RANOVA 4.0

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About

RANOVA Version 4.0

Produced by Peter Rostron

Based on papers from the Analytical Methods Committee[5,6,7]; the Eurachem/CITAC guide (2019)[1]; Rostron & Ramsey (2012)[4]; Rostron Fearn & Ramsey (2020)[8]; Rostron Fearn & Ramsey (2024)[11]

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1.0 Introduction

RANOVA 4.0 is an Excel application to assist in estimation of uncertainty by the **duplicate method***, which is fully described in the Eurachem guide[1]. It is intended for use by analytical chemists, or for anyone else who needs to measure concentrations within a bulk of material through the analysis of a number of discrete samples.

Its primary function is to perform **robust and classical analysis of variance** on sets of duplicated measurements at selected sampling targets that have been specifically acquired for the purpose of uncertainty estimation. It achieves this by splitting the variance in a set of duplicated measurements between three encompassing components as follows:

a) Variance in measured concentrations between the designated sampling targets;
b) Variance in concentrations between duplicated samples (sampling variance) acquired within these sampling targets;
c) Variance between duplicated analyses (analytical variance) that have been conducted on some or all of the samples acquired in (b).

RANOVA is presented as an easy-to-use Excel application, which allows measurement data from other Excel spreadsheets to be easily pasted into the program, and output to be pasted into other documents as required.

The latest version (RANOVA V 4.0) was released in April 2024.

*Items in bold are described in more detail in Principles of operation.

2.0 Principles of Operation

2.1 Robust ANOVA

*Estimation of measurement uncertainty using **balanced** and **unbalanced designs***

In an experiment to characterise concentration levels of an analyte within a bulk of material, it is usual to designate a number of discrete locations at which measurements will be made. These may either be made *in situ*, or performed *ex situ* on smaller portions of material that have been physically removed from the whole. In some cases, it may only be necessary to know the average concentration in the whole bulk. The **sampling target** is then the entire mass of material, and the standard uncertainty on the mean concentration is simply estimated as:

$$s/\sqrt{N}$$

where s = the standard deviation of the measured concentration values, and N = the total number of measurements taken.

In many situations, additional information about the distribution of analyte concentrations throughout the material is also required. For example, in an investigation of contaminated land it is often necessary to identify and delineate "hotter" areas that require some sort of remediation process. In this case, the **sampling target** can be considered to be a discrete volume, mass or area within the whole. In such an investigation, it is desirable to be able to estimate the uncertainty on individual measurements, so that this can be taken into account in subsequent decision making. It can also be useful to estimate the contributions to the overall uncertainty that are made by a) the sampling process, and b) the analytical process. This information enables the practitioner to make cost-effective decisions on where best to allocate resources in subsequent experiments (e.g. whether to use a different analytical procedure, or whether to adjust the sampling protocol).

The Eurachem guide *Measurement Uncertainty Arising From Sampling* recommends the use of the **duplicate method** for the empirical estimation of measurement uncertainty[1,2,3]. This involves taking duplicated samples at a number (usually 10%, with a minimum of eight) of randomly assigned measurement locations, where each measurement location is intended to represent a single sampling target. Duplicated analyses of these samples is then carried out, according to one of two protocols. In the **balanced design**. both of the duplicated samples are analysed twice (Fig 1). In the **unbalanced design** only one of the duplicated samples undergoes duplicated analysis (Fig 2). Application of the balanced design requires three extra measurements to be made at each of the designated duplicate measurement locations, while application of the unbalanced design requires only two extra measurements. So use of the unbalanced design reduces the analytical component of the cost of uncertainty estimation by one third.

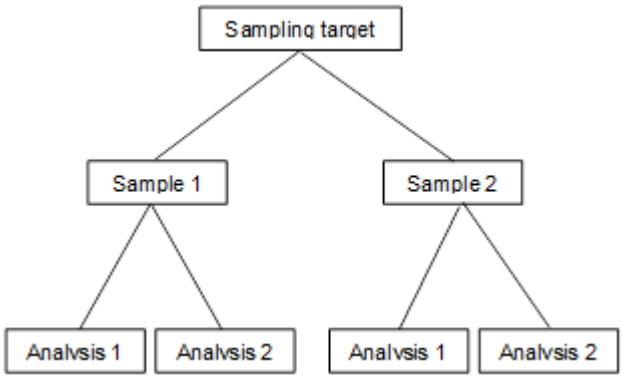


Fig. 1 The balanced experimental design, which can be used to estimate the sampling and analytical components of measurement uncertainty using the duplicate method. Two samples are taken at a proportion (e.g. 10%) of the primary sampling points, and each of these samples is chemically analysed twice[4]

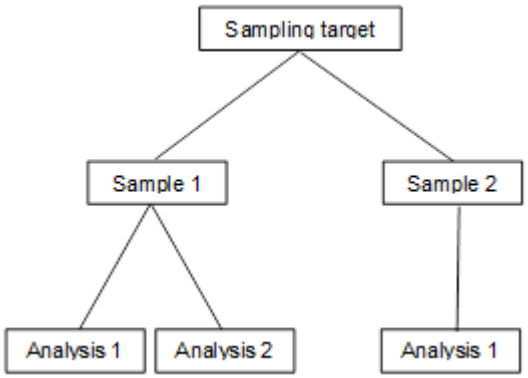


Fig. 2 The unbalanced experimental design, where only one of the two samples from a duplicated primary sample undergoes duplicate analysis. This reduces the total number of chemical analyses required[4]

Analysis of variance (ANOVA) is then used to separate the total variance between measurements into components that arise from three different sources:

a) Variance in concentration between the sampling targets;
b) Variance in concentration between the duplicated samples (sampling variance, s^2samp);
c) Variance in concentration between the duplicated analyses (analytical variance, s^2anal).

