Renishaw plc
Spectroscopy Products Division
New Mills, Wotton-under-Edge,
Gloucestershire GL12 8JR
United Kingdom

Tel +44 (0) 1453 524524
Email raman@renishaw.com

www.renishaw.com

RENISHAW
apply innovation™

TM023 – Micro-plate Mapping WiRE™ 5

This document aims to show the WiRE™ 5 user how to set up and use Micro-plate mapping. It assumes the Micro-plate mapping software has been installed correctly, and the presence of a Micro-plate mapping stage insert.

Micro-plate definition

1. Clip the stage insert into place, and slot the Micro-plate into it, noting the position of well A1 (e.g. bottom right or top left).
2. Open the Micro-plate mapping program.
   Start…..Programs….Renishaw WiRE 5.0…Micro-plate Mapping

If a new Micro-plate is to be added, go to 3a. If the Micro-plate has been previously defined, use 3b.

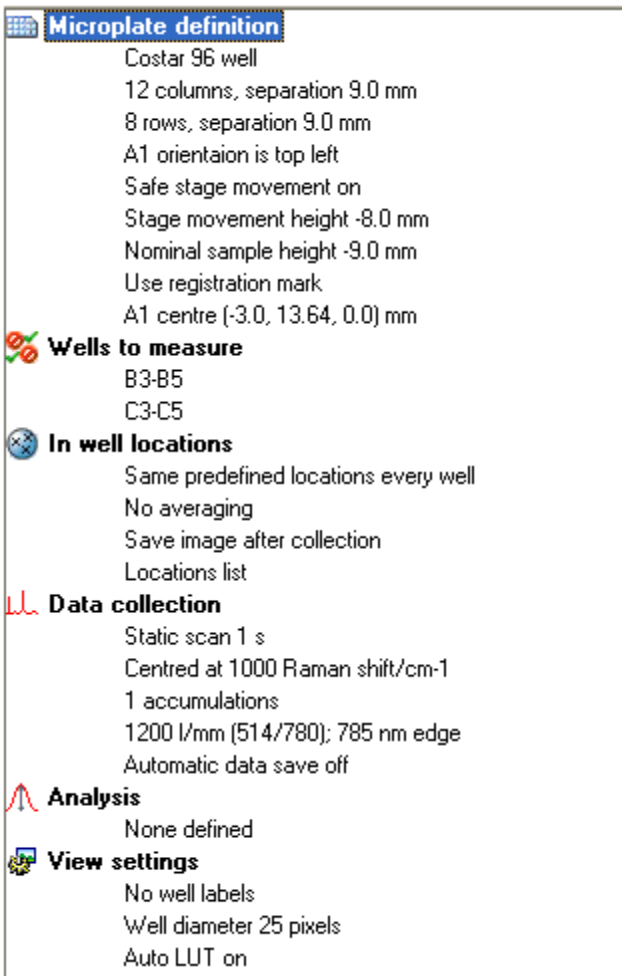

Figure 1 Micro-plate mapping menu

3a. Right click on Micro-plate *definition*, in the measurement tree on the left-hand side of the screen (Figure 1), and select *Configure*. Click on *Add*, and enter a name for the Micro-plate. Enter the number of rows and columns on the Micro-plate and the distances between wells. Select the correct orientation of the Micro-plate at the bottom of the Micro-plate *definition* window, as determined in 1.

3b. Select the required Micro-plate from the drop-down menu at the bottom of the Micro-plate *definition* window.

