.

Graphs for individual metabolites are the best way to see differences:

- Double-click a metabolite name: this gives a column chart with mean peak intensity and standard deviations as error bars
- Double-click a specific cell in column I: this gives a column chart showing the peak intensity for every individual sample. Each sample is coloured according to it's study group

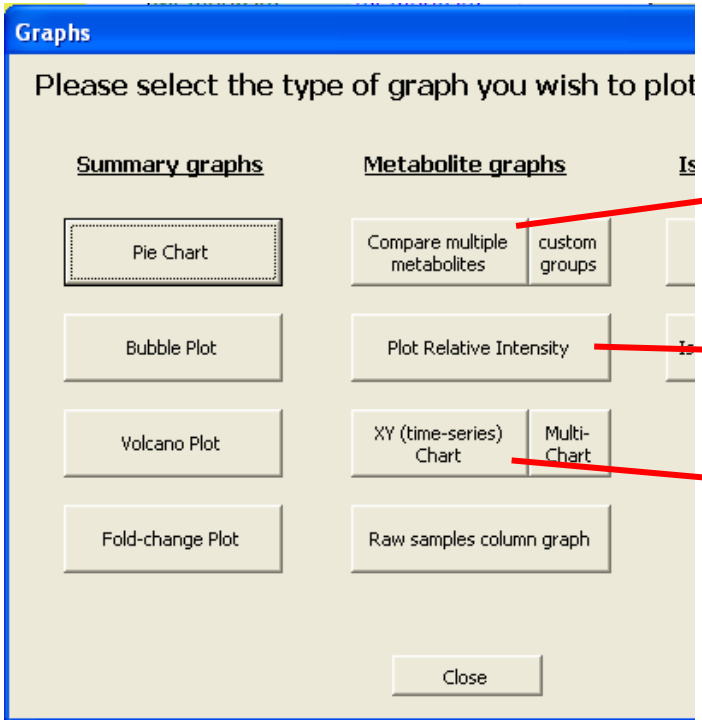


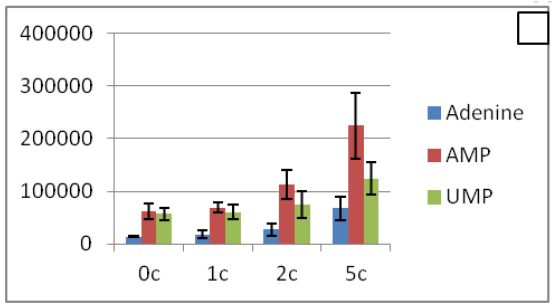


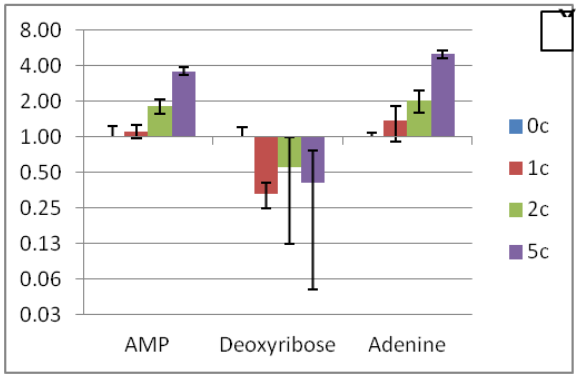
Checking differences

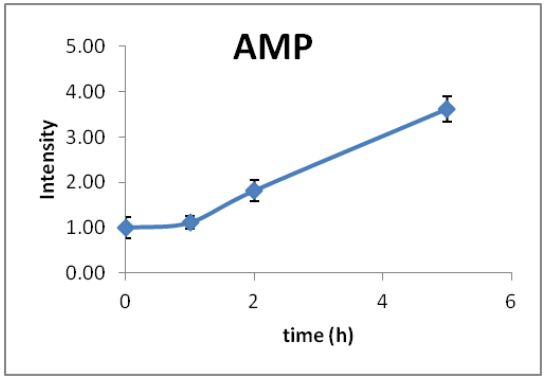
Is the difference real?

Additional graphs for individual metabolites are available from the ‘Graphing’ button:









Checking differences

Technical note on copying graphs

It is often nice to copy Ideom charts to a Word or Powerpoint file for reports or presentation

Be careful… Office links charts, so that if you do further processing in Ideom (i.e. sorting or filtering) the chart in Word/Powerpoint will change without you knowing!!

Two ways to avoid this are:

1. After pasting the chart in Word/Powerpoint 'break the link' to the Excel chart

   2007: Office button >> Prepare >> Edit links to files >> select chart and click 'Break link'

   2010: Office button >> File >> Edit links to files >> select chart and click 'Break link'

2. Paste as a picture (however in this case you can't edit it later)

   Paste special >> picture (PNG)

Troubleshooting:

If you have trouble copying a chart, deselect it (by selecting another random cell), then select it again and then copy.

Checking differences

Is the difference real…? Ion suppression?

Occasionally in LCMS an intensity difference is apparent for one metabolite, which is actually due to altered ionisation (enhanced or suppressed) caused by another chemical (metabolite, salt, solvent, or contaminant).

Internal standards

The best way to avoid this problem is to use isotope-labelled internal standards for every metabolite to normalise peak intensities. (see new features later in this tutorial)

External standards

Ideom currently supports inclusion of up to 9 external standards for quality control purposes (see Settings sheet). Intensities for these is plotted for each sample (if present) on the mzMatch page(s) after uploading data from mzMatch. Normalisation by these standards is not recommended (unless one of them co-elutes with your metabolite of interest).

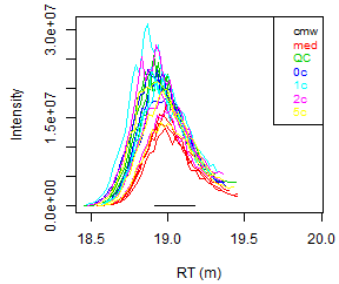
Sort by retention time

Sort your results by retention time (column B), and if there are numerous co-eluting compounds (i.e. with similar retention times) that show the same intensity trend across sample groups then your differences may be due to ion suppression.

Checking differences

Is the difference real…? Show me the data!

For absolute confirmation (i.e. before publishing a significant finding), double-check the peaks in raw data: (in case the intensity difference is due to odd peak shapes, or a peak was missed or not grouped correctly in the data processing)



Mouse-over cells in column A to see the extracted chromatograms

Double-click the retention time for a specific metabolite (in column B):

This gives you a graph showing the retention time and intensities of all peaks with the same mass

Alternatively (not in tutorial):

- Ctrl-Shift-X : activates the macro to view a selected mass in Xcalibur (mass must be selected first)

Ideom Tutorial: Part 2

Data Interpretation/Visualization Tutorial plan

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Metabolite Identification

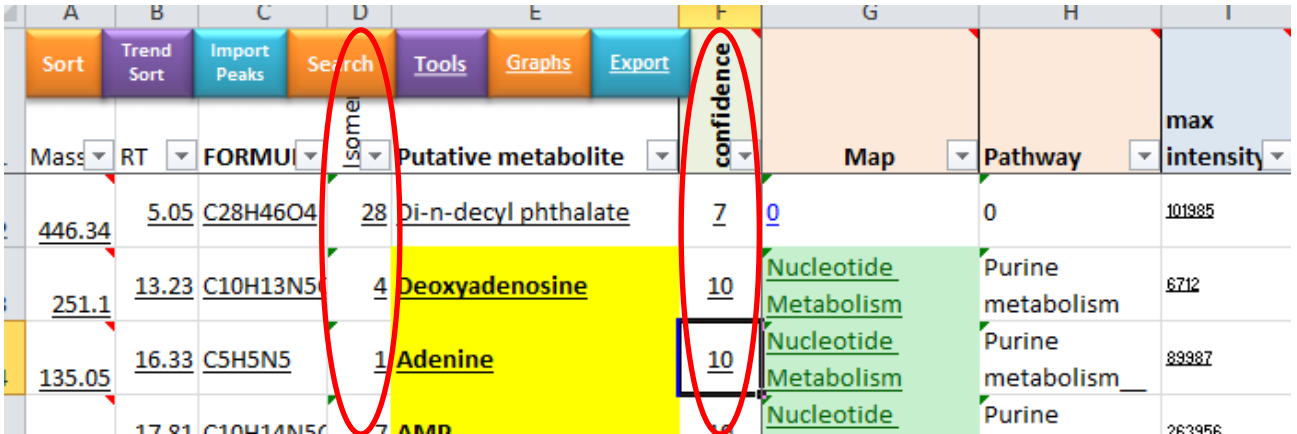
Metabolite identification is the biggest bottleneck in untargeted metabolomics

Ideom provides the best possible automated identification, however it is not possible to be absolutely certain.

False identifications are usually due to:

1. LCMS artefacts (e.g. adducts, fragments, peak shoulders, noise)
2. Isomers (e.g. leucine, isoleucine)

Metabolite identifications should always be interpreted in the context of the confidence level (column F), and the number of isomers in the database (column D).



Metabolite Identification

Identification verification

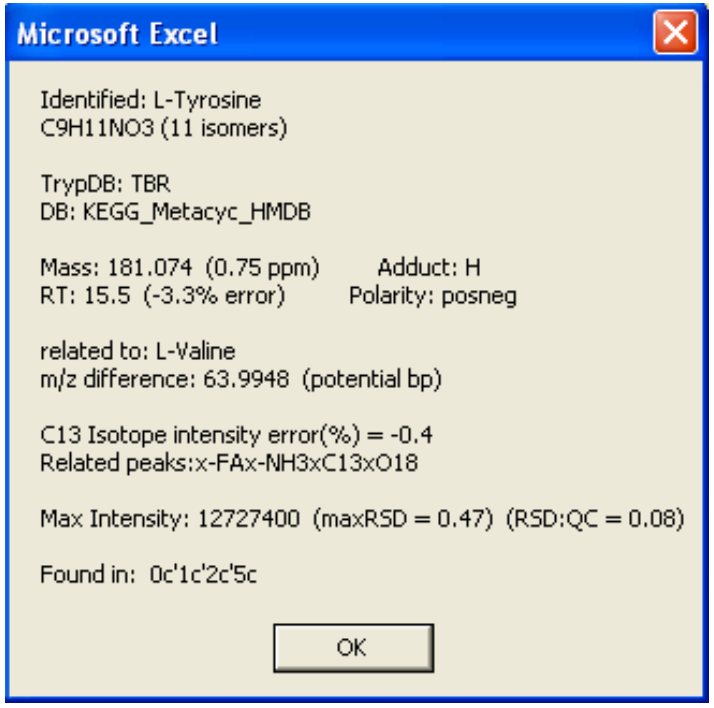
1. Confidence score and information box
2. Related Peaks (MS artefacts)
3. Isomers
4. EIC: Other peaks in the chromatogram
5. Alternate formulas (including adducts)
6. Sample intensities and reproducibility
7. Peak shape
8. Presence of associated metabolites (KEGG pathways)

Metabolite Identification

1. Confidence score and information box

The confidence level (column F) gives an indication of the confidence of identification.

Double-click a cell in column F to get background information about each metabolite to help confirm or reject the identification (e.g. find L-Tyrosine and double-click on the 8 in column F)



This metabolite is identified as L-Tyrosine according to the formula, but there are 11 possible isomers.
L-Tyrosine is expected in these samples according to the KEGG Tbr annotations, and this metabolite is in MetaCyc and HMDB.
The detected mass is correct for this formula within 0.75ppm and it was detected in both pos and neg ionisation, predominantly as the protonated adduct.
The retention time is 3.3% earlier than the authentic standard RT.
mzMatch suggests this is related to L-Valine, but the mass difference is 63.9948, which is not a likely adduct.
The C13 isotope confirms the presence of 9 carbon atoms (-0.4% error), and there is also an O18 isotope (confirming oxygen atoms are present). Fragments for loss of ammonium and loss of formic acid are consistent with this metabolite containing a primary amine and carboxylic acid.
The maximum peak intensity is 12727400, which is large (i.e. it is not background noise), and the maximum RSD is 0.47 (i.e. Some variability in some samples), but the QC samples are reproducible (RSD: 0.08). This peak is found at significant levels in all study groups (0c, 1c, 2c and 5c).

In summary, it is highly likely that this peak is indeed L-Tyrosine, but to be absolutely certain you would need to rule out the 10 other possible isomers

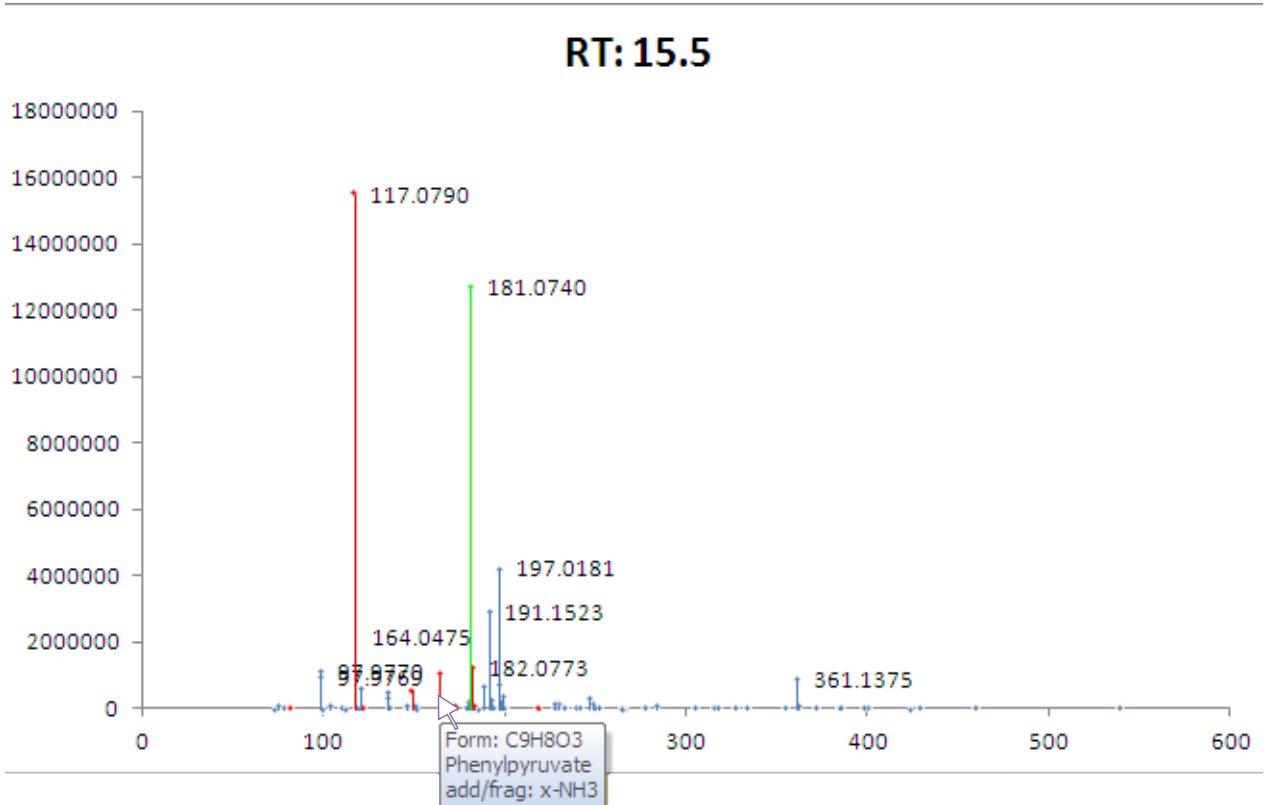
NOTE: This information is stored in the ‘Identification’ sheet. Change the header in column I to access this information directly on the ‘comparison’ sheet.