The standard deviations corresponding to (b) and (c) above are then estimates of the sampling and analytical precision (e.g. the repeatability)[1]. The random components of the *expanded relative uncertainties* at ~95% confidence can be estimated (traditionally using a coverage factor of 2) as:

$$U_{samp} = \frac{100 \times 2s_{samp}}{mean} \%$$

$$U_{anal} = \frac{100 \times 2s_{anal}}{mean} \%$$

These can then be combined in an estimate of the total expanded measurement uncertainty as

follows:

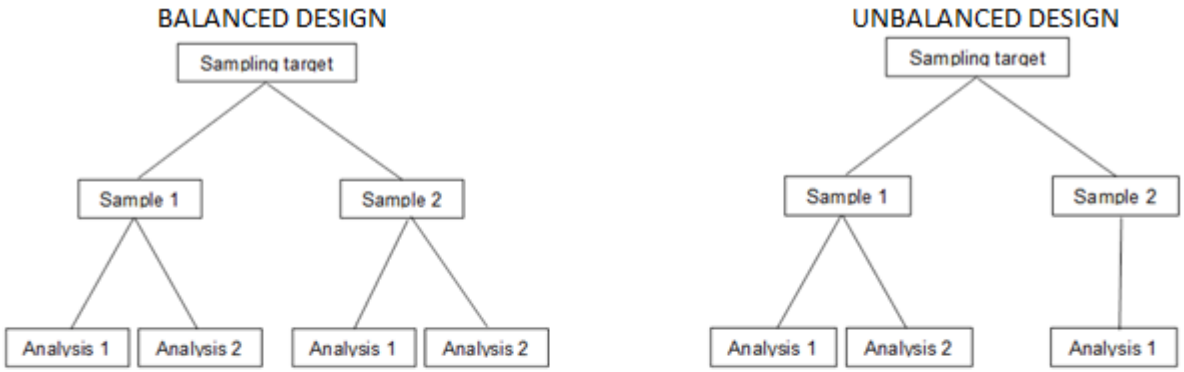
$$U_{meas} = \sqrt{U_{samp}^2 + U_{anal}^2}$$

Equation 1

Note that the *expanded relative analytical uncertainty* is strictly an estimate of analytical repeatability and does not therefore include all components of analytical uncertainty that might be estimated by a commercial laboratory.

Robust analysis of variance *(including example, see AMC Tech. Brief No. 64)*

Using this method of acquiring duplicate measurements results in a table of measured concentrations, such as those in Fig 3. This shows simulated measurements from 10 duplicate measurement locations for the balanced design (left) and the unbalanced design (right).



S1A1	S1A2	S2A1	S2A2
13.7	14.6	13.8	14.5
10.1	9.4	5.7	5.7
5.2	5.4	10.9	9.2
11.7	12.5	10.4	9.9
9.5	10.0	9.3	8.2
11.8	11.0	12.0	12.7
8.7	8.7	13.0	12.4
13.4	12.1	14.9	15.8
5.3	5.3	5.1	5.4
12.6	12.9	17.2	18.6

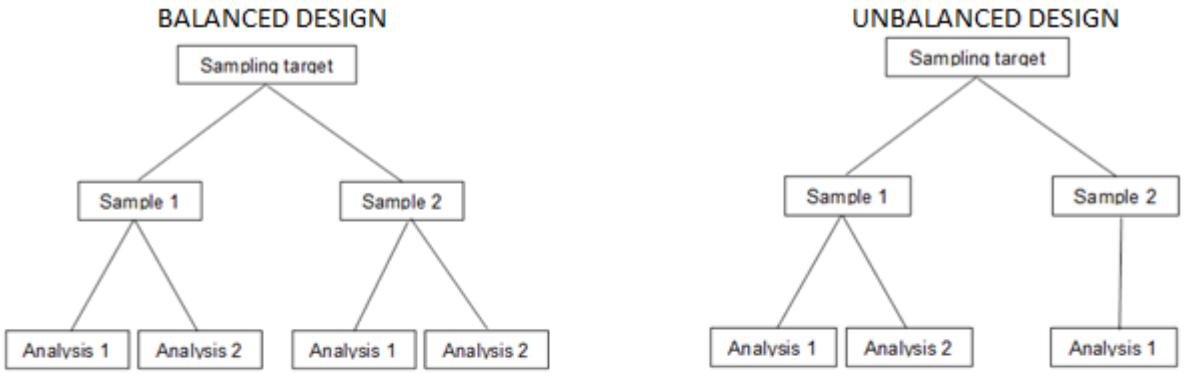
S1A1	S1A2	S2A1
13.7	14.6	13.8
10.1	9.4	5.7
5.2	5.4	10.9
11.7	12.5	10.4
9.5	10.0	9.3
11.8	11.0	12.0
8.7	8.7	13.0
13.4	12.1	14.9
5.3	5.3	5.1
12.6	12.9	17.2

Fig 3 Examples of data generated by the duplicate method using the ***balanced design*** *(left) and the* ***unbalanced design*** *(right). This shows simulated concentration data (units are arbitrary) drawn from a population with mean = 10, between-target standard deviation = 2, sampling uncertainty (2s) = 40% and analytical uncertainty (2s) = 10%*

These datasets could be processed using **classical ANOVA**, provided by Excel and other software packages, and the components of the total variance that arise from differences between the measured values made from *Sample 1* and *Sample 2* used to estimate the random component of uncertainty in the measurements that results from the sampling protocol used. In a similar way,

the variances that arise from differences between the measured values made from *Analysis 1* and *Analysis 2* could be used to estimate the random component of uncertainty due to the analytical procedure.

In real data, a small proportion of outlying measurements (i.e. less than 10%) are often found to exist[1]. These can seriously distort the variances estimated by this method. An example of the above dataset with one outlying value is shown in Fig 4.