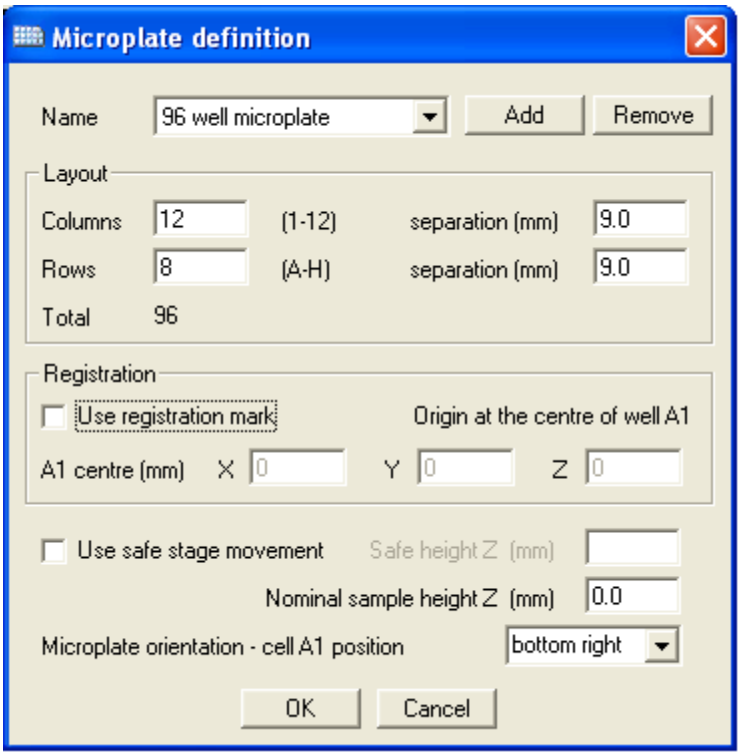

Figure 2 Defining a new Micro-plate

Setting a registration mark

A registration mark is a physical reference point on the plate defined by the user. This allows the system to find other positions on the plate relative to the mark. The registration point may be selected as the centre of well A1, but it can be difficult to find this with a high degree of accuracy unless the centre is marked. Instead, a feature on the plate, such as part of a letter or number can be used as the registration mark. This must then be defined relative to the centre of well A1.

Using bottom of A1 centre as registration mark

This is the fastest and most convenient method for setting the registration. The advantages of using this method are that it minimises additional steps defining the reference relative to A1 centre; and the reference Z value is very close to the well bottom. The only disadvantage is well A1 must be empty.

1. Ensure the bottom of well A1 has a suitable mark close to the centre

2. Open the video and focus the crosshair on the A1 central feature with the objective to be used for data collection

3. Right click on the video window, and select *Set origin*, this centres the video axis on this point

4. Select *register* from the same menu, and click *OK*.

Using separate reference mark defined relative to A1 centre (XY)

This method allows well A1 to be used for sample analysis, but does require additional information to ensure that the A1 well centre is known well, relative to the user defined reference.

1. Open the video and focus the crosshair on the desired feature with the objective to be used for data collection (Figure 3)

2. Right click on the video window, and select *Set origin*, this centres the video axis on this point

3. Select *register* from the same menu, and click *OK*.

4. Focus on the top edge (in the y direction) of the circular rim of well A1, and record the x and y positions, which will be the positions relative to the registration mark