Metabolite Identification

2. Check Related Peaks

To see the mass spectra of all co-eluting peaks double-click the mass (column A)

The green peak is this peak. The red peaks are related (according to mzMatch). The blue peaks are co-eluting, but probably not related.



Example: Double-click the mass for L-Tyrosine.

Hover over a mass in the graph to see annotations and click to go to the 'alldata' sheet to interrogate these peaks in detail

NOTE: This is the only Ideom graph that cannot be easily re-sized. The MS peaks do not move to scale with the axes.

Metabolite Identification

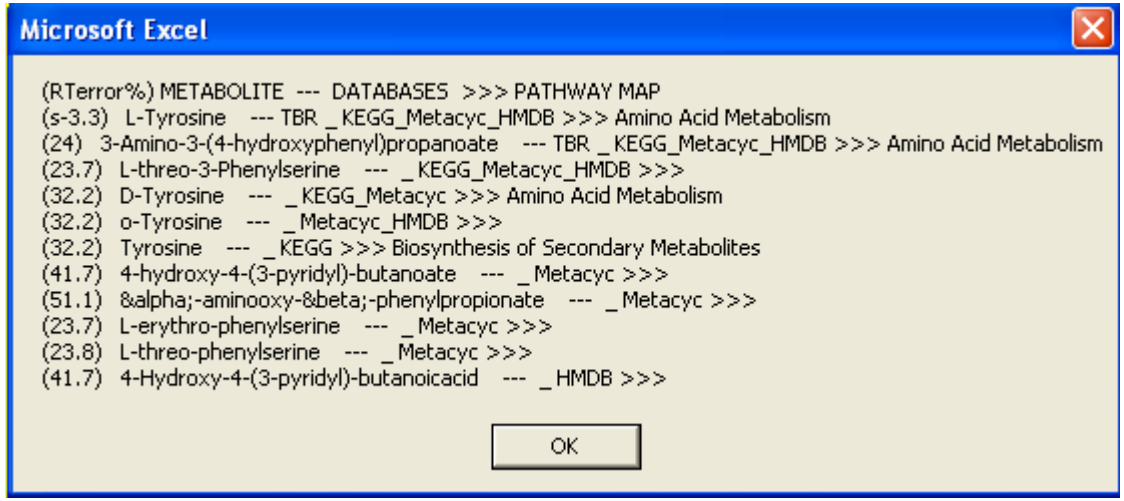
3. Check Isomers

The isomer count (column D) gives the number of isomers with this formula in the database

Double-click a cell in column D to get the list of isomers for a particular formula

Looking at predicted retention time errors and database information you may be able to exclude some alternatives based on chemical (retention time) or biological (presence in a given organism) factors

(e.g. find L-Tyrosine and click on the 11 in column D)



- This metabolite is identified as L-Tyrosine, based on the retention time of an authentic standard.
- Nevertheless, it could be a tyrosine or phenylserine isomer, or 3-Amino-3-(4-hydroxyphenyl)propanoate.
- The aminoxy and pyridyl compounds are much less likely based on their physicochemical properties (Rt).
- L-Tyrosine remains the most likely based on genome annotations for this organism.

NOTE: RT errors are expressed relative to the predicted retention time for that metabolite structure. If an authentic standard retention time is in the database, the error is prefixed by an “s”.

Metabolite Identification

3. Check Isomers

To find out more information about a particular metabolite (or structure), use the weblinks in columns G and H

Column G weblinks run a search in Chemspider for that particular metabolite

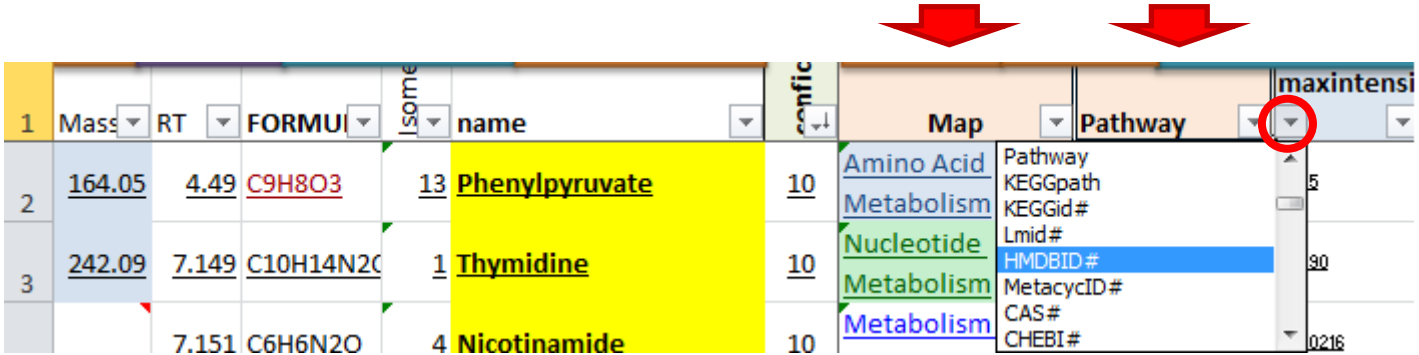
If you prefer another website (e.g. Pubchem, Google, Wikipedia, BioCyc, KEGG) you can change this in the settings sheet, cell AG29 (scroll across to the right).

Double-click column H to open a form with links to many common metabolite databases

Columns G and H can be changed by selecting another header (cells G1 & H1)

The hyperlinks in column G remain the same (e.g. Chemspider) regardless of the header.

NOTE: You may change the metabolite by selecting the metabolite name in column E, then clicking the down-arrow that appears to the right, then select the alternative isomer from the dropdown list.



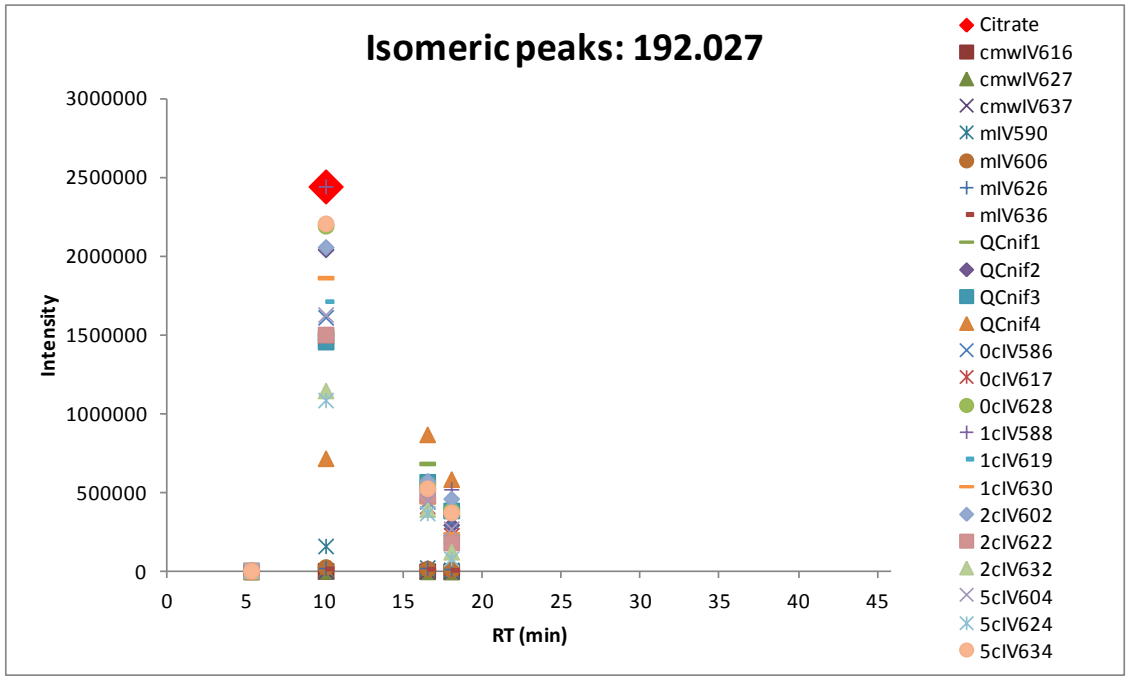
NOTE: If your computer is running slowly you may switch off hyperlinks in the tools menu

Metabolite Identification

4. EIC: Other peaks in the chromatogram

If there is more than 1 isomeric peak detected in the dataset, the formula will be coloured red.

Assignment of each isomeric peak to the correct metabolite is initially based on retention time. Alternatively it is based on the assumption that the 'most biologically likely' isomer is the most intense peak in the data. Double-click on the retention time to see the intensities of all isomeric peaks in the dataset. E.g. double-click the retention time for Citrate



This shows a very large peak citrate, in addition to some other smaller peaks. They could be chromatographic artefacts, or artefacts from other metabolites, but may be less abundant isomers.

Follow the prompts to double-check the signal in raw data.

Metabolite Identification

5. Check alternative formulae

Double-click the in the formula cell to see if this mass might be another formula (within the ppm error) or an adduct other than H^+

E.g. L-Cystathionine
This shows that the cystathionine formula for the H+ adduct is the best alternative. $C_{15}H_{10}O_2$ is also within 4ppm, but much less accurate than $C_7H_{14}N_2O_4S$

Alternatively, it could be an acetonitrile adduct of DL-Methionine sulfone, but the retention time is not very accurate.

- Try changing the entry to $C_5H_{11}NO_4S$ by selecting this formula and the ACN adduct from the boxes at the top, and click 'update formula'.
(then change it back to cystathionine)

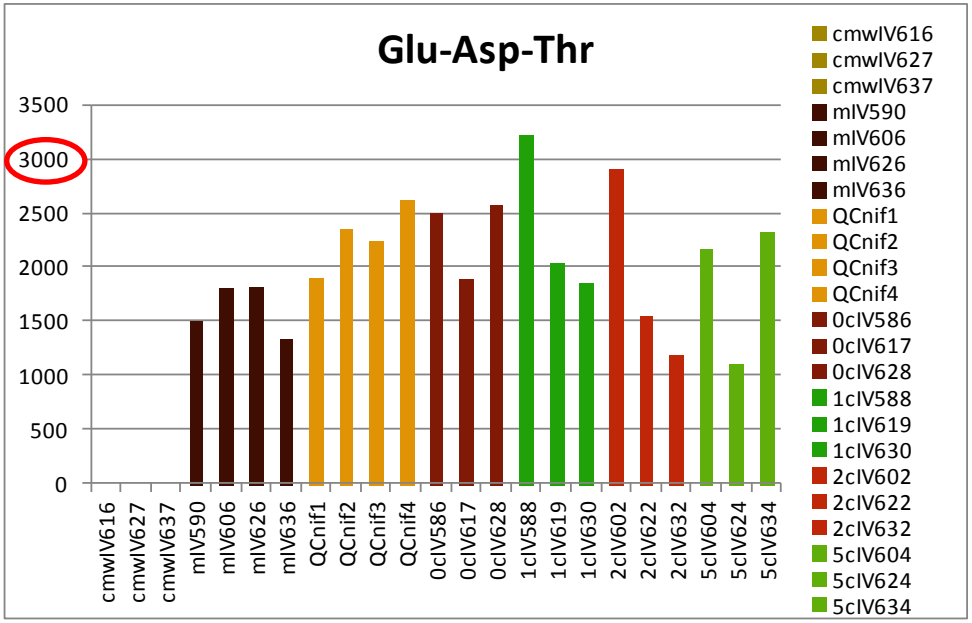
- To look up other possible formulae, not in the Ideom database, use one of the external links at the top right.
RCDK will give an unbiased list of possibilities (may take a few minutes).
Chemspider and Xcalibur simply copy the mass for you to use in their own search engines.

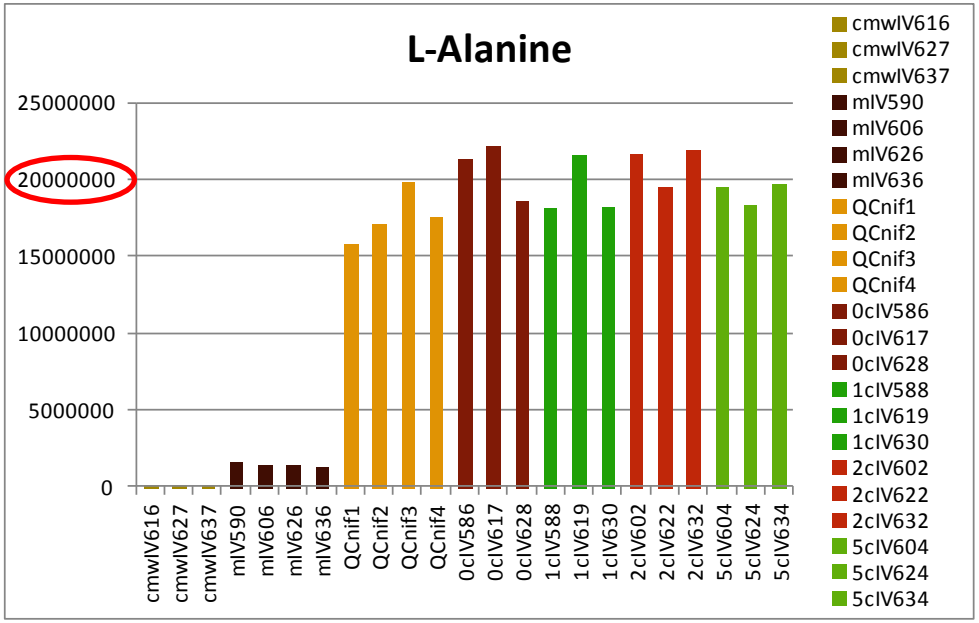
Metabolite Identification

6. Check sample intensities and reproducibility

Double-click the in column I to see plot of individual samples with raw intensities

- check peak intensities relative to LOQ
- check reproducibility in your samples of interest



