Method Sheet 107

Plotting column charts with error bars using Microsoft Excel

Overview

This method sheet explains how to plot a bar or column chart with error bars using Microsoft Excel. When preparing charts for presentation in an assessed report or dissertation, it is good practice to prepare them to the same standard of quality expected by journals that publish articles in the Life Sciences. The steps shown below explain how to format your charts to a standard similar to that of the expected style.

Arrangement of data

1) Microsoft Excel is quite flexible in terms of the arrangement of data within the worksheet necessary to plot a chart, so they can be in rows or in columns as you prefer.
2) You should aim to bring together separate groups of numbers, with each value reflecting the result from a whole, independent experiment.
3) For example, if you have completed 4 separate experiments, you would have 4 values for each of the different conditions, including the control, arranged without gaps in rows or in columns as shown in the example below:

	A	B	C	D	E	F	G
1							
2		DMSO	Extract 1	Extract 2	Extract 3	Extract 4	Extract 5
3	Exp 1	96%	26%	7%	6%	19%	19%
4	Exp 2	85%	22%	4%	0%	1%	13%
5	Exp 3	73%	16%	0%	1%	0%	0%
6	Exp 4	98%	22%	5%	3%	11%	11%

4) We do not give the results of individual experiments in separate charts, rather, we must calculate the **mean** (average) of the multiple separate experiments, and plot those mean values on a single chart.
5) Copy and paste the column headings from the first row of your data table (i.e. the row showing ‘DMSO’, ‘Extract 1’, etc. as shown in the example above) into a spare row just below your data table.
6) To calculate the mean of each column, type the following formula in a cell just below the first data column heading:

```
=AVERAGE(B3:B6)
```

7) Note that your own data may be in different locations, so adjust the cell references in the formula accordingly to fit your own data.
8) We must also present some indication of how variable the data were on the chart - it is necessary to show this because results with low variability mean high reproducibility, giving readers more confidence in conclusions drawn from the results.
9) When plotting a bar chart, we most commonly show the variability in the form of error bars.
10) We begin by calculating the standard deviation of each group of experimental replicates.

11) Type the following formula into a cell just below where you calculated the mean:

```
=STDEV(B3:B6)
```

12) Now select both cells with these calculations and drag the green fill button at the bottom right of the selected squares, and copy across to fill the whole row, your data table should now look something like this:

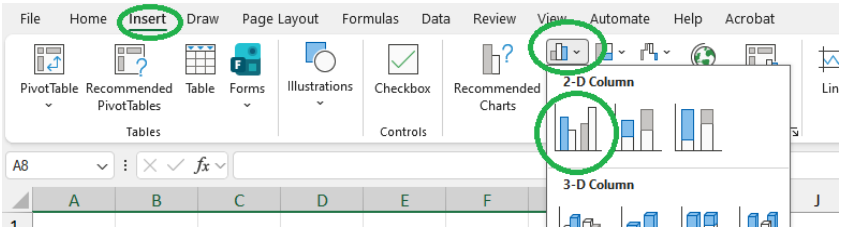
2		DMSO	Extract 1	Extract 2	Extract 3	Extract 4	Extract 5
3	Exp 1	96%	26%	7%	6%	19%	19%
4	Exp 2	85%	22%	4%	0%	1%	13%
5	Exp 3	73%	16%	0%	1%	0%	0%
6	Exp 4	98%	22%	5%	3%	11%	11%
7							
8		DMSO	Extract 1	Extract 2	Extract 3	Extract 4	Extract 5
9	Mean	88%	22%	4%	2%	8%	11%
10	SD	12%	4%	3%	2%	9%	8%

13) Make sure the top left cell of the new data table is empty - Excel gets confused and does not plot charts properly if there is anything in this cell.

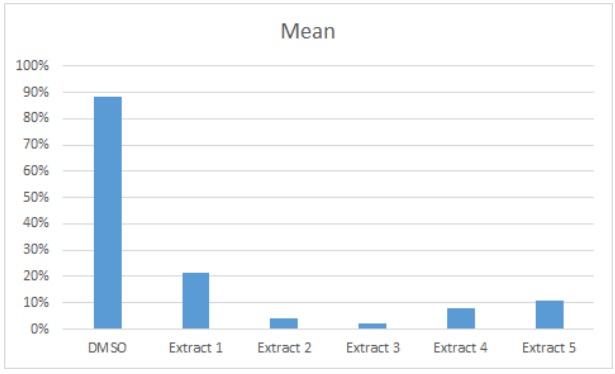
Plotting a column chart in Excel

1) Now select all the cells in the first two rows of the new data table - be careful to NOT select the row containing the SD values at this stage.