5. Focus on the bottom edge of well A1. The x value should be the same, and the y different (Figure 4)

6. Calculate the mid-point between the two y values. This gives the position of the centre of the well

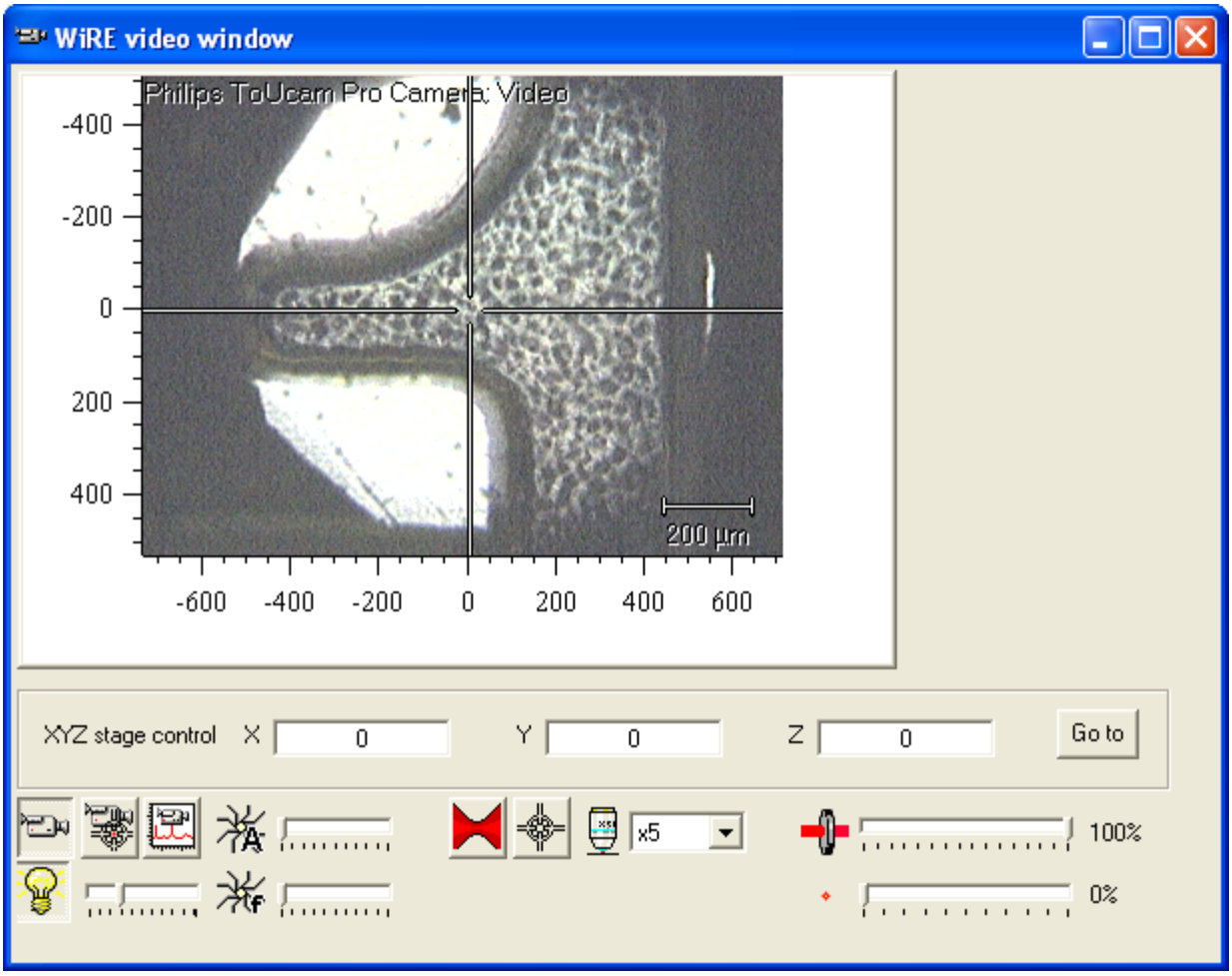

Figure 3 An example registration mark

7. Go back to Micro-plate *definition,* and select *Configure.* Check the *use registration mark* box, and enter the values of x and y obtained in '6' (Figure 5).

Note that the values must be entered in mm, while the stage operates in units of µm, so the values must be divided by 1000.

This procedure can be illustrated using the example below, based on Figure 4.

	x /µm	y /µm
Registration mark	0	0
Top of well A1	68	2358
Bottom of well A1	68	10906
Centre of well A1	68	6727