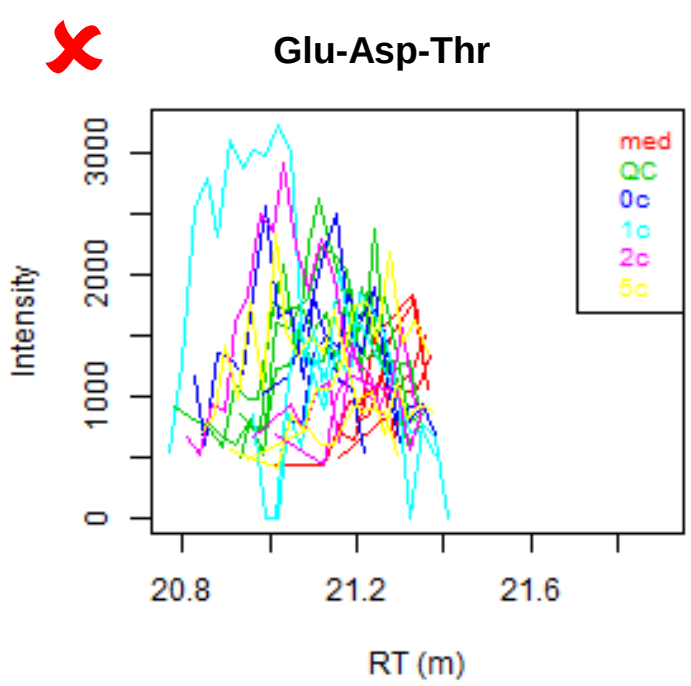


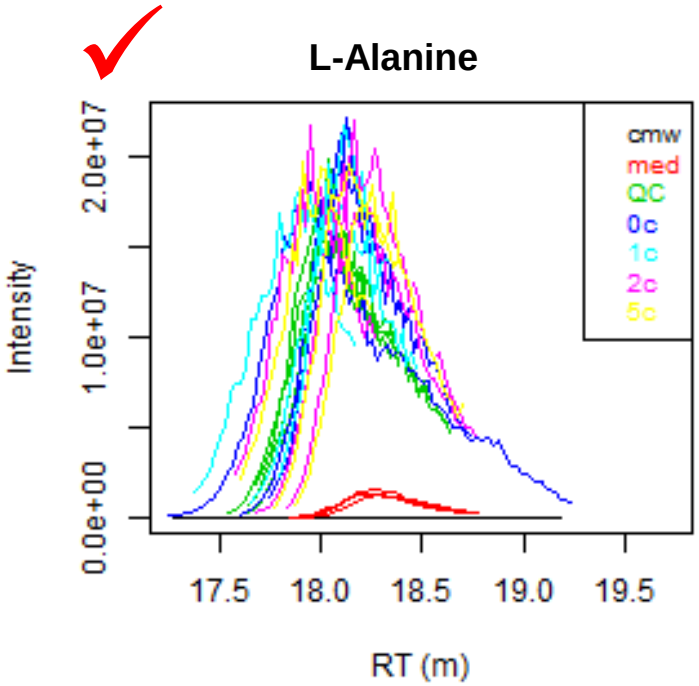
Metabolite Identification

7. Check peak shapes

Hover mouse over the mass in column A to see extracted peaks

- Ideally guassian peaks, some subjective judgement required





Metabolite Identification

8. Presence of associated metabolites (KEGG pathways)

The presence of biochemically related metabolites increases the confidence of identification

131.095	14.04	C6H13NO2	12	L-Leucine	10	Amino Acid Metabolism	Valine, leucine and isoleucine
130.063	5.499	C6H10O3	17	(S)-3-Methyl-2-oxopentanoic acid	8	Amino Acid Metabolism	Valine, leucine and isoleucine
116.047	5.744	C5H8O3	9	3-Methyl-2-oxobutanoic acid	8	Amino Acid Metabolism	Valine, leucine and isoleucine
117.079	15.44	C5H11NO2	16	L-Valine	10	Amino Acid Metabolism	Valine, leucine and isoleucine
173.069	15.36	C7H11NO4	6	(2S,5S)-carboxymethylproline	5	Biosynthesis of Secondary Metabolites	(5R)-carbapenem biosynthesis
240.147	15.67	C12H20N2O3	2	Slaframine	5	Biosynthesis of Secondary Metabolites	Alkaloid biosynthesis II
446.339	5.05	C28H46O4	22	3-Dehydroteasterone	8	Biosynthesis of Secondary Metabolites	Brassinosteroid biosynthesis
202.095	19.87	C8H14N2O4	5	Proclavaminic acid	5	Biosynthesis of Secondary Metabolites	Clavulanic acid biosynthesis
270.053	5.406	C15H10O5	24	Apigenin	6	Biosynthesis of Secondary Metabolites	Flavonoid biosynthesis Isoflavon
259.046	17.2	C6H14NO8P	8	alpha-D-Glucosamine 1-phosphate	8	Carbohydrate Metabolism	Aminosugars metabolism
389.072	17.87	C11H20NO12I	1	N-Acetylneuraminate 9-phosphate	8	Carbohydrate Metabolism	Aminosugars metabolism
179.079	25.04	C6H13NO5	10	D-Glucosamine	10	Carbohydrate Metabolism	Aminosugars metabolism

Or, use the ‘Targeted’ tool to check all metabolites in a pathway

Metabolite Identification

Identification verification

IDEOM

		Column
1.	Confidence score and information box	F
2.	Related Peaks (MS artefacts)	A
3.	Isomers	D
4.	EIC: Other peaks in the chromatogram	B
5.	Alternate formulas (including adducts)	C
6.	Sample intensities and reproducibility	I
7.	Peak shape	A
8.	Presence of associated metabolites (KEGG pathways)	G/H
9.	MSMS data – see part 3	

Ideom Tutorial: Part 2

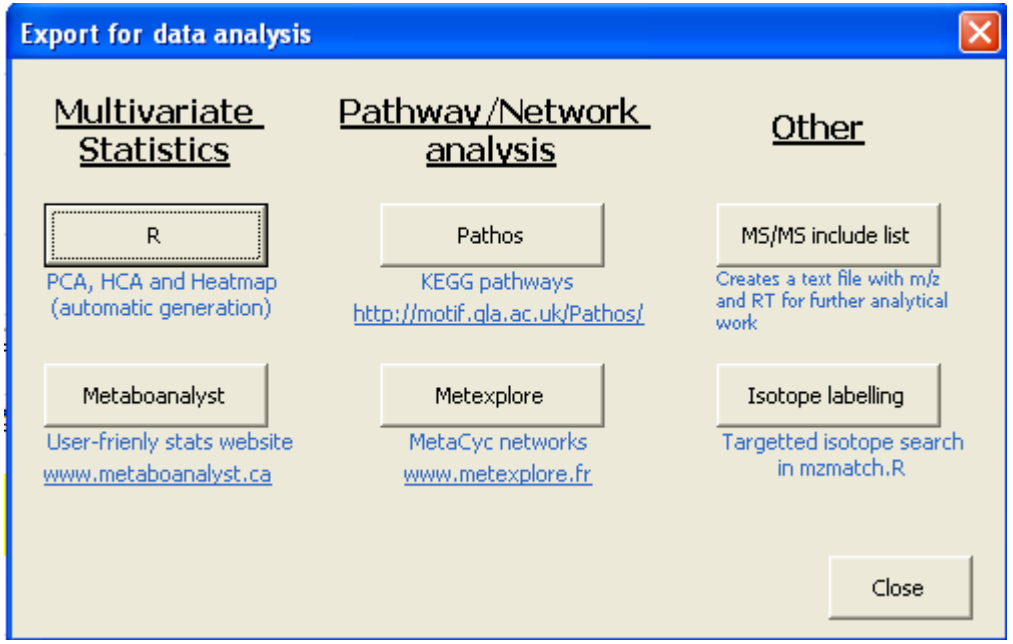
Data Interpretation/Visualization Tutorial plan

- Getting Started
- Finding differences
  - sort
  - filter
  - graph
- Checking data integrity “Is it a real difference”
- Metabolite Identification
- Exporting to external programs or websites
- Changing groups for comparison

Export for analysis

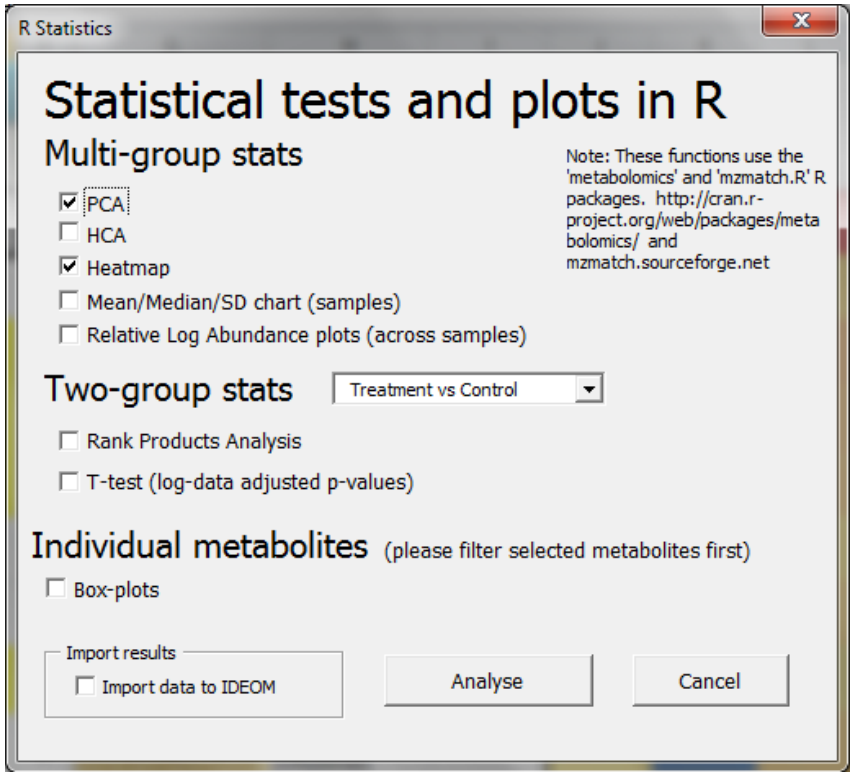
Export data to use external metabolomics applications

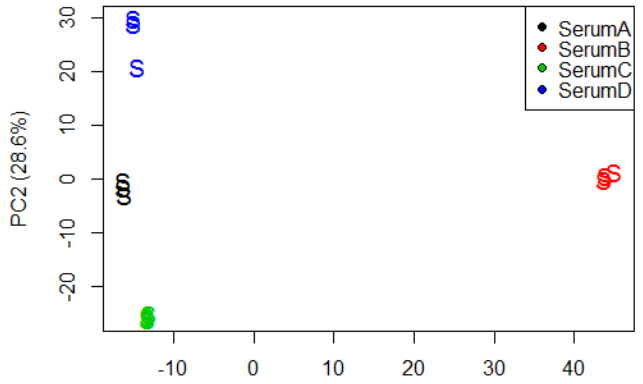
Click on the “Export for analysis” button to access export options

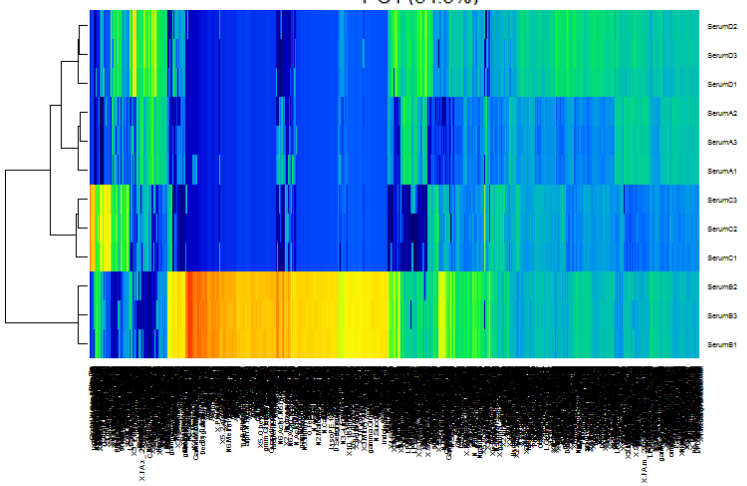


Export for analysis

Export data to do multivariate statistics in R







If you are familiar with R you may do additional analyses:

- The peak intensity data are in “PeakTable”
- Samples in rows
- Metabolites in columns
- Group names in “sampleclasses”

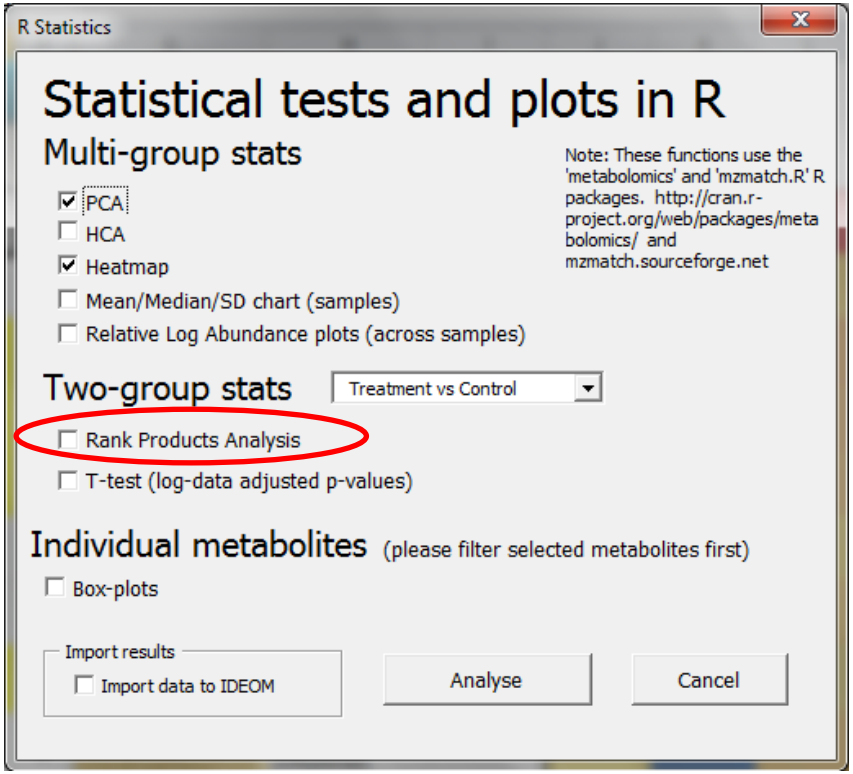
Export for analysis

Rank Product analysis

Option to do a two-sample comparison (treatment vs control) or for each study group to be compared to control group

Results are imported to IDEOM in the columns to the right of existing data

Each two-sample comparison has a test for increased abundance and a test for decreased abundance



wikipedia.org/wiki/Rank_product

Export for analysis

Export data: Metaboanalyst

Metaboanalyst is a very useful, and user-friendly, website for metabolomics analysis (particularly multivariate statistics).