2) Then click on the 'Insert' tab of the ribbon, select the 'Insert column chart' icon, and click on the first of the 2D column chart options as shown below:



3) Excel will plot your data straight away using its default format for column charts, which should look something like this:



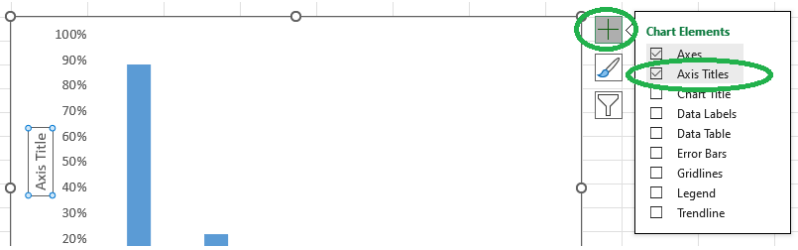
4) This format has a number of weaknesses that mean it would score poorly in a dissertation, and would not meet the standards for publication in a journal article.

5) Take the following steps to format the chart to the standards expected in the field.

6) Remove the title from the top of the chart by clicking on it and pressing delete - the chart title should never be in this location, but rather in a separate figure title below the chart written in the Word document of your report later (not Excel).

7) Click on any horizontal or vertical gridlines that are shown within the main area of the chart and press the delete or backspace key to remove them.

8) Click on the main body of the chart and a large 'Plus' icon appears at the right of the chart - click on this button to reveal a menu of options, and select 'Axis titles' (or select 'Add chart element' from the ribbon if the plus button does not appear).

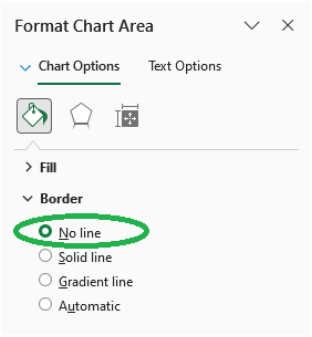


9) In this example, we do not require an X-axis title since there are already explanatory headings for each column, so you can click on it and press delete to remove it.

10) Click on the Y-axis title and rename with an appropriate label, such as 'Cell viability (% Max)'.

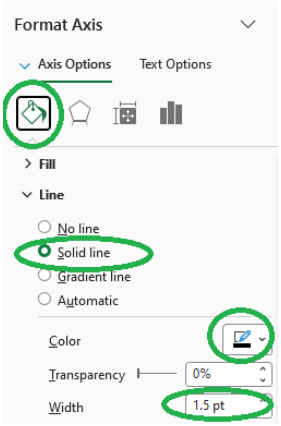
11) Double click on the white space of the outer part of the chart, (not the inner main part of the chart where the columns are), and a new 'Format Chart Area' menu will appear at the right of the screen.

12) Select 'Border' then 'None' to remove the outer border line.



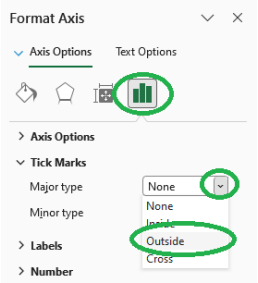
13) Professional charts should have black 1.5 point axis lines, to set this, double click on the text of one of the axes, then a new 'Format Axis' menu will appear.

14) Click on the 'Paint' icon, then 'Solid line' select black for colour and 1.5 point for width.



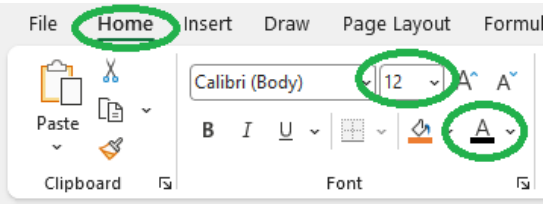
15) Do the same thing for the other axis, then double click on the axis text again to reveal the ‘Format Axis menu, and select the chart icon at the right of the upper section.

16) Click on ‘Tick marks’, then ‘Major type’ and ‘Outside’.