	x /mm	y /mm
Values to enter	0.068	6.727

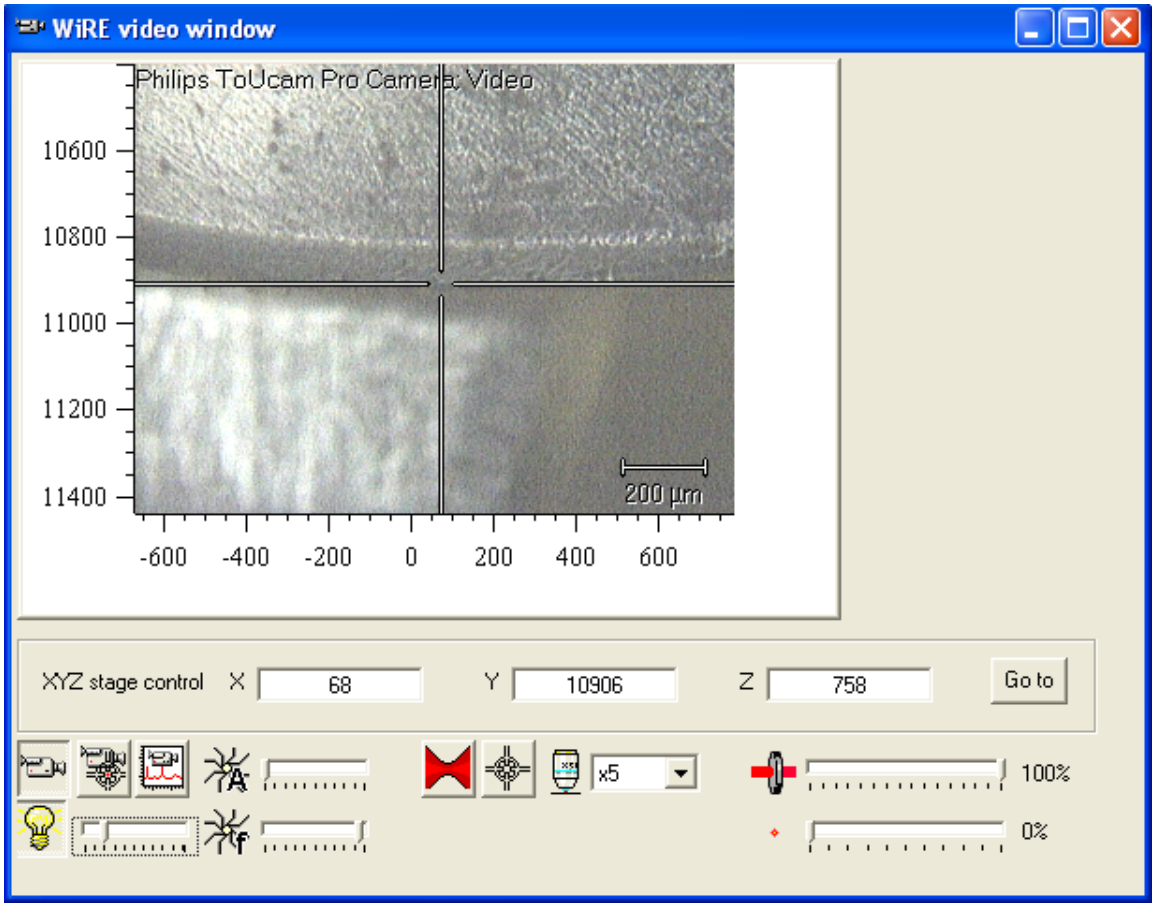

Figure 4 The bottom edge of well A1

Calculated registration settings

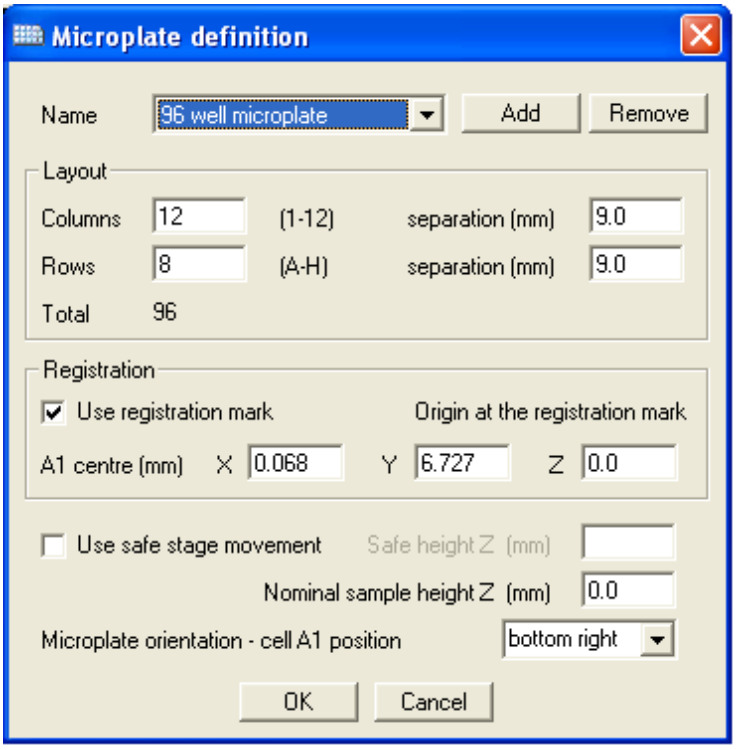

Figure 5 Registration mark settings

Z-axis settings

1. The option *Use safe stage movement* protects the objective against hitting the Micro-plate by moving the stage down (in the z direction) before moving it in x and y. The safe stage movement is only applied when using the *Go to* option on the video viewer (Figure 3), or on the *In well locations* window, for movements over 500 μm. This may be useful when short working distances are being used, but adds additional time to the measurement. At longer working distances, this is not required. If using this setting, check whether the value entered should be positive or negative. This depends upon the z axis set -up, and can be checked by moving the stage up or down with the joystick control, and noting the change in the z value in the video viewer window (Figure 3).