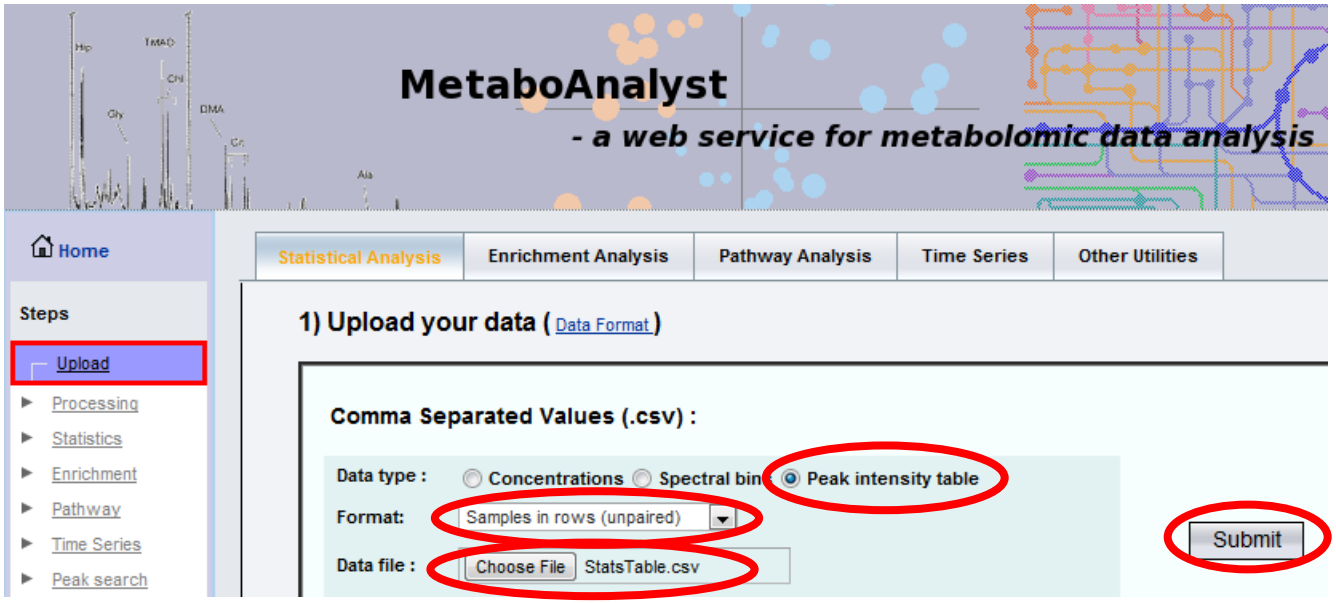
This website is managed by the metabolomics group in Alberta, Canada. Contact them (Jeff Xia) if you have any questions or problems.

After going to the analysis website, select "Concentrations" or "Peak Intensity Table" then upload the "StatsTable.csv" file (in the same folder as your Ideom file) and submit.

The website will guide you through the data checking and normalisation steps.

If unsure, skip the data filtering, select no row-wise normalisation, and autoscaling (or paretoscaling) for column-wise normalisation.

Select the analyses of your choice from the menu on the left



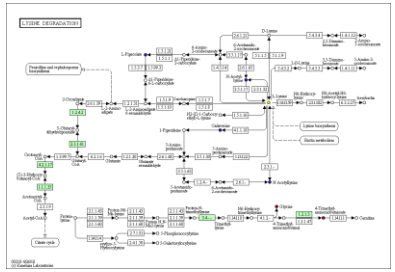
Export for analysis

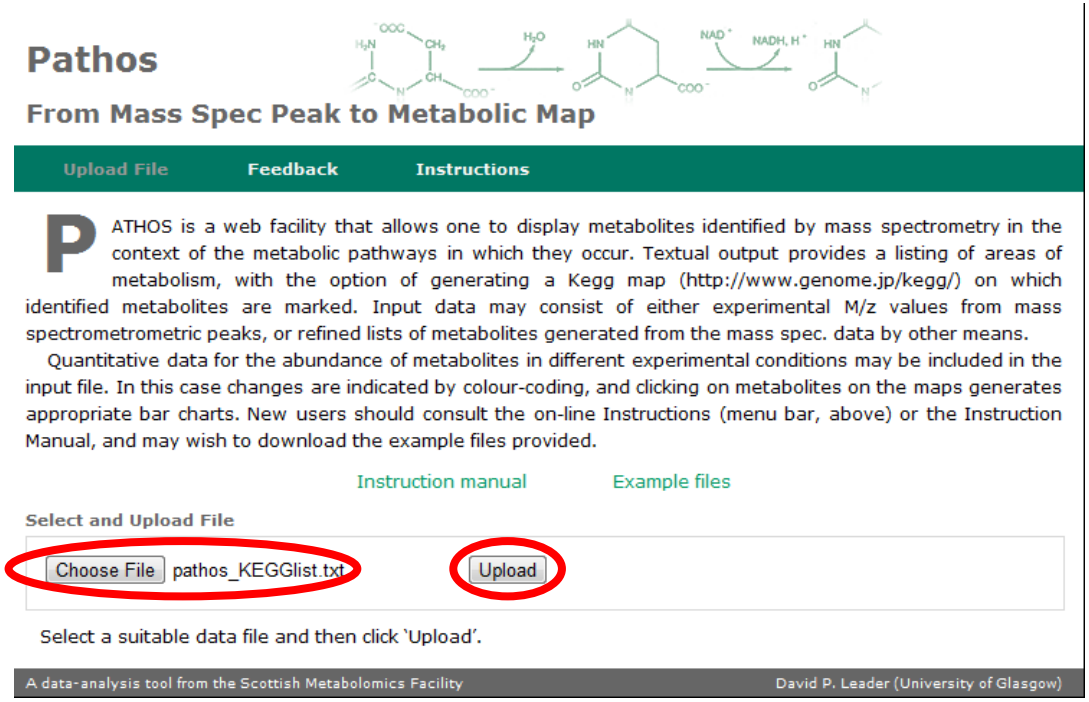
Export data: Pathos

Pathos is a user-friendly website for mapping metabolomics data onto KEGG pathways

This website was developed in Glasgow.

Contact David Leader if you have any questions/problems/suggestions.





After going to the Pathos website, choose the "pathos_KEGGlist.txt" file (in the same folder as your Ideom file) and 'Upload'.

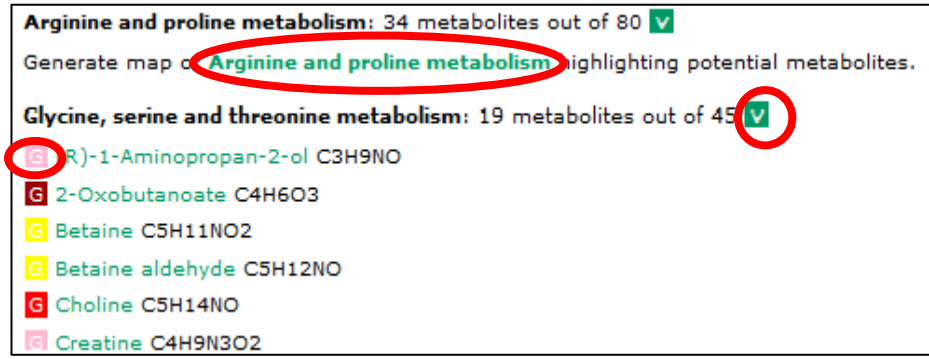
Follow the steps on the website (i.e. select your organism) and hit 'Run'.

Browse data by pathway by clicking the V buttons, or generating each map

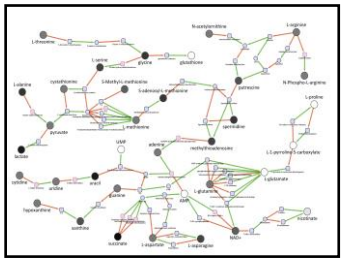
Click G buttons to view graphs of metabolite levels (or click the coloured metabolite spots on the maps)

Click metabolite names to view metabolite info

Mouse-over chemical formula to see isomers in the pathway



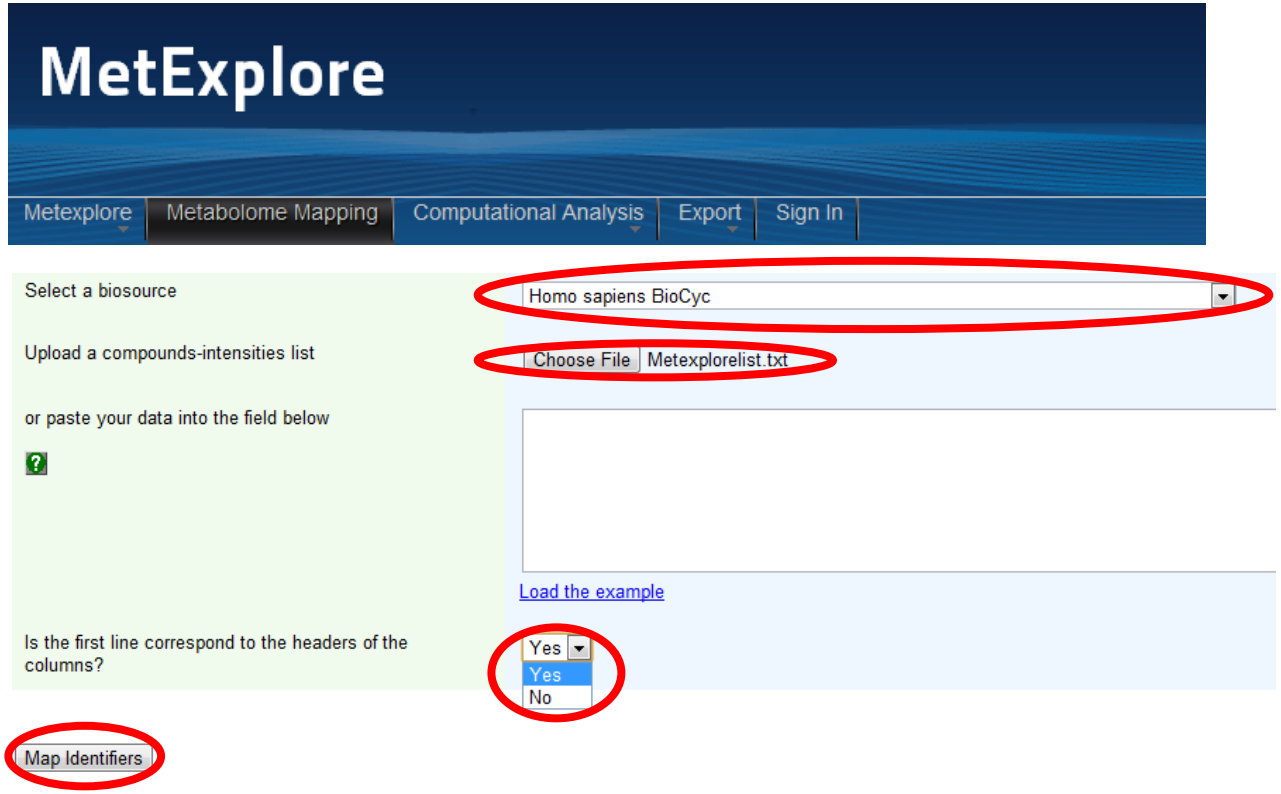
Export for analysis



Export data: Metexplore

Metexplore is a powerful website for mapping metabolomics data onto MetaCyc networks, which can be viewed with Cytoscape

This website is was developed in Toulouse, France. Contact Fabien Jourdan if you have any questions/problems/suggestions.



After going to the Metexplore website, select the biosource (organism), choose the “metexplorelist.txt” file (in the same folder as your Ideom file), select ‘Yes’ for the first line corresponding to headers, and ‘Map Identifiers’

NOTE: To start from the Metexplore home-page select “Metabolome Mapping” >> “Map Identifiers”

Additional options are available in the ‘Filter’ tabs at the top of the screen (e.g. filtering pathways or metabolites)

Select ‘Launch Cytoscape’ to view network

Ideom Tutorial: Part 2

Data Interpretation/Visualization Tutorial plan

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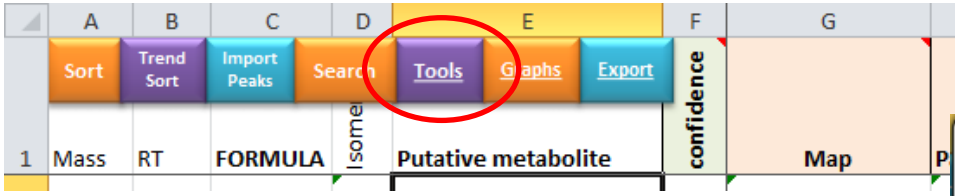
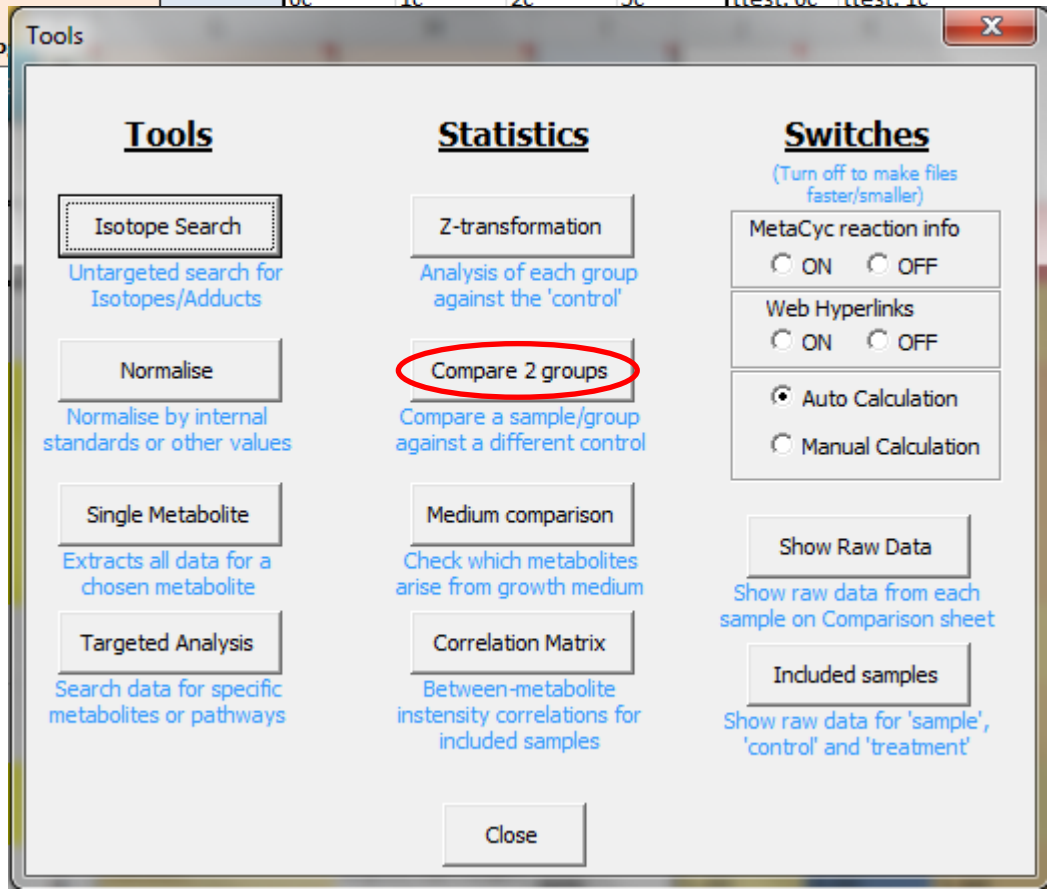
  - sort
  - filter
  - graph
- Checking data integrity “Is it a real difference”
- Metabolite Identification
- Exporting to external programs or websites
- Changing groups for comparison