17) Do this for both axes, they should now both be black, 1.5 point and with tick marks on the outside of the line.

18) The font size is a little difficult to read, so to make it more clear select the whole chart by clicking on the white space of the outer region of the chart (not the area with columns) and set the font size to 12 and black using the Font section of the home ribbon.



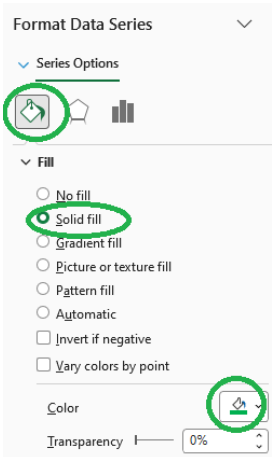
19) Double click on one of the columns in the central area of the chart, a new ‘Format Data Series’ menu should appear.

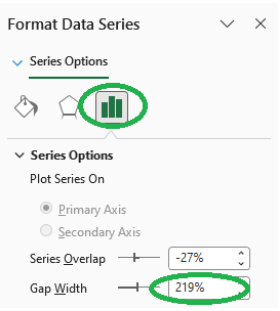
20) Click on the ‘Paint’ icon, then ‘Solid fill’, then select the colour for your columns - journals often prefer solid black but this is up to your own preference.

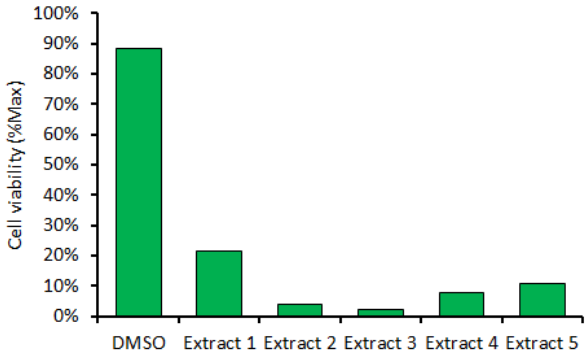
21) Click on ‘Border’ then ‘Solid line’ and select black as the colour.

22) Click on the ‘Chart’ icon of the same menu, then adjust the ‘Gap width’ setting to a lower value, to set the width of your columns to be more visually appealing.

23) Your chart should now look something like that shown at right:

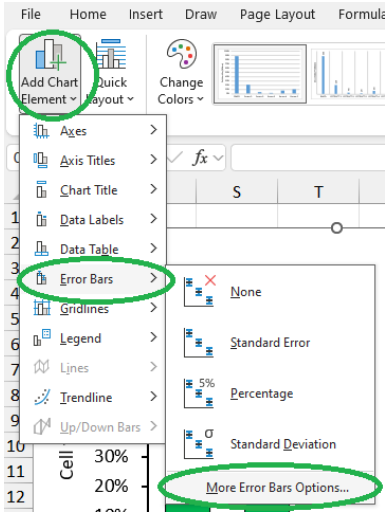


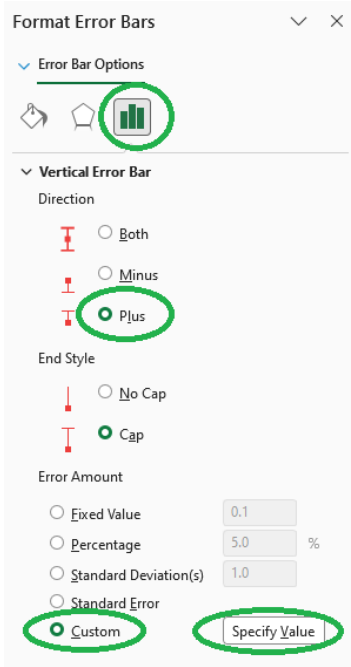


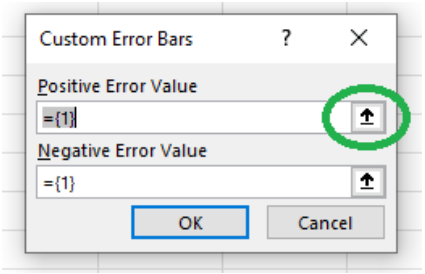


Adding error bars to your chart

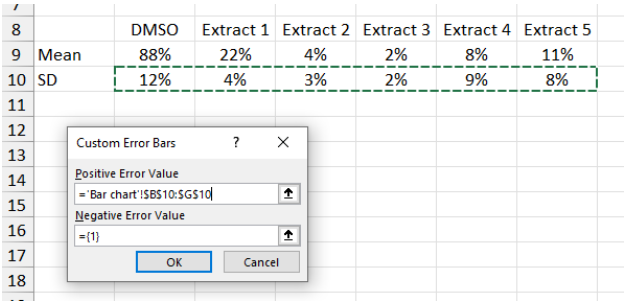
1) We must now add error bars to the chart to show how variable the individual experimental replicates were.
2) Click anywhere on the chart, then from the ribbon at the top of the screen, select the 'Chart Design' tab.
3) Click on the 'Add Chart Element' button at the left of the ribbon, then select 'Error bars' then select 'More Error Bar Options'.
4) Note: DO NOT select the 'Standard Deviation' option from this menu - Excel does not know where your SD values are and it will get these wrong, introducing error bars that are not correct if you accidentally select this option - you must instead select 'More Error Bar Options'.
5) A new 'Format Error Bars' menu appears at the right of the screen.
6) Select the Chart icon, then select 'Plus' for Vertical error bar direction, in the 'Error Amount' section select 'Custom' then click the 'Specify Value' button.
7) In the new box that appears mid scren, click on the upward pointing arrow to the right of the 'Positive Error Value' box.





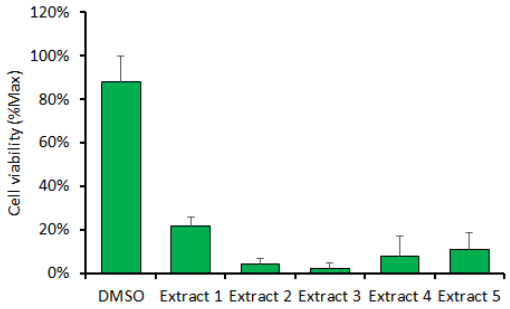


8) Now use the mouse to select all of the standard deviation values from the third row (the SD row) of your data table, but do not include the row heading.
9) This tells Excel where the SD values are in your worksheet and to use these values to draw your error bars in the upward direction.



10) Click ‘OK’, or press the ‘Enter’ key twice to confirm selection of these cells to draw the error bars.

11) Your chart should now have error bars on top of the columns, as shown in the example below:



Adding stars to indicate statistical significance

1) It is common practice to indicate on the chart which of the treatments yield a result that is significantly different from the control condition.

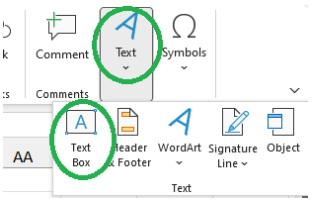
2) After completing an appropriate statistical test (such as a one way ANOVA), you will have p-values for each of these comparisons.

3) We typically draw one star (usually as an asterisk *) above any columns with a p-value of less than 0.05.

4) We typically draw two stars (**) or three stars (***) above any columns where the treatments meet $p<0.01$ or $p<0.001$, respectively.

5) There is no automated way to do this using Excel directly, but you can add them manually.

6) Click on the ‘Insert’ tab, then select the ‘Text’ button at the right hand side of the ribbon, and choose ‘Text box’.

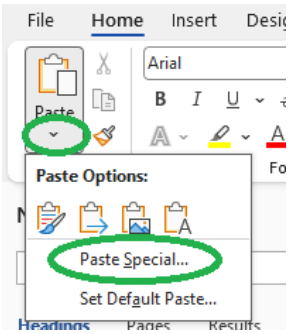


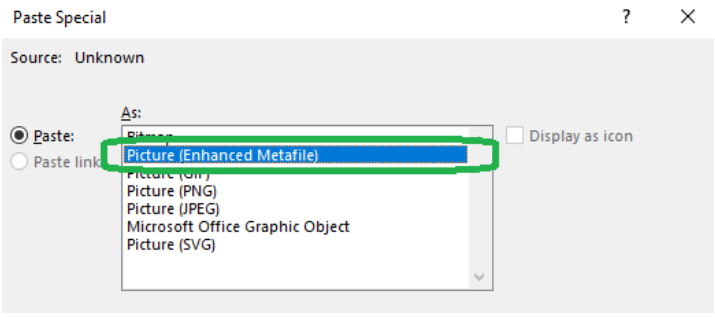
7) Type one or more asterisks (press the shift key and number 8 together) into the text box.