2. *The nominal sample height Z* setting allows the user to set a default height for the microscope stage for each well. This is useful if the samples are all at similar heights in the wells. If the height of the registration mark is significantly different to the heights of the samples, setting a nominal sample height avoids repetitive large movements in z to focus on each sample.

Note that both these values should be entered in units of mm.

Setting up the locations for spectral acquisition

1. Right click on *Wells to measure* (Figure 1), and select *Configure*. Check the boxes of the wells to measure. The column and row headings can be clicked on to select or deselect all the boxes in that row or column. **This mask overrides the *In well locations* menu, so that only wells selected here will be measured.**

   Masks can be exported or imported (as *.xml) from the file menu.

2. Right click on *In well locations* (Figure 1), and select *Configure*. One of three methods of defining locations can be selected (Figure 6).

   - *Define locations as part of data collection* allows the user to manually define the location in the well before each scan.
   - *Same predefined locations for every well* allows the user to acquire data at the same offset from well centre in each well. This is particularly useful if, for example, the wells contain accurately pipetted volumes of liquids.
   - *Independent predefined locations for each well* allows the user to select all the locations prior to data acquisition, but select different locations in each well. This is the most useful option if the wells do not contain similar samples. Once the locations have been defined, the system can be left to run automatically.

It is important to have the correct option selected before setting locations, as the locations may be lost if the option selected is altered later.

3. The video image check boxes allow the automatic capture of video images of the sample either before or after the data acquisition, or both. The latter image can be useful to check that no sample damage or modification has occurred. The video viewer can be loaded during data collection from the *View* menu, by selecting *Show video image*. Before or after shots of the sample can be viewed.

The procedure for defining In well locations is as follows:

1. Use the *Navigate* Micro-plate box in the *In well locations* window to select the desired well. This is done by selecting the well from the drop-down menus, and clicking *Go to well* / *Prev* / *Next.*

2. Focus on the sample in the well. This can be done using the control on the joystick for fine adjustments. For larger adjustments, enter the required z value in the *Z* field in the *locations for*… box, then click *Go to offset*. Use the joystick to select the desired location in the cell.

Note: The box will be named *locations for all wells* if the *Same predefined locations for every well* Option is selected, or *locations for well XX,* if the *Independent predefined locations for each well option is selected,* where XX is the currently selected well, e.g. B3. If the *Define locations as part of data collection* option is selected, this box will be greyed out, as in this mode the locations are selected immediately prior to each scan.