Changing Comparison groups

Compare 2 Groups

Allows you to choose different control and treatment groups for a pairwise comparison





Samples to compare are chosen from alldata sheet

Results are written to columns at the right

- Relative intensity
- P-value (t-test)
- Mean intensities
- Optional:
  - Standard deviations
  - Fisher ratio

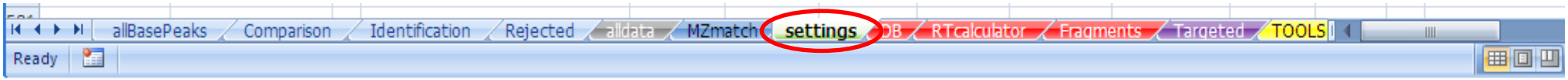
Changing Comparison groups

Compare all groups against another control group

You may change the control group and re-run the 'Comparison' (step 8)

You may also include/exclude other groups from the new 'Comparison'

1. Go to the 'Settings' sheet



2. In column H, change the set-type for each group

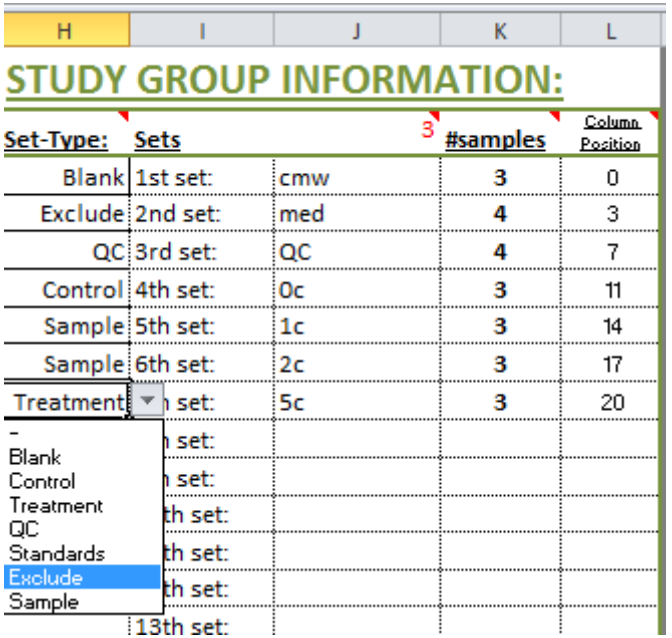
You should always assign 1 'control' group

NB: you cannot assign more than 1 'control' group

Assign a 'treatment' group for two-group comparisons/exports

All other included groups should be assigned as 'sample'

'Exclude' groups that you don't wish to compare



Click step 8 'Compare all sets' to re-calculate the comparison sheet based on the new group settings

Caution: if you choose 'yes' to including all base peaks it will take much longer

Part 3

Additional features of IDEOM

Additional Features

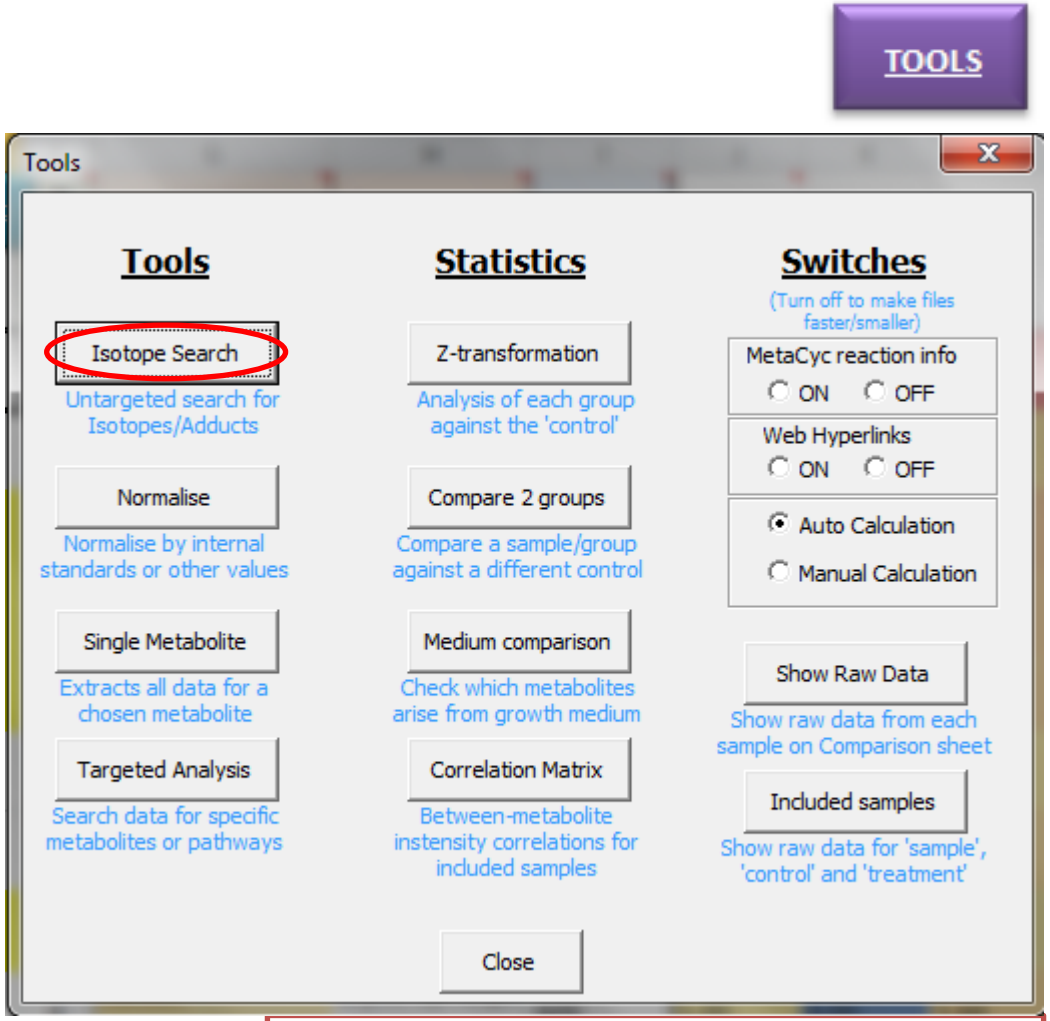
New and additional IDEOM features

- **Further Data Analysis**
  - **Isotope search** (for stable-isotope tracing)
  - Single metabolite data extraction
  - Medium comparison
  - Heat maps in IDEOM
- **Normalisation**
  - Fully labelled internal standards
  - Signal based (eg. TIC)
  - User-defined
- **Targeted Analysis**
  - Standards (3 mixtures)
    - Show all charts
  - Calibration
  - Quantification
  - Pathway profile
- **Reporting**
  - Metabolights export
- **MSMS annotation**
- **General IDEOM tools**
  - Annotate DB
  - Import experimental methods
  - Import data from old IDEOM file
- **General R Scripts**
  - TIC checker
  - Get all chromatograms
  - Filter peakml file (create pdf)
  - XCMS processing
- **Excel functions**
  - Function formulas
  - Mass names
- **New applications**
  - GCMS (low res)

Additional Features

Further Data Analysis

- **Isotope search** (for stable-isotope tracing)
  - Untargeted search for all possible isotopomers of a list of metabolites (or unidentified features)
  - Supports:
    - ^{13}C
    - ^{15}N
    - ^{18}O
    - ^{2}H
    - User-defined mass difference
  - User manually selects the samples or groups of samples from mzMatch sheet (imported peaks)
- Export menu allows **targeted isotope** search in raw data through mzMatch.R

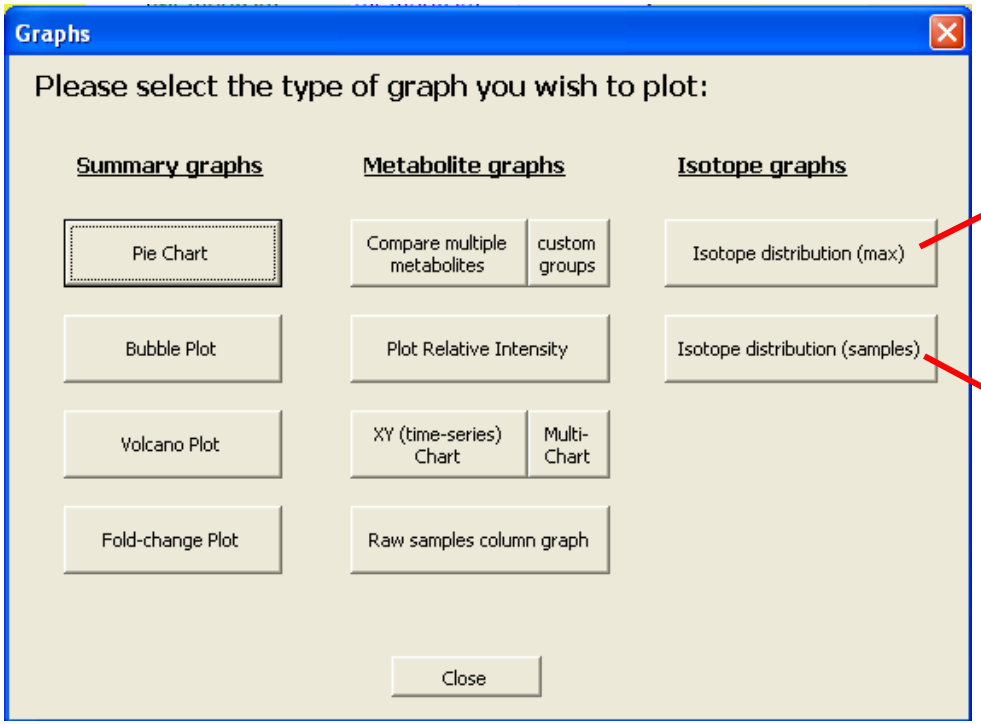


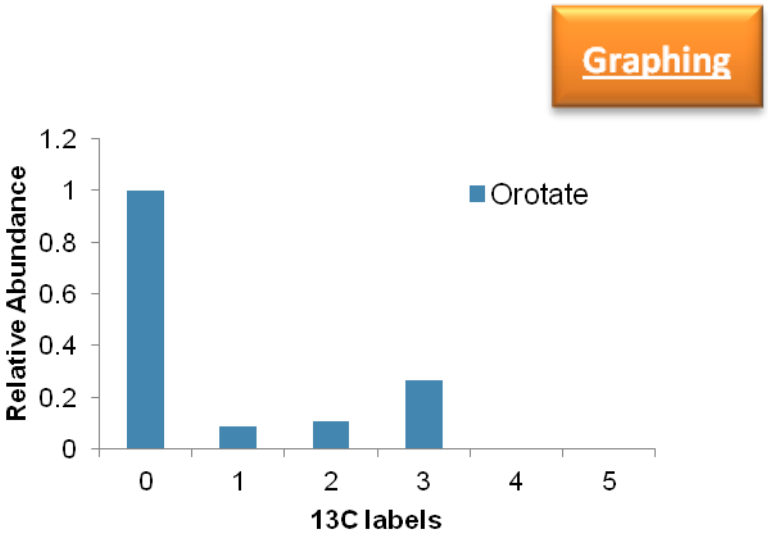
The data in the workshop doesn't contain isotope tracers, but you can still search for the natural isotopomers.

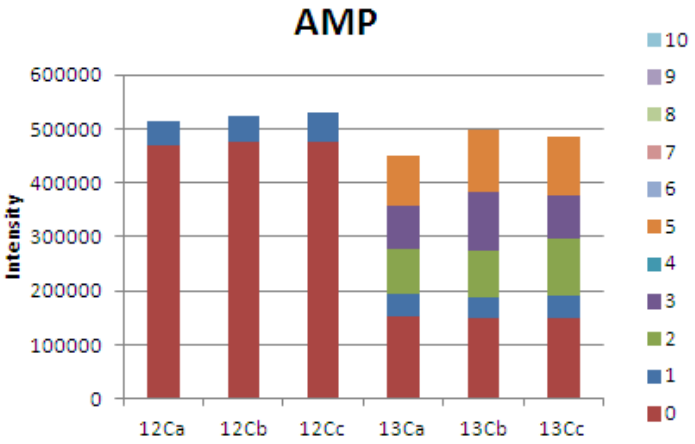
Additional Features

Further Data Analysis

- **Isotope graphs** (for stable-isotope tracing)
  - Specific plots to view labelled isotope patterns





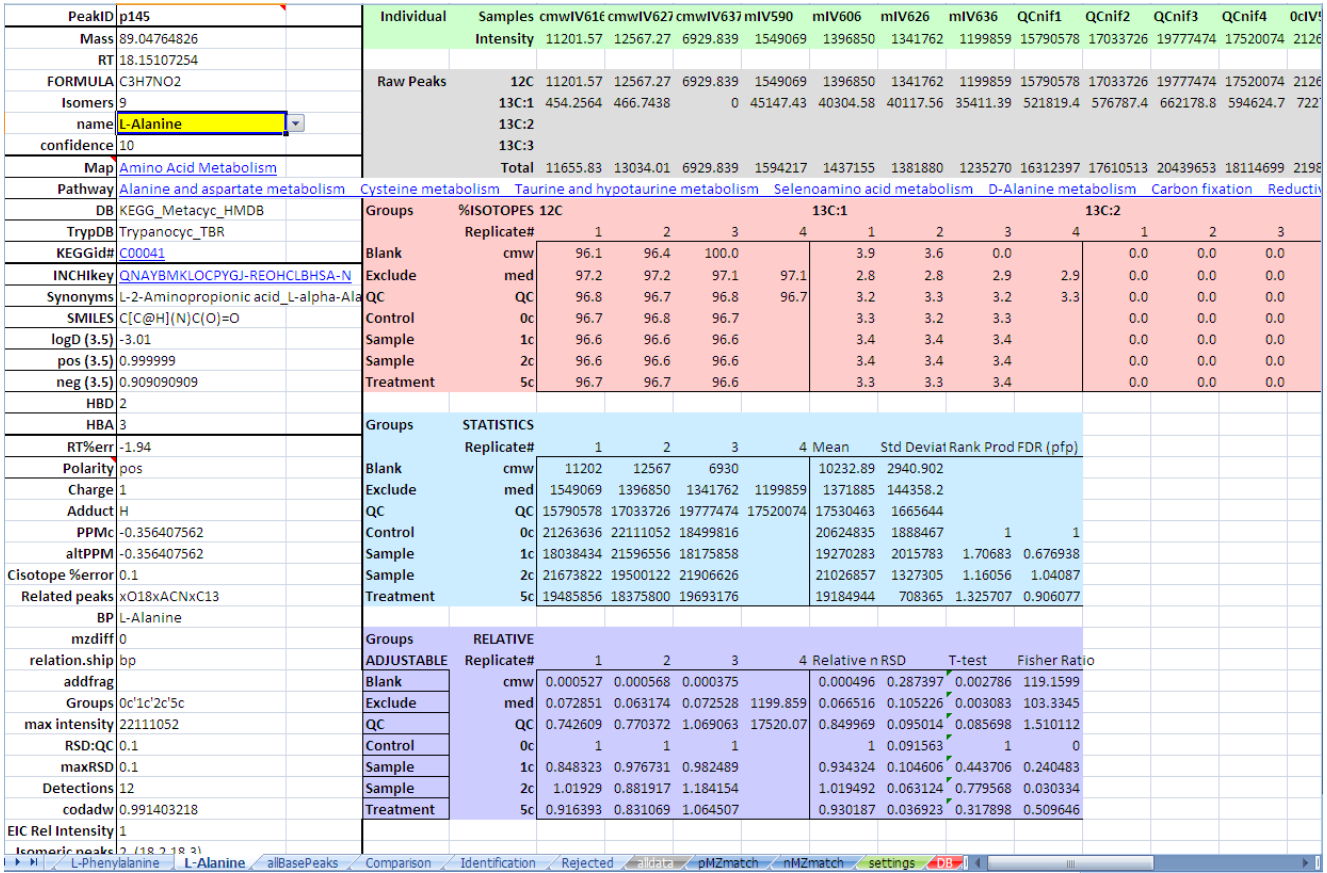


Additional Features

Further Data Analysis

TOOLS

- **Single metabolite** data extraction
  - Extracts data for chosen metabolite onto a fresh sheet for you to edit/analyse/graph/etc