8) Click on the text box and select the ‘Shape format’ tab from the ribbon.

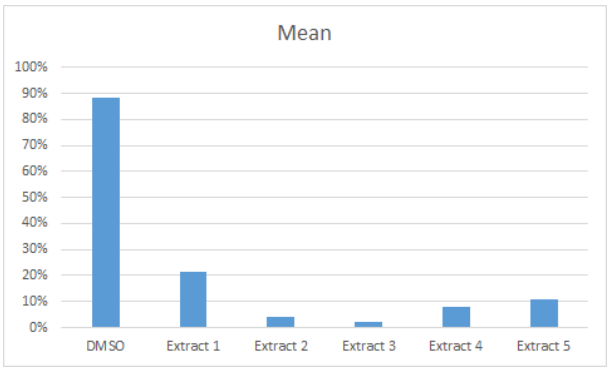
9) Use the ‘Shape fill’ and ‘Shape outline’ buttons to format the text box to have no border line and no background colour (not even white in case it obscures an error bar).

10) Repeat to insert stars reflecting p-value for each of the treatments above each column.
11) If there are treatments with responses that do not differ significantly from the control condition (i.e. p>0.05) then we would not insert anything above those columns, but if you want to clarify this you can type ‘n.s.’ for not-significant
12) You will normally want to copy and paste the resulting chart into a Word document when writing your dissertation.
13) Be careful to NOT use a standard copy and paste for this transfer.
14) Instead, you must first select the chart you want to copy by clicking on it, then pressing the shift key and keeping it held down while you then click on each of the ‘star’ text boxes until the chart and all of the text boxes are selected simultaneously.
15) Now press control C, or right click then select ‘Copy’, and open your Word document.
16) Do NOT simply press control V or a standard Paste option.
17) Instead, in Word, click on the downward arrow below the large ‘Paste’ button at the left hand side of the home ribbon.
18) Select the ‘Paste Special’ option, and a new menu appears mid screen.
19) Click on the ‘Picture Enhanced Metafile’ option and click ‘OK’.

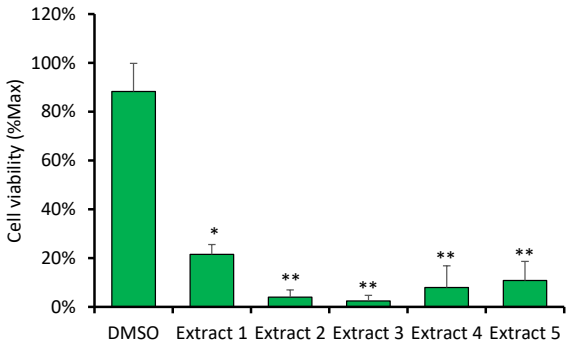




20) Now the chart and its text box overlays are pasted into the Word document.
21) The advantage of this approach is that when you resize the chart to make it fit in your document, everything within the chart will resize appropriately too so it looks correct.
22) If you only perform a standard paste at this stage without using the ‘Picture Enhanced Metafile’ option, then resize it, the formatting will be corrupted as the font sizes will not resize correctly.
23) Your completed chart should now look something like the example shown at right:



Before improvements to formatting



After improvements to formatting

Notes

- Remember that it is not appropriate to chart the means or display error bars using results arising from multiple replicates from within only one experiment.
- For example, you should not plot data on triplicate measurements from within the same plate - these are called technical replicates, which are not the same as experimental replicates.
- Instead, you should plot the means and calculate standard deviation from multiple, separate experiments.
- If you do have data from only one experiment and want to report it in your dissertation, you must make clear in the figure legend that the chart represents the results of only one experiment (n=1).
- If you would like to show smaller error bars, you can plot Standard Error of the Mean (SEM, which is simply the SD divided by the square root of the ‘n’ (number of separate experiments), but be sure to indicate in your figure legends that SEM is shown instead of SD in your error bars.
- If you want to prepare a single figure containing multiple panels, perhaps several similar charts, or mixing charts and images, a convenient way of doing this is to paste the charts as enhanced metafiles into Powerpoint, then add text boxes and images as preferred, then copy the whole group from Powerpoint and paste into Word as an enhanced metafile.