S1A1	S1A2	S2A1	S2A2
13.7	14.6	13.8	14.5
10.1	9.4	5.7	5.7
5.2	5.4	10.9	9.2
11.7	12.5	10.4	9.9
9.5	10.0	9.3	8.2
11.8	11.0	12.0	12.7
18.2	8.7	13.0	12.4
13.4	12.1	14.9	15.8
5.3	5.3	5.1	5.4
12.6	12.9	17.2	18.6

S1A1	S1A2	S2A1
13.7	14.6	13.8
10.1	9.4	5.7
5.2	5.4	10.9
11.7	12.5	10.4
9.5	10.0	9.3
11.8	11.0	12.0
18.2	8.7	13.0
13.4	12.1	14.9
5.3	5.3	5.1
12.6	12.9	17.2

Fig 4 The same datasets shown in Fig 3 but with one outlying values (highlighted) in both the balanced and the unbalanced designs

In this case, **classical** ANOVA estimates values of the expanded relative sampling and analytical uncertainties (2s) that are not representative of the main population sampled (Table 1). Using these uncertainty estimates could result in a serious error in subsequent decision making.

	Original seed distribution parameters	BALANCED DESIGN	UNBALANCED DESIGN
Mean	10	11	12
Standard deviation	2	3.8	3.6
Relative sampling uncertainty (2s)	40%	30%	8%
Relative analytical uncertainty (2s)	10%	29%	36%

*Table 1 Results of running **classical** ANOVA on the datasets shown in Fig 4. The single outlying value that is highlighted in Fig 4 has seriously distorted the estimates of expanded relative measurement uncertainty resulting in estimates of analytical uncertainty that exceed that of the original simulated distribution in Fig*

3 by a factor of approximately 3.

One way of dealing with this would be to remove outlying values from the datasets, either by judgment or by using statistical significant tests. Such an approach would, however, lead to an underestimation of the variance that is characteristic of the analytical method[1]. An alternative method is to use **robust** statistics in the ANOVA calculation. This uses an iterative approach to *accommodate* rather than reject outlying values, while down-weighting their overall impact on the final result. The RANOVA program allows **robust ANOVA** to be performed on both balanced and unbalanced designs. The results of these calculations for the same data (Fig 4) are shown in Table 2.

	Original seed distribution parameters	BALANCED DESIGN	UNBALANCED DESIGN
Mean	10	11	11
Standard deviation	2	4	3.8
Relative sampling uncertainty (2s)	40%	37%	34%
Relative analytical uncertainty (2s)	10%	12%	12%

*Table 2 Results of running **robust** ANOVA on the datasets shown in Fig 4. It can be seen that the estimates of the random components of sampling and analytical uncertainty are much more representative of the original distribution parameters than were achieved by **classical ANOVA** (Table 1)*

Although the relative sampling and analytical uncertainties are greater than those used to create the simulated measurements prior to adding the outlying measurements, the results of the **robust ANOVA** shown in Table 2 are much better estimates of the uncertainties in the original dataset for both the balanced and the unbalanced designs (Fig 3). Some increase in the uncertainties would be expected, because robust ANOVA accommodates, rather than rejects, the outlying values.

Simplified experimental design

The Eurachem guide *Measurement Uncertainty Arising From Sampling (Appendix D)*[1] suggests a simplified design that might be used if costs prohibit the use of the balanced or unbalanced designs discussed above. ANOVA will then give an estimate of the total measurement uncertainty only. If the analytical uncertainty can be estimated by some other means (e.g. by the laboratory), then the sampling uncertainty can be re-calculated by removing the analytical uncertainty using a re-arrangement of Equation 1. The simplified experimental design can be applied by copying the measurements in the A1 column to the A2 column for both S1 and S2 (Fig 5).

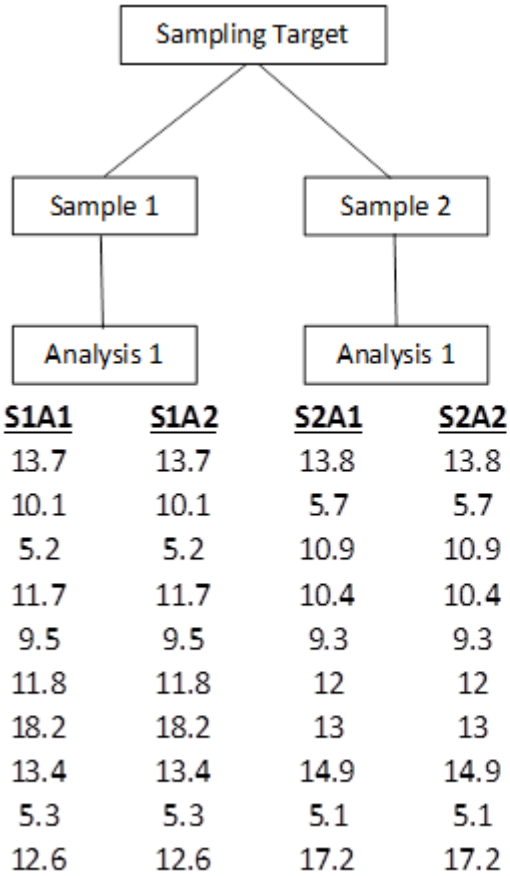


Fig 5 The data from Fig 3 but with only one analysis per sample. S1A2 and S2A2 are copied from S1A1 and S2A1 respectively

RANOVA will result in the following estimate of measurement uncertainties (Table 3). The analytical uncertainties are reported as zero. Note that although this is a correct calculation from the data, analytical uncertainty will always exist, and it is recommended that the balanced or unbalanced experimental designs are used whenever possible, as these provide empirical estimates of the random components of both sampling and analytical uncertainties that are derived from the reported measurements.