PeakID	p145
Mass	89.04764826
RT	18.15107254
FORMULA	C3H7NO2
Isomers	9
name	L-Alanine
confidence	10
Map	Amino Acid Metabolism
Pathway	Alanine and aspartate metabolism
DB	KEGG_Metacyc_HMDB
TrypDB	Trypanocyc_TBR
KEGGid#	C00041
INCHIkey	QNAYBMKLOCPYGJ-REOHCLBHSA-N
Synonyms	L-2-Aminopropionic acid_L-alpha-Ala
SMILES	C[C@H](N)C(O)=O
logD (3.5)	-3.01
pos (3.5)	0.999999
neg (3.5)	0.909090909
HBD	2
HBA	3
RT%err	-1.94
Polarity	pos
Charge	1
Adduct	H
PPMc	-0.356407562
altPPM	-0.356407562
Cisotope %error	0.1
Related peaks	xO18xACNxC13
BP	L-Alanine
mzdiff	0
relation.ship	bp
addfrag	
Groups	0c'1c'2c'5c
max intensity	22111052
RSD:QC	0.1
maxRSD	0.1
Detections	12
codadw	0.991403218
EIC Rel Intensity	1
Isomeric peaks	2 (18.2 18.3)

Individual	Samples	cmwIV61(	cmwIV627	cmwIV637	mIV590	mIV606	mIV626	mIV636	QCnif1	QCnif2	QCnif3	QCnif4	0cIV
	Intensity	11201.57	12567.27	6929.839	1549069	1396850	1341762	1199859	15790578	17033726	19777474	17520074	2126
Raw Peaks	12C	11201.57	12567.27	6929.839	1549069	1396850	1341762	1199859	15790578	17033726	19777474	17520074	2126
	13C:1	454.2564	466.7438	0	45147.43	40304.58	40117.56	35411.39	521819.4	576787.4	662178.8	594624.7	722
	13C:2												
	13C:3												
	Total	11655.83	13034.01	6929.839	1594217	1437155	1381880	1235270	16312397	17610513	20439653	18114699	2198

Cysteine metabolism | Taurine and hypotaurine metabolism | Selenoamino acid metabolism | D-Alanine metabolism | Carbon fixation | Reductiv

Groups	%ISOTOPES	12C				13C:1				13C:2		
	Replicate#	1	2	3	4	1	2	3	4	1	2	3
Blank	cmw	96.1	96.4	100.0		3.9	3.6	0.0		0.0	0.0	0.0
Exclude	med	97.2	97.2	97.1	97.1	2.8	2.8	2.9	2.9	0.0	0.0	0.0
QC	QC	96.8	96.7	96.8	96.7	3.2	3.3	3.2	3.3	0.0	0.0	0.0
Control	0c	96.7	96.8	96.7		3.3	3.2	3.3		0.0	0.0	0.0
Sample	1c	96.6	96.6	96.6		3.4	3.4	3.4		0.0	0.0	0.0
Sample	2c	96.6	96.6	96.6		3.4	3.4	3.4		0.0	0.0	0.0
Treatment	5c	96.7	96.7	96.6		3.3	3.3	3.4		0.0	0.0	0.0

Groups	STATISTICS								
	Replicate#	1	2	3	4	Mean	Std Deviat	Rank Prod	FDR (pfp)
Blank	cmw	11202	12567	6930		10232.89	2940.902		
Exclude	med	1549069	1396850	1341762	1199859	1371885	144358.2		
QC	QC	15790578	17033726	19777474	17520074	17530463	1665644		
Control	0c	21263636	22111052	18499816		20624835	1888467	1	1
Sample	1c	18038434	21596556	18175858		19270283	2015783	1.70683	0.676938
Sample	2c	21673822	19500122	21906626		21026857	1327305	1.16056	1.04087
Treatment	5c	19485856	18375800	19693176		19184944	708365	1.325707	0.906077

Groups	RELATIVE								
ADJUSTABLE	Replicate#	1	2	3	4	Relative n	RSD	T-test	Fisher Ratio
Blank	cmw	0.000527	0.000568	0.000375		0.000496	0.287397	0.002786	119.1599
Exclude	med	0.072851	0.063174	0.072528	1199.859	0.066516	0.105226	0.003083	103.3345
QC	QC	0.742609	0.770372	1.069063	17520.07	0.849969	0.095014	0.085698	1.510112
Control	0c	1	1	1		1	0.091563	1	0
Sample	1c	0.848323	0.976731	0.982489		0.934324	0.104606	0.443706	0.240483
Sample	2c	1.01929	0.881917	1.184154		1.019492	0.063124	0.779568	0.030334
Treatment	5c	0.916393	0.831069	1.064507		0.930187	0.036923	0.317898	0.509646

L-Phenylalanine | L-Alanine | allBasePeaks | Comparison | Identification | Rejected | pMZmatch | nMZmatch | settings | DB

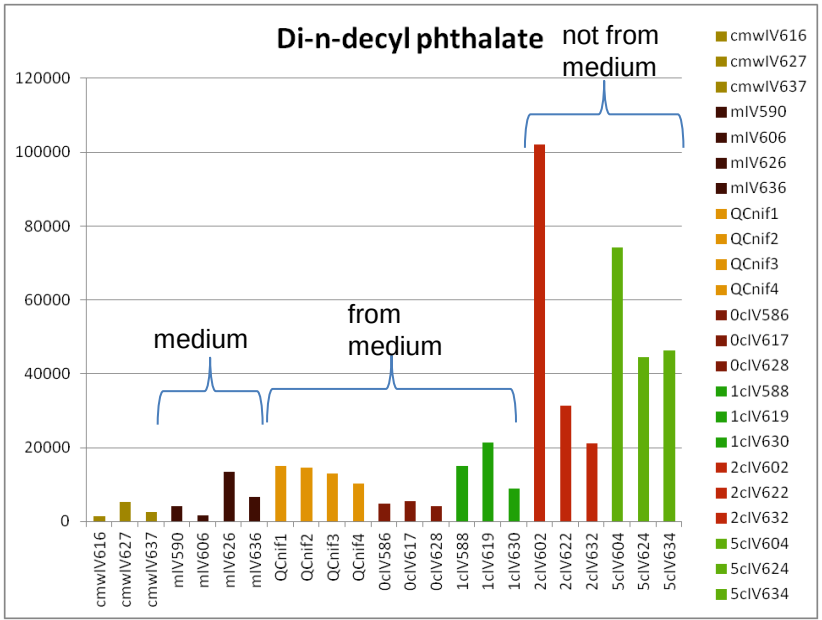
Additional Features

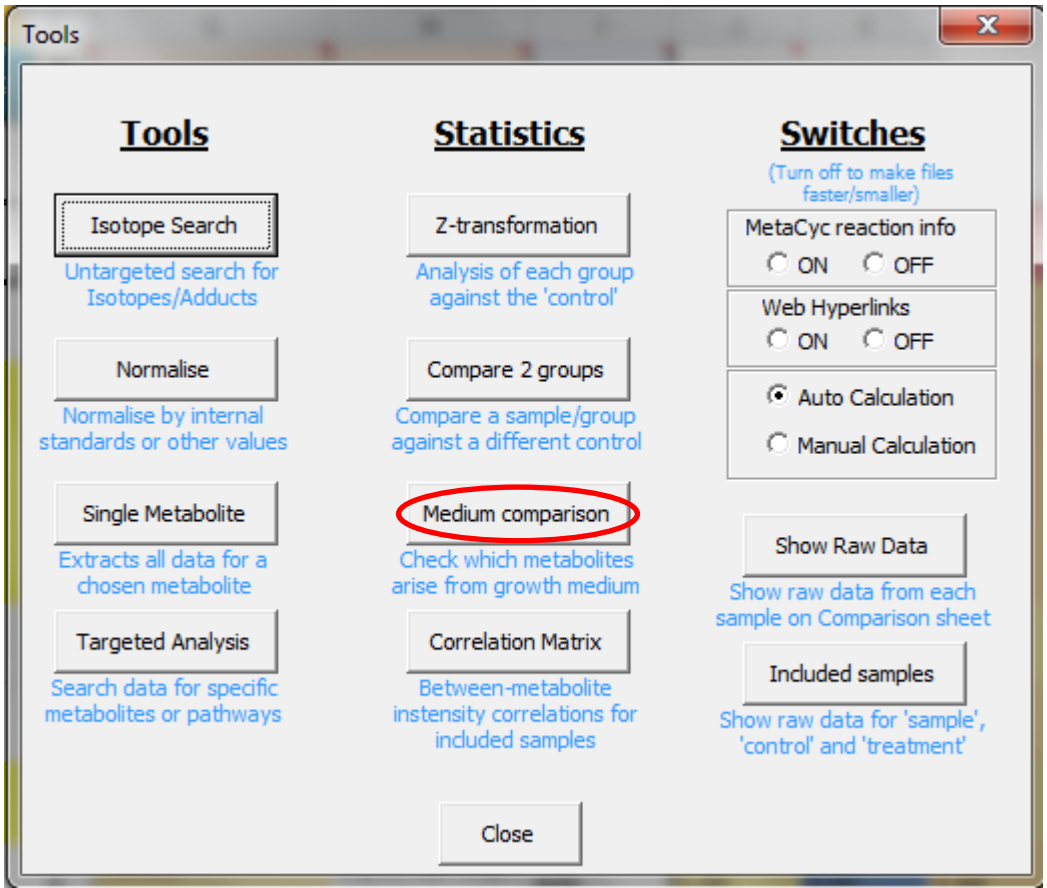
Further Data Analysis

- **Medium Comparison**

Mostly the same as the ‘Compare 2 groups’ function

Additional feature annotates if lowest sample intensity is below the highest medium intensity



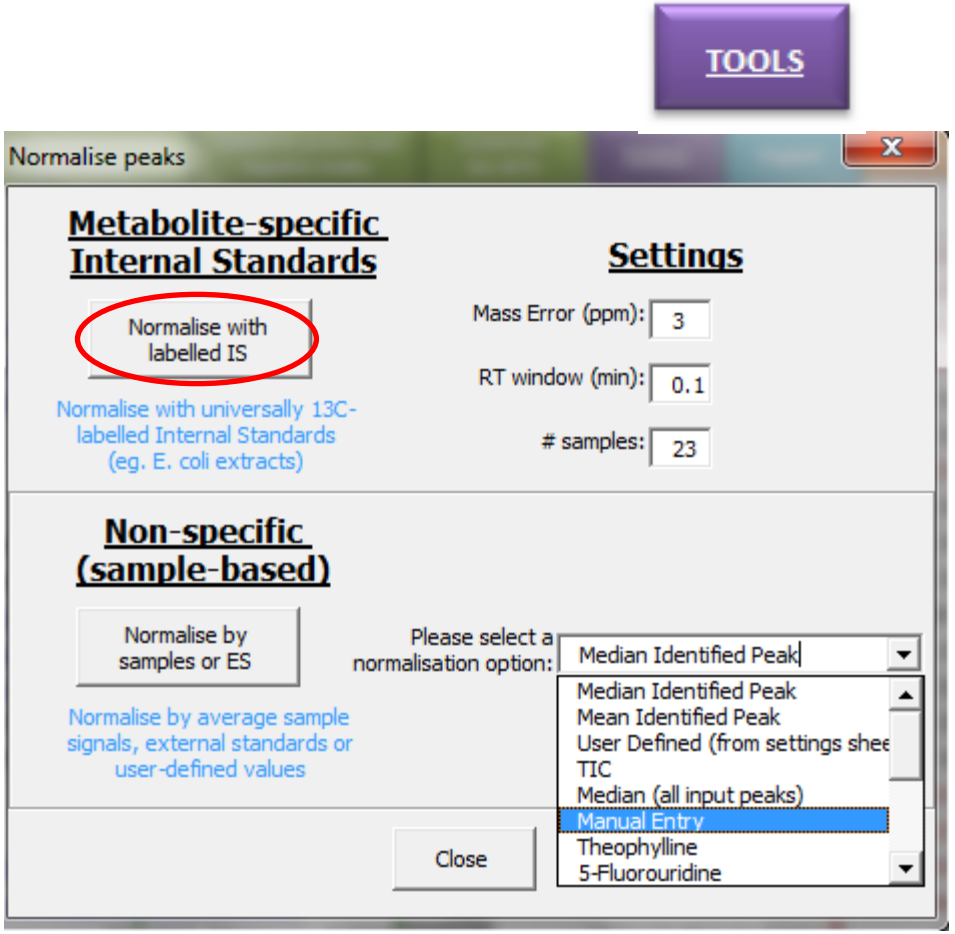


Additional Features

Normalisation:

Fully labelled internal standards (IS)