3. Name the location, and click *Add Location.* Locations can be edited or deleted using the *edit* or *delete* buttons.

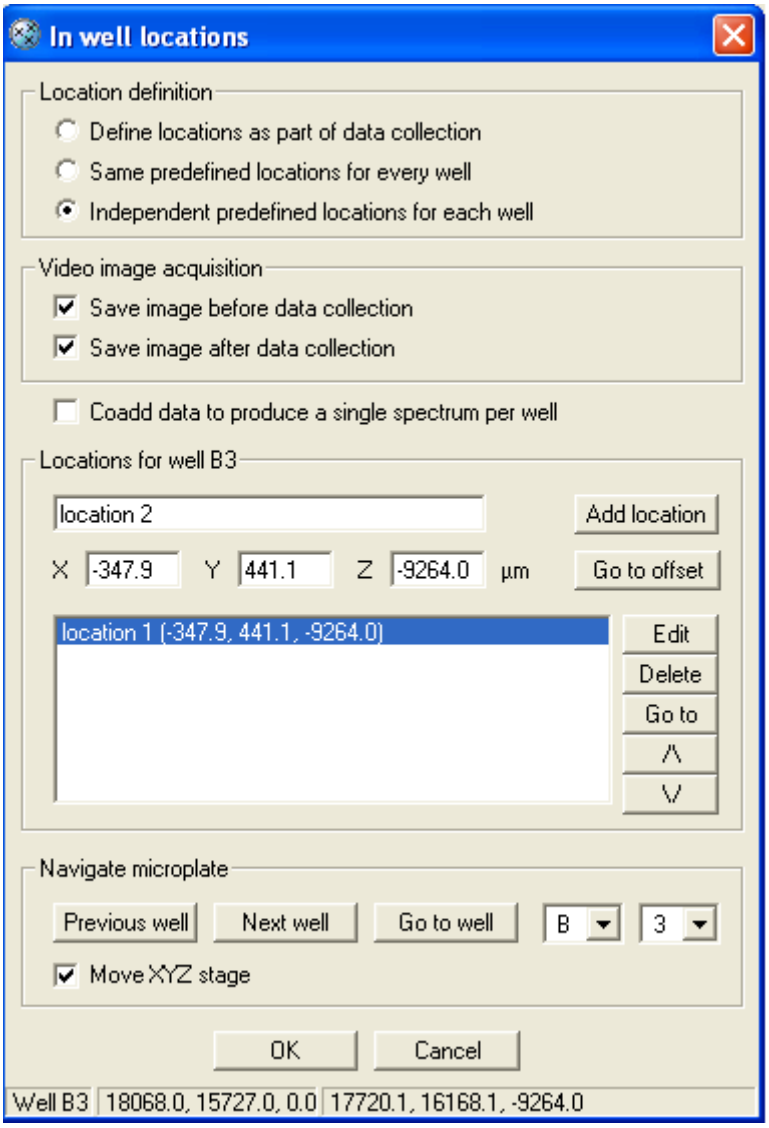

Figure 6 Defining the In well locations

Procedures for Spectral Acquisition

If the first method, *Define locations as part of data collection*, is used, the following procedure applies:

1. Click *OK* in the *In well locations* window. Select the scan conditions required by right clicking *Data collection* (Figure 1), then selecting *Configure*.

2. Start data collection using the run button  on the tool bar.

3. When prompted, focus the microscope on the desired location in the first well to be measured.

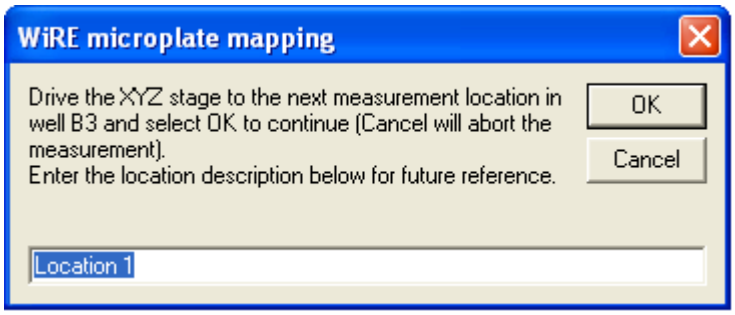

Figure 7 Prompt Screen

4. There will then be the option to select more locations in the same well, or to move on the next well. Continue until spectra have been obtained from all the desired locations in all the selected wells.

If the second method, *Same predefined locations for every well* is used, the following procedure applies:

1. Use the procedure described in the **Setting up the locations for spectral acquisition** section to define the In well locations. Repeat this for as many locations as required. These locations will be applied to all wells.

2. Select the scan conditions required by right clicking *Data collection* (Figure 1), then selecting *Configure*.

3. Start data collection using the run button  on the tool bar. The same locations will be scanned in all the selected wells.

If the third method, *Independent predefined locations for each well,* is selected, the following procedure applies:

1. Use the procedure described in the **Setting up the locations for spectral acquisition** section to define the In well locations. Repeat this for as many locations as required in the well.

2. Move to the next well, and repeat the process. Continue until all locations in all wells have been defined.

3. Select the desired scan conditions by right clicking *Data collection* (Figure 1), then selecting *Configure*.

4. Start data collection using the run button  on the tool bar. The locations will be scanned automatically, in turn.

Analysis

To display the data, the program opens a spectrum viewer window, and a Micro-plate diagram window. A video image window can be opened as described in **Setting up the locations for spectral acquisition**.

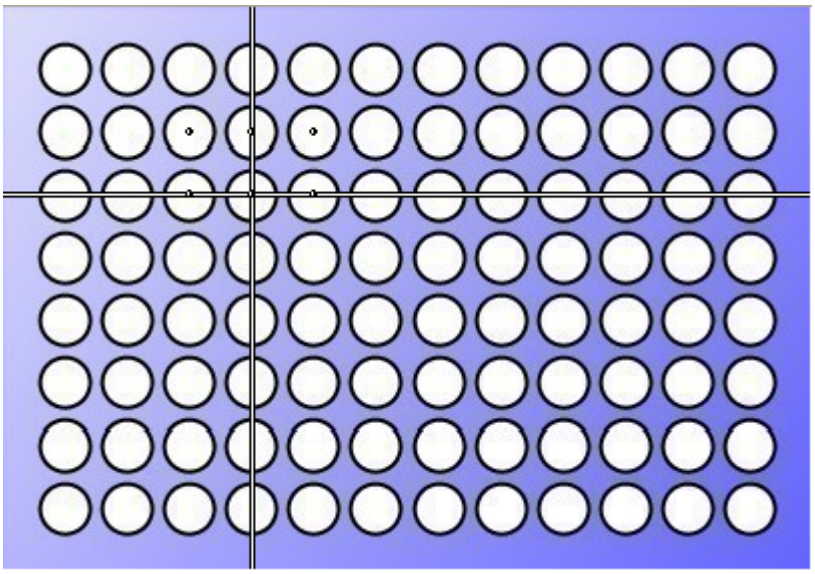

Figure 7 Micro-plate diagram

Clicking on a well in the Micro-plate brings up the spectra for that well. When multiple spectra are collected from a single well, these can be cycled through using the buttons on the right of the toolbar. 

The data is obtained as a multifile, which can be used to produce a map overlaid on the Micro-plate image. See the mapping training module for further information.

The data can also be exported to WiRE™ 3, by selecting *export to WiRE* in the *File* menu. Analysis such as baseline correction, curve fitting etc can then be applied in WiRE™ 3 if desired.

View settings

If the measurement is cleared (*Measurement……Clear……All or Analysis*) the view settings must be entered prior to data collection as this sets the well diameter in the image. The image label control is also controlled through the view settings. To observe the image labels the control must be on before data collection.

Multiple data sets can be saved as individual spectra if required, with the labelling nomenclature user definable. To export data use *File…..Export to singles*, and chose the file name and data type.

The labelling nomenclature is set as default to; 1 to 12 (columns), and A to H (rows). Actual values will depend on the well size.

This nomenclature can be changed in *File…Options*.

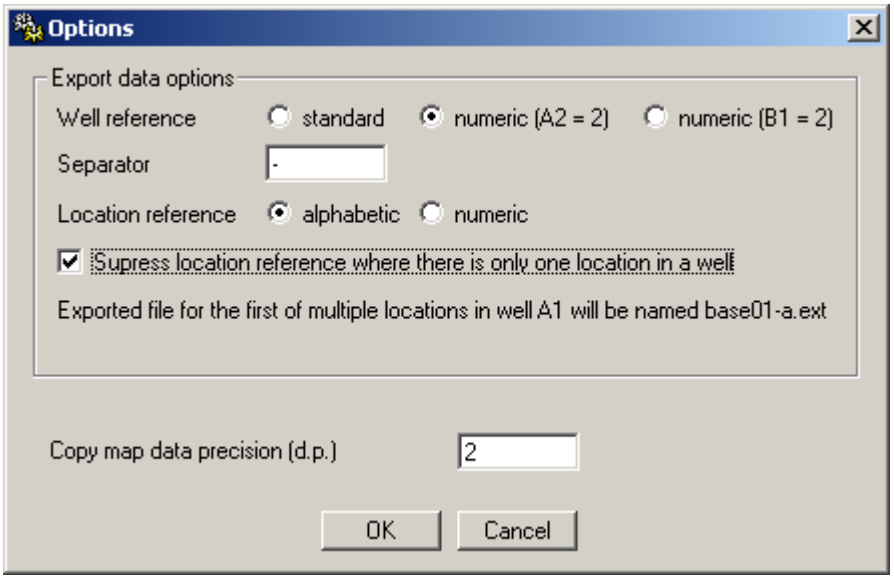

Figure 8. Labelling nomenclature options

So, the exported data can been given specific values depending on the well, and the number within the well.

Figure 8 shows the options available.

The W*ell reference* refers to the value given to a specific well, the options for this are;

- Standard – where the letter and number are given for each well
- Numeric (A2 = 2) – where each well is given a number only, ordered in rows (horizontally)
- Numeric (B1 = 2) – where each well is given a number only, ordered in columns (vertically)

The *Separator* allows the user to define the separation in filename between the *Well reference* and *Location reference*.

The *Location reference* refers to the value given to a specific location within a well, the options for this are;

- Alphabetic – where a lowercase letter is given as a suffix for each well
- Numeric – where a number is given as a suffix for each well

These options allow data to be stored in other standard formats, if desired.