	Original seed distribution parameters	Classical ANOVA	Robust ANOVA
Mean	10	11	11
Standard deviation	2	4	4
Relative sampling uncertainty (2s)	40%	41%	36%
Relative analytical uncertainty (2s)	10%	0%	0%

*Table 3 Results of running **classical** and **robust** ANOVA on the datasets shown in Fig 5, where S1A2 = S1A1 and S2A2 = S2A1*

More examples and details of the robust ANOVA calculations can be found in Rostron *et al* (2012)[4], AMC (2001)[5], AMC (1987a)[6] and AMC (1987b)[7] See References.

2.2 Uncertainty Factors

*Estimation of the **Uncertainty Factor***

This section was added with the release of RANOVA 2.0 (September 2015)

Traditionally, measurement uncertainty has been expressed as the measured result plus/minus a value or fraction. For example, if 10 targets were randomly selected from a sample of 100 targets for measurement using the *duplicate method*[1], and ANOVA was performed on the duplicate measurements, producing the following output:

Classical ANOVA

Mean	*10.953*		No. Targets	*10*
Total Sdev	*3.7994*			
	Btn Target	Sampling	Analysis	Measure
Standard deviation	*3.0359*	*1.6226*	*1.6082*	*2.2845*
% of total variance	*63.85*	*18.24*	*17.92*	*36.15*
Expanded relative uncertainty (95%)		*29.63*	*29.37*	*41.72*

Then the value of each target, including uncertainty, could be written in the form (e.g.) 10.0 (units) ± 41.72%

This does not take into account that it is often found that the distribution of repeated measurements of a target is lognormal rather than Gaussian. When the uncertainty is small, the traditional method might be considered a reasonable approximation. However, when for example the random component of uncertainty due to sampling is estimated in a contaminated land investigation, then the combined measurement uncertainty might be quite large, perhaps approaching or even exceeding 100%. In these cases there may be an advantage to expressing the uncertainty in a way takes into account the asymmetric distribution of values that could be attributed to the measurand.

It has been suggested[1] that a multiplicative factor be used in a *times/over* (*,/) expression that can be calculated as

$$^{F}U = \exp(2 * s_G)$$

Where ^{F}U is the Uncertainty Factor and s_G is the standard deviation of the $\log_e$-transformed distribution.

RANOVA 4.0 calculates and displays the component and combined expanded uncertainty factors obtained by performing classical ANOVA on the $\log_e$-transformed distribution of input measurements (see example below).

Classical ANOVA

Mean	*10.953*		No. Targets	*10*
Total Sdev	*3.7994*			
	Btn Target	Sampling	Analysis	Measure
Standard deviation	*3.0359*	*1.6226*	*1.6082*	*2.2845*
% of total variance	*63.85*	*18.24*	*17.92*	*36.15*
Expanded relative uncertainty (95%)		*29.63*	*29.37*	*41.72*
Uncertainty Factor (95%)		*1.4653*	*1.2894*	*1.5823*

Using this approach the value of an individual target including uncertainty could be written in the form 10.0(units) *,/ 1.58

2.3 Confidence Intervals

Estimation of Confidence Intervals for a Nx2x2 balanced design

This section was added with the release of RANOVA 3.0 (June 2020)

It may be useful in some situations to calculate confidence intervals for variance estimates produced using the duplicate method. An example would be when a researcher estimates uncertainties of measurements on the same targets by more than one method. Comparison of confidence intervals on these uncertainties would indicate whether the uncertainty estimates themselves were significantly different between different analytical methods[8].

Confidence limits for individual variances from **classical ANOVA** are calculated using the following formulae[8,9,10]:

Target Level

$$\left(\frac{I-1}{\chi^2_{\alpha/2,I-1}}\right)\left[\hat{\sigma}^2_{\text{Target}} + \frac{MS_{\text{Sample}}}{JK}\left(1-F_{\alpha/2,I-1,I(J-1)}\right)\right] \le \sigma^2_{\text{Target}} \le \left(\frac{I-1}{\chi^2_{1-\alpha/2,I-1}}\right)\left[\hat{\sigma}^2_{\text{Target}} + \frac{MS_{\text{Sample}}}{JK}\left(1-F_{1-\alpha/2,I-1,I(J-1)}\right)\right]$$

Sample Level

$$\left(\frac{I(J-1)}{\chi^2_{\alpha/2,I(J-1)}}\right)\left[\hat{\sigma}^2_{\text{Sample}} + \frac{\hat{\sigma}^2_{\text{Analysis}}}{K}\left(1-F_{\alpha/2,I(J-1),IJ(K-1)}\right)\right] \le \sigma^2_{\text{Sample}} \le \left(\frac{I(J-1)}{\chi^2_{1-\alpha/2,I(J-1)}}\right)\left[\hat{\sigma}^2_{\text{Sample}} + \frac{\hat{\sigma}^2_{\text{Analysis}}}{K}\left(1-F_{1-\alpha/2,I(J-1),IJ(K-1)}\right)\right]$$

Analysis Level

$$\hat{\sigma}^2_{\text{Analysis}}\left(\frac{IJ(K-1)}{\chi^2_{\frac{\alpha}{2},IJ(K-1)}}\right) \le \sigma^2_{\text{Analysis}} \le \hat{\sigma}^2_{\text{Analysis}}\left(\frac{IJ(K-1)}{\chi^2_{1-\frac{\alpha}{2},IJ(K-1)}}\right)$$

Where I = the number of targets = N
J = the number of samples = 2
K = the number of analyses = 2
MS_{Sample} = the mean square of the middle (sampling) level from ANOVA
$F_{p,\,v1,\,v2}$ = the inverse cumulative distribution function of the F probability distribution with degrees of freedom v$1,v2$ for probability p
$\chi^2_{p,v}$ is the inverse cumulative distribution function of the chi-squared distribution with degrees of freedom v for probability p

For combined variances, if we have k mean squares S^2_i with degrees of freedom v_i for i = 1,...,k then we can estimate approximate 100(1-α)% confidence limits for θ from[10]:

$$\sum_{i=1}^{k} c_i S_i^2 - \left(\sum_{i=1}^{k} L_i^2 c_i^2 S_i^4\right)^{\frac{1}{2}} \le \theta \le \sum_{i=1}^{k} c_i S_i^2 + \left(\sum_{i=1}^{k} H_i^2 c_i^2 S_i^4\right)^{\frac{1}{2}}$$

Where

$$L_i = 1 - \frac{\nu_i}{\chi^2_{1-\alpha/2,\nu_i}}$$

$$H_i = \frac{\nu_i}{\chi^2_{\alpha/2,\nu_i}} - 1$$

In the case of the *N*x2x2 balanced design,

$$\text{For } \sigma^2_{Meas} = \sigma^2_{Samp} + \sigma^2_{Anal} : \; c_1 = k-1, c_2 = 1$$

$$\text{For } \sigma^2_{Total} = \sigma^2_{Targ} + \sigma^2_{Samp} + \sigma^2_{Anal} : \; c_1 = J(k-1), c_2 = J-1, c_3 = 1$$

The confidence limits so obtained are divided by *K* and *JK* respectively.

Confidence limits for individual and combined variances from **robust ANOVA** are calculated using a bootstrapping method. A large number (2000) independent bootstrap samples are generated. A bootstrap sample is a data set, of the same size and structure as the observed one, generated by random sampling with replacement from the observed data set. Robust ANOVA is run for each bootstrap sample. Confidence intervals are estimated from the empirical distributions of these results.

A more detailed description of the bootstrapping method can be found in Rostron *et al* (2020)[8]

2.4 Improved Uncertainty

Improved uncertainty estimates for a Nx2x2 balanced design, using calculated coverage factors

This section was added with the release of RANOVA 4.0 (April 2024)

Common practice is to derive an Expanded MU by multiplying a standard deviation by a coverage factor *k* = 2 for a desired confidence level of approximately 95 %. This will be an underestimate when the standard deviation is estimated from a limited amount of data, e.g. *n* << 30. Ideally we would use Student's *t*-distribution to provide a value for *k*, but this requires an evaluation of the degrees of freedom (df). This is not straightforward when two variances from an ANOVA (classical or robust) need to be combined to estimate the measurement variance. RANOVA 4.0 provides an estimated coverage (*k*-factor) for both classical and robust ANOVA, with corresponding improved estimates of MU. These are additions to previous versions of RANOVA, the locations of these improved values are show in the red boxes below.

RANOVA 4.0 - Analysis of variance (ANOVA) for *n* targets and 2 samples, including calculated coverage factors

BALANCED DESIGN — **Classical ANOVA**

Mean	10.953		Targets (*n*): 10	
Total SD (std dev)	3.7994			
	Btn Target	Sampling	Analysis	Measure
SD (or *u*)	3.0359	1.6226	1.6082	2.2845
% of total variance	63.85	18.24	17.92	36.15
U' (Exp rel uncertainty *k*=2)		29.63	29.37	41.72
^{F}U (Uncertainty Factor *k*=2)		1.4653	1.2894	1.5823
U' (Exp rel uncertainty) for 95% confidence (*k* = 2.11)				44.01
^{F}U (Uncertainty Factor) for 95% confidence (*k* = 2.11)				1.6340

BALANCED DESIGN — **Robust ANOVA** — HELP!

Mean	11		Targets (*n*): 10	
Total SD (std dev)	4.012			
	Btn Target	Sampling	Analysis	Measure
SD (or *u*)	3.3739	2.0608	0.6824	2.1709
% of total variance	70.72	26.39	2.89	29.28
U' (Exp rel uncertainty *k*=2)		37.47	12.41	39.47
U' (Estimated exp rel uncertainty) 95% confidence (*k* = 2.54)				50.13

u = standard uncertainty, applies to Sampling Analysis and Measure
k = coverage factor. Please refer to HELP! for more details

Methodology - Classical ANOVA

Satterthwaite[12] provides a method for estimating df (v) for variances that have been derived as linear combinations of mean-squares. Applying this method to the experimental design in Fig. 1 a simplification can be derived for the $n \times 2 \times 2$ design, where v_M is an estimate of df for the MU (derived as a linear combination of variances of sampling and analysis), I is the number of sampling targets, MS_A is the mean-square value atthe analytical level, and MS_S is the mean-square value at the sampling level[11].

$$v_M \approx \frac{I(MS_A+MS_S)^2}{\frac{MS_A^2}{2}+MS_S^2}$$

This value of v_M can then be used to obtain a modified k-factor from Student's t-distribution.

Methodology - Robust ANOVA

For Robust ANOVA, as described in Section 2.1, and using the algorithm described further in references [5,6,7], a different approach is needed, because it is not possible to obtain a corrected estimate of the k-factor mathematically. The methodology used by RANOVA 4.0 is based on a bootstrapping approach. This is further described in Rostron, Fearn and Ramsey (2024)[11].

Please note that there will be small differences in the Estimated Expanded Relative Uncertainty between successive runs on the same data (hence the inclusion of the word 'Estimated' in the robust case). This is because the calculations are based on bootstrap samples derived from the input data, which include a random component (random sampling with replacement).

3.0 Using RANOVA4.0

3.1 Overview

The original RANOVA program was arranged on a single Excel spreadsheet, allowing ANOVA to be performed on simple experimental designs with 2 samples per N targets, and 2 analyses per sample (known as Nx2x2 balanced designs). It also allowed **unbalanced** experimental designs to be used, where one of the samples was analysed once only.

RANOVA v2.0 was launched in 2015 with the following additional functions:

1. A new "Extended Balanced Design" sheet was included. This allows measurement

data for **balanced** designs with 2-8 samples each with 2-8 analyses to be input;

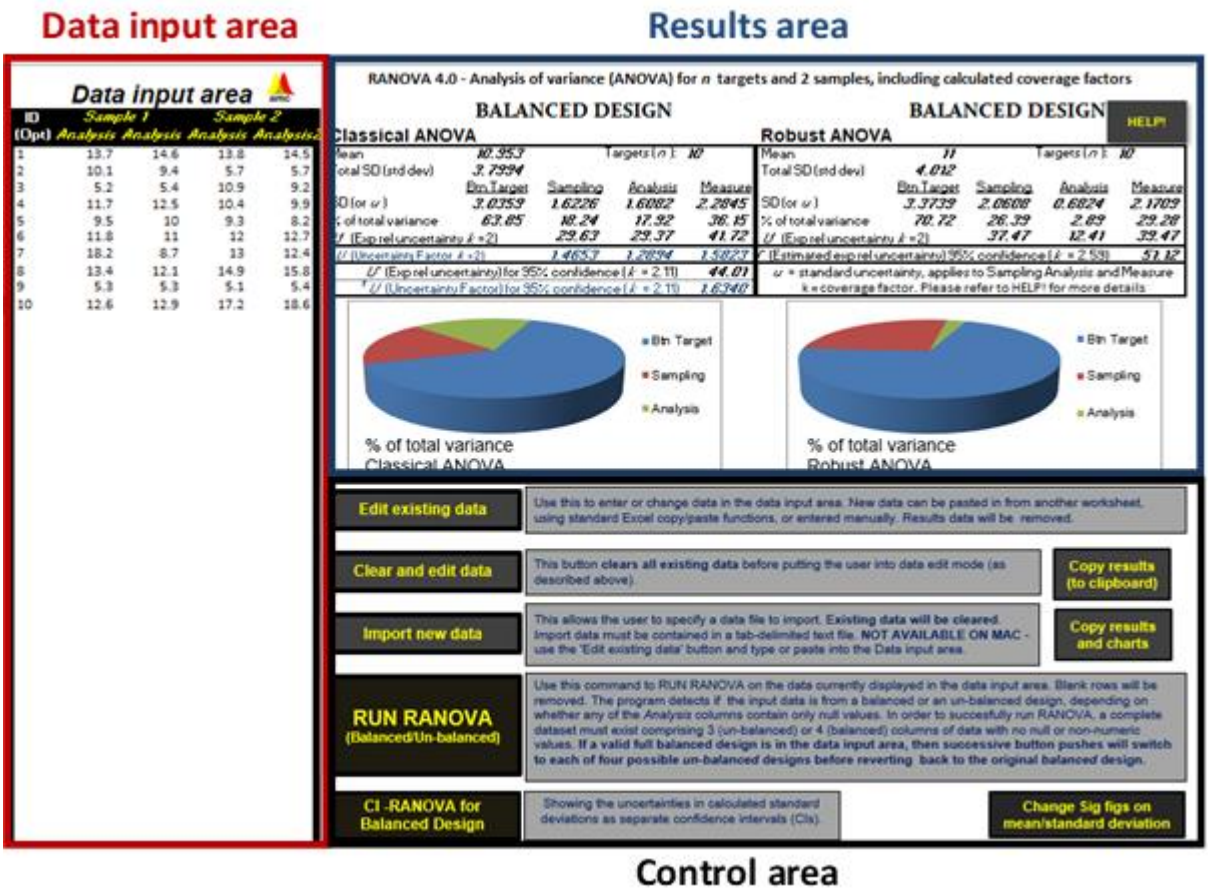
2. The Results area was modified to provide the following additional information:
   a) The number of targets (*N*) input by the user;
   b) The component and combined expanded uncertainty factors now displayed in the Classical ANOVA section of the Results area (Section 2.2).

Further versions (RANOVA v3.0 and v4.0) were launched in 2020 and 2024 respectively. RANOVA v3.0 included an option to calculate confidence limits on the *N*x2x2 balanced design which is anticipated to be the most commonly used experimental design (Section 2.3). Version 4.0 adds an improved estimate of the measurement uncertainty (MU) for the *N*x2x2 design if using values of *N* that might typically be used, when the common method of applying a coverage factor of 2 for an approximate 95 % confidence interval will result in an under-estimate of the MU.

The remaining text in this section and sections 3.2 - 3.6 describe operation of the **RANOVA** sheet for basic 2 sample, 1-2 analyses experimental designs (as in the original RANOVA). Section 3.7 explains how to use the **Extended Balanced Design** sheet for those who wish to use experimental designs that contain more than 2 samples and/or more than 2 analyses. Section 3.8 explains the use of the CI RANOVA button to obtain confidence limits on the standard deviations calculated by RANOVA 3.0. Section 3.9 describes the improved estimate of MU provided in V3.1.

The contents of the **RANOVA** sheet are grouped into three areas:

1) A *data input area (in red)*, into which duplicate measurement input data is entered;
2) A *results area (in blue)*, which presents the results of ANOVA calculations, and visual representations of the relative sizes of the three different variances that are calculated;
3) A *control area (in black)* with 4 main command buttons to the left.



Apart from the 4 main command buttons (explained below), an additional three buttons are available:

1) An additional button at the top right of the results area calls RANOVAHELP 4.0 (This function is not available on Excel for Mac, instead a pdf help file is provided). If HELP is not available to the RANOVA 4.0 workbook, then clicking this button will explain how to access HELP.
2) Two buttons to the right of the control area allow the user to copy the ANOVA results to the clipboard. The upper of these buttons copies the numerical results only, while the lower copies both the numerical results and the charts.

Note that the data input area extends off the bottom of the page that is visible when RANOVA 4.0 is first opened. This allows up to 1000 rows of duplicated measurement sets to be entered. You can view or edit data in this lower area by using the usual Excel commands, e..g. scroll bar, page up/page down keys, or the arrow keys.

When RANOVA 4.0 is first opened, the entire screen will be locked to the user with the exception of the command buttons (and the "HELP!" button). If the sheet has been saved during a previous run of the program (using the standard Excel save /save as commands) then any data from that save will also be displayed.

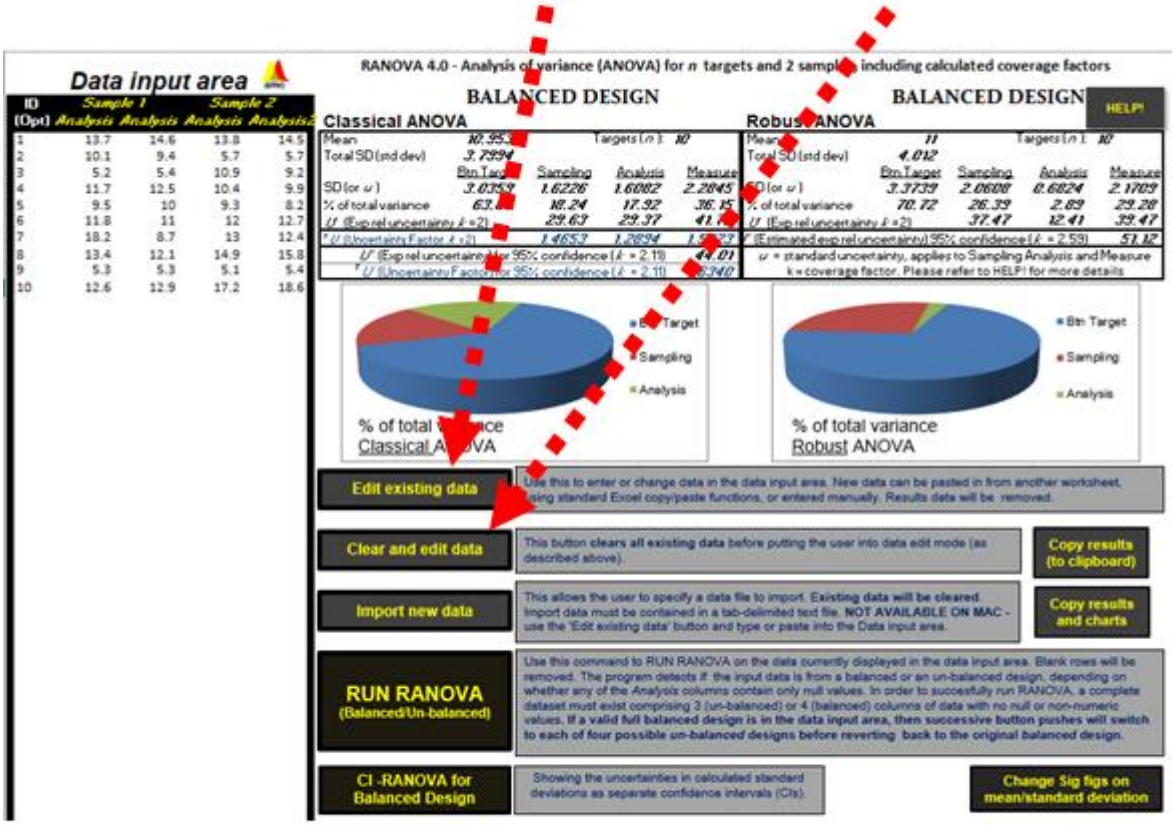
Note that it is only possible to add or edit data in the *<u>data input area</u>* after one of the top three buttons to the left of the *control area* has been pressed. Once one of them is pressed, any existing results in the *<u>results area</u>* will be blanked out. This is done to ensure data integrity, so

that is never possible to view results that do not correspond with the input measurement data.

To ensure essential macros are preserved, it is not possible to copy, delete, rename or create new sheets in the RANOVA 4.0 workbook.

3.2 Entering Data

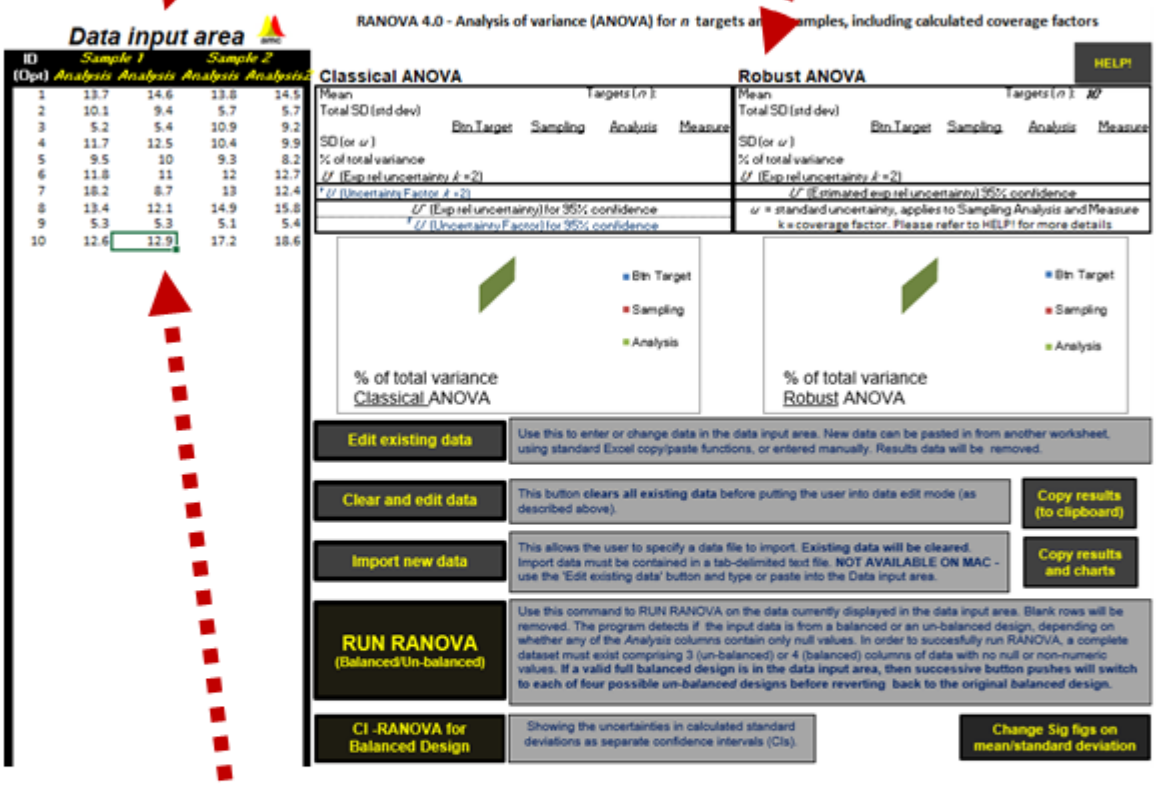
To manually enter, or copy and paste, measurement data into the input area you must first press the *Edit existing data* or the *Clear and edit data* button.



Note that the *Clear and edit data* button will clear out any existing measurement data in the *data input area*.

You will then be able to manually add, edit, or paste in measurement data from other Microsoft applications using the standard Excel paste commands. If pasting data from another location then the Edit or Clear buttons must be activated before copying data to the clipboard, as these buttons will empty the contents of the clipboard.

The *results area* will be cleared to ensure there is no inconsistency between the measurement data and the results.



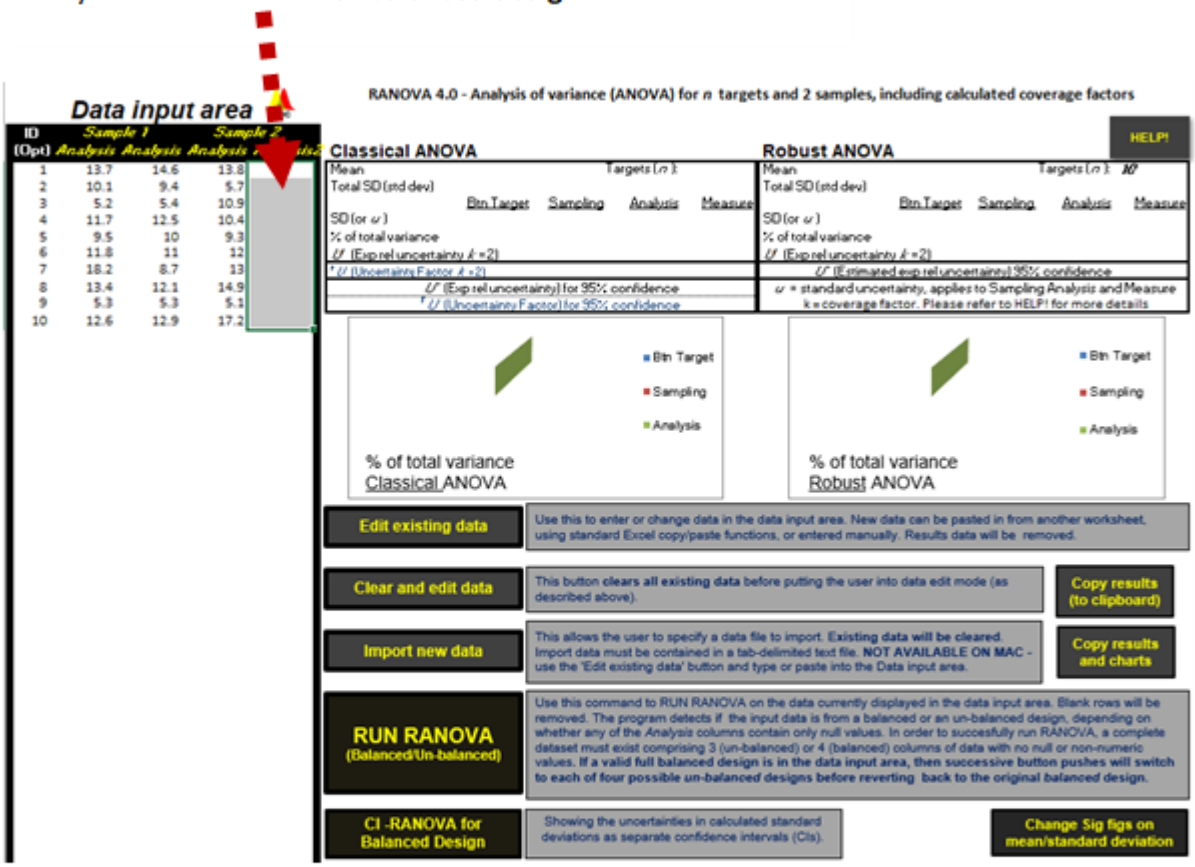
Any formatting (e.g. borders, colours) that are pasted in will be retained until one of the command buttons is pressed, when all formatting will be cleared.

To set up RANOVA 4.0 for a full **balanced design**, four matched columns of numbers must be entered in the *data input area* for *Analysis 1* and *Analysis 2* of *Sample 1* and *Sample 2*, as shown above (the *ID* column is optional and does not affect the calculations).

If you wish to run RANOVA 4.0 for both **balanced** and **unbalanced designs** on the same data, then single columns of measurement data can be alternately removed from the design at a later stage.

If you want to set up RANOVA 4.0 specifically for an **unbalanced design** only, then three matched columns of measurement data must be entered in the appropriate columns.