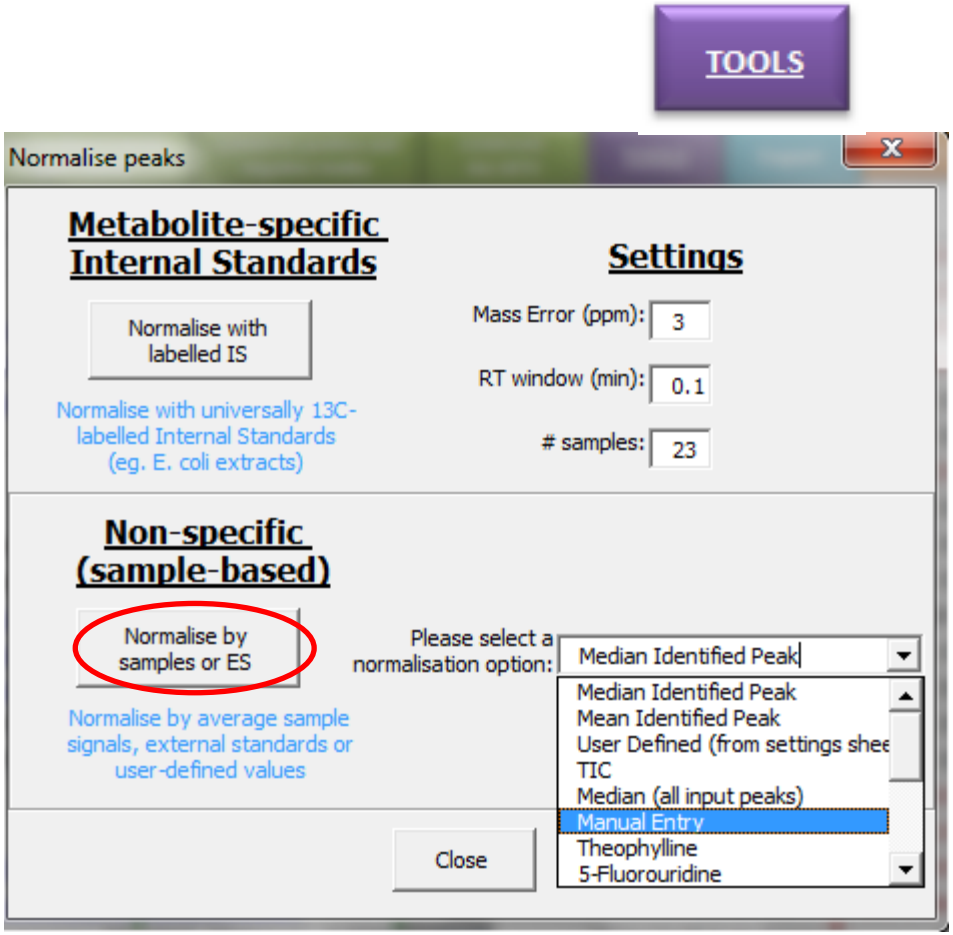
- Mass error and RT windows define search parameters to locate fully labelled internal standards (NB: features must have a formula in column C)
- All peak intensities are divided by the corresponding IS intensity for each sample
- IS intensities are shown on the alldata sheet to allow you to check that they are correctly identified
- Features without a detected IS are normalised by the nearest two IS based on retention time
- Users may enter a specific IS to be used (or skip the normalisation) for each metabolite



The data in the workshop doesn't contain fully labelled internal standards.

Additional Features

Normalisation:



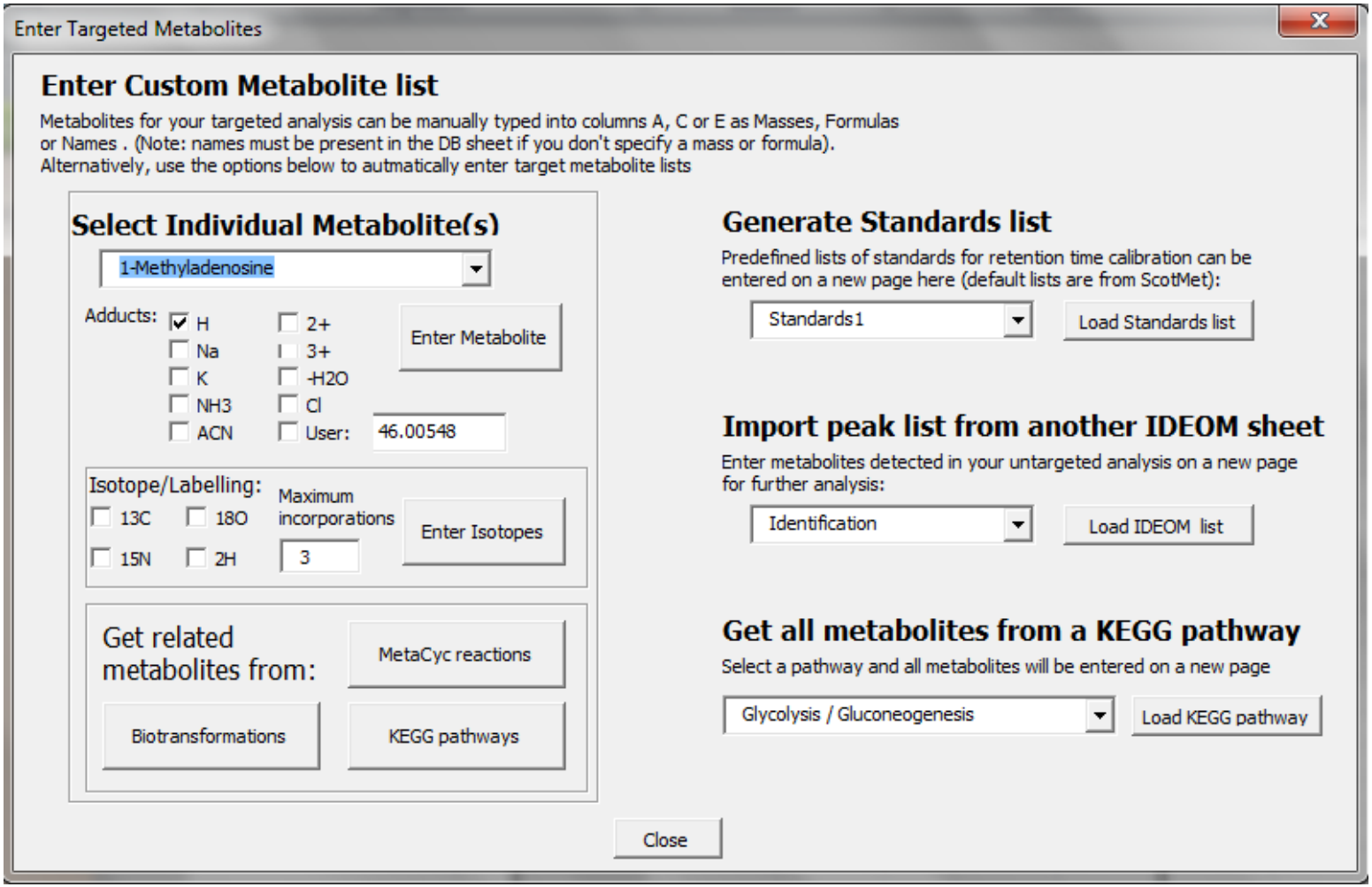
Non-specific normalisation
NB: This normalisation is not recommended for LCMS data except in unavoidable, well-defined circumstances

- Median/mean identified peak, only uses metabolites from the identification sheet (i.e. less bias from noise)
- TIC and median uses all input peaks
- User-defined divides intensities by the values in column R of the Settings sheet (e.g. enter cell counts or protein content)
- Manual Entry allows you to enter values for each sample on the fly
- Further normalisation options are based on the external standards present in the Settings sheet (columns U:AD)

Additional Features

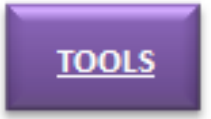
Targeted Analysis

TOOLS



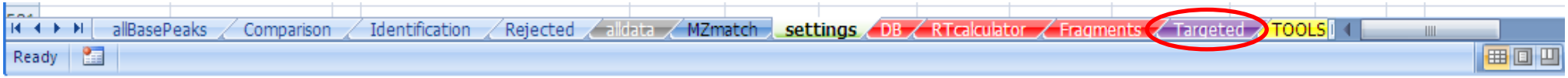
Additional Features

Targeted Analysis

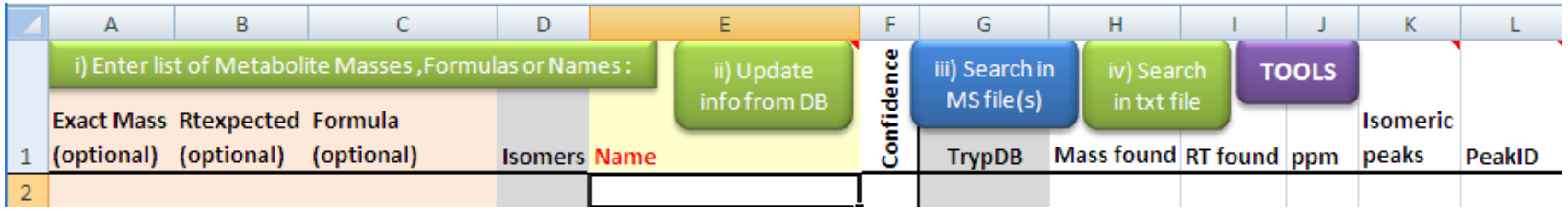


Detect and Measure specific metabolites

Select the 'Targeted' sheet, or choose 'Targeted' from the TOOLS menu on the Settings sheet



Follow steps (i) to (iv) (skip step 3 if using data already uploaded to IDEOM)



i) Metabolites can be entered directly as Exact mass, Formula or Name (in columns A, C or E)

Button (i) allows import of:

- individual metabolites from the DB
- Standards lists used by Scotmet for analysis of standard retention times
- Metabolite lists from results pages in IDEOM

Additional Features

Targeted Analysis

ii) Update info from DB

If metabolites are present in the database (with correct spelling), the formula, RT and mass will be obtained automatically.

If any metabolites are not in the database they will appear in red and you are required to enter the formula and/or exact mass (and retention time, optional).

(NOTE: Manually entered masses will over-ride existing masses in the database)

iii) Search in MS file(s) [optional] if looking in raw data (.raw, .mzXML or .peakML)

This step uses mzMatch to extract 'peaks' into peakML files, which is very greedy and results in a lot of noise. This approach avoids the problem of XCMS not detecting metabolites with poor peak shapes, but means you need to manually check the peaks.

The output from this step includes peakml file, txt files (for step iv) and pdf with peaks

iv) Search in txt file

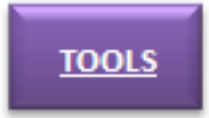
Either use the mzMatch_output.txt file from step (iii) or any data sheet within IDEOM (e.g. alldata or mzMatch)

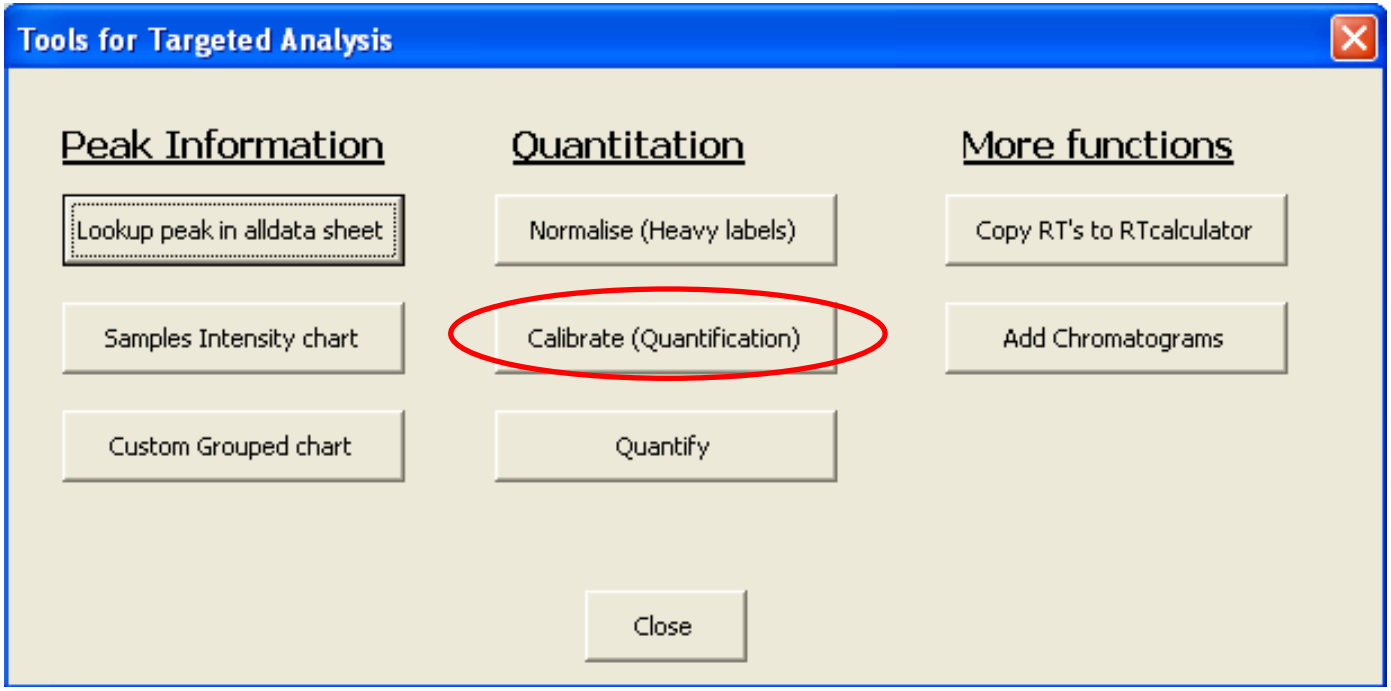
Any text file in the same format as mzMatch output text files should work

Additional Features

Targeted Analysis

- Tools for analysis on targeted sheet





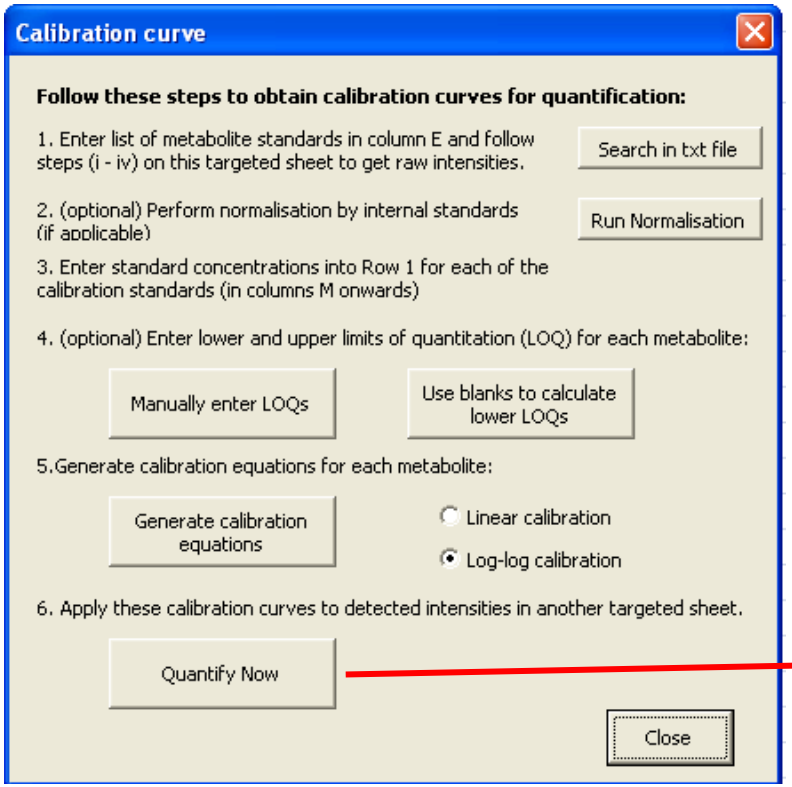
Additional Features

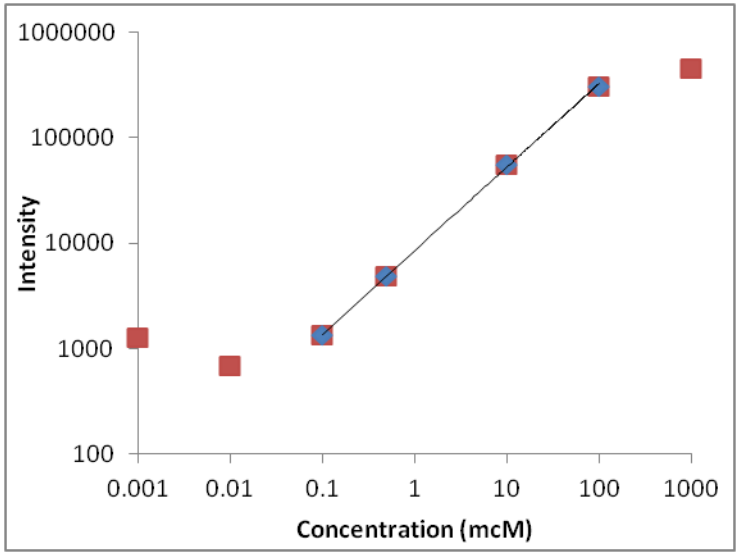
Targeted Analysis

TOOLS

This function is still undergoing testing. No data is available for the workshop.

- **Calibration**
  - Generates calibration curves (linear or log-log) and equations for quantification





Quantification (UNDER DEVELOPMENT)

- Quantification of list of targeted metabolites based on calibration standards

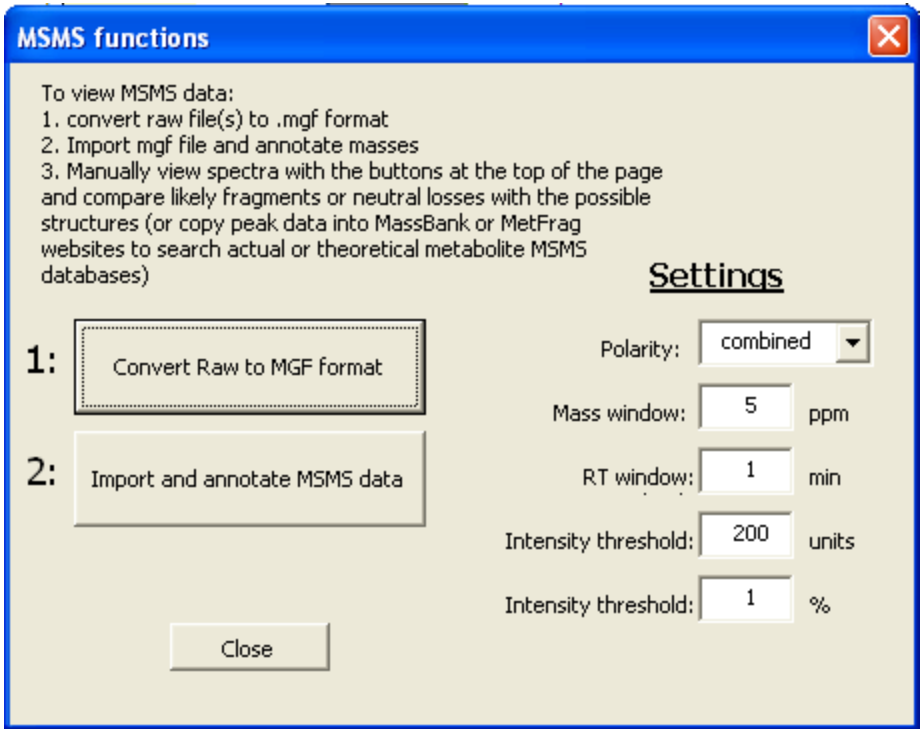
Additional Features

MSMS analysis

- Import and annotate MSMS data
  - Convert Raw to mgf file format (with msconvert through R)
  - Imports mgf file into fresh page in IDEOM
  - Matches precursor ions with peaks in IDEOM data from untargeted analysis
  - Lists fragment masses and neutral losses
  - Lists likely formula for each fragment and neutral loss (if formula in fragment list)
  - Plots MSMS spectra with peak annotation where available
  - Links to Massbank and Metfrag for individual verification

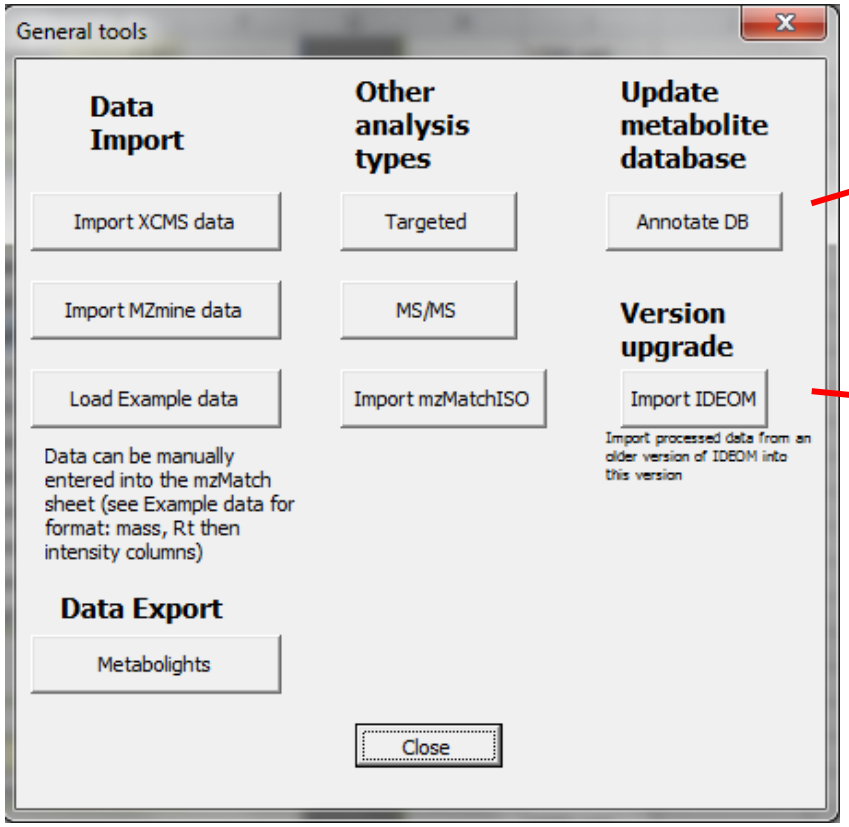
MSMS

No MSMS data is available for the workshop.



Additional Features

General IDEOM tools (settings sheet): TOOLS

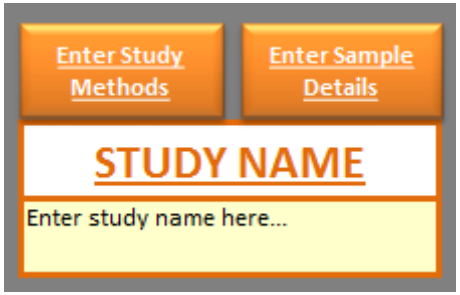


Annotate DB

- Adds annotations to the database for new organisms/studies
- This can also be used as a generic matching algorithm between IDEOM and other Excel/txt/csv files

Import IDEOM

- Allows upgrade of data from old IDEOM files (to allow access to new automated functions).



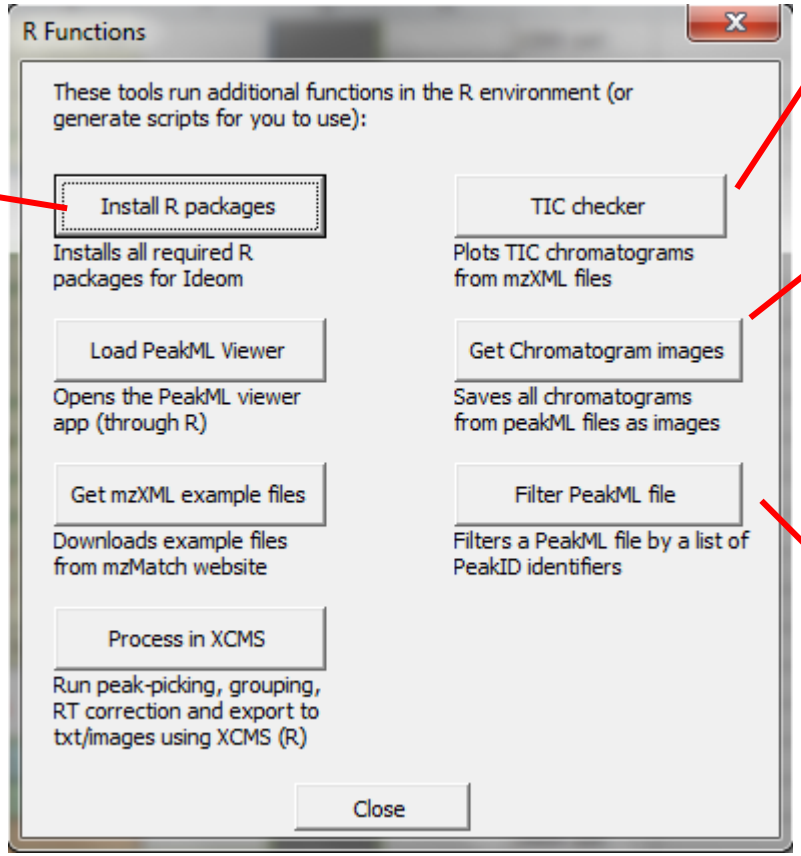
Enter Study Methods / Sample Details

- Experimental metadata can be stored here
- These sheets are only for recording purposes and don't affect Ideom processing.

Additional Features

Other R scripts (settings sheet): R script menu

Install R packages
For first-time installation and subsequent updating of XCMS, mzMatch, etc



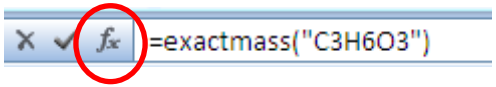
TIC checker
- R script to generate pdf files of overlaid TICs from raw mzXML files (for manual QC check)
- Usually best to group replicates before running this

Get Chromatogram images
- R script to extract all chromatogram images from a peakml and saves them as small png files
- Useful if you cannot use the normal 'add chromatograms' feature (e.g. If the peakml file is too big to load on your computer you can get the chromatogram images using another machine)

Filter PeakML file
- Creates a pdf file to allow viewing of all peaks in a peakML file filtered by a list of metabolites
- NB: filtering is based on metabolite formula (i.e. masses). No Rt is taken into account so multiple isomeric peaks may appear for each mass.

Additional Features

Excel functions



‘User-Defined Formulas’ can be used in any Excel cell

fx =**ExactMass(**formula, Clabels,Nlabels,Olabels,Dlabels)

- Calculates the exact mass from a formula
- Supports heavy isotope labelled metabolites
- Only common atoms supported: C,H,N,O,S,P,Cl,F,I,Br,Se

fx =**ppmcalc**(mass,theoreticalmass, formula)

- Calculates the mass difference (in ppm) between a given mass and a theoretical formula or mass

fx =**formulaMATCH**(mass, ppm, masslist, formulalist)

- Finds a matching formula in a database of ascending masses (e.g. the DB sheet).
- If two masses either side of the search mass are within the allowable ppm error the answer is *italicised*

fx =**Formulavalid**(formula)

- Checks the validity of a proposed chemical formula against 5 of Kind & Fiehn's 7 golden rules

fx =**IsotopeAbundance**(formula, atom)

- Calculates the theoretical natural isotope abundance of a specified atom in a given formula (relative to basepeak)

fx =**Pos**(pH, cation, pka1,pka2,pka3,...) & **Neg**

- Calculates the average number of charges on a molecule at a given pH

fx =**FormulaReactor**(formula1,formula2,formulaloss)

- Adds the atoms of two formulas to give the formula (e.g. for adduct prediction)
- Also allow subtraction of one formula from another (e.g. for fragment prediction)

fx =**proton** & **Naadduct** & **Kadduct** & **Cladduct**

- Returns the exact mass (these are Excel names)

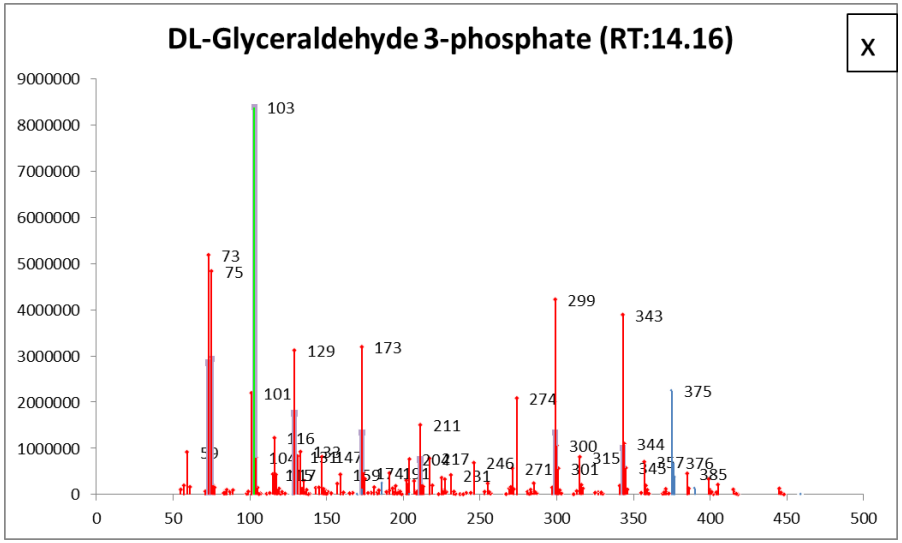
Additional Features

GCMS processing (low resolution)

MS mode:	Mass Resolution
GCMS (EI)	Low

- Automated identification of metabolites in standards database
  - Identified by quant ion and Rt
  - Spectral match score provided (but not essential for ID)
  - Qual ions #1 & #2 relative intensity provided (but not essential for ID)

- Links from individual metabolites (spectra) to Golm metabolite DB for identification of unknowns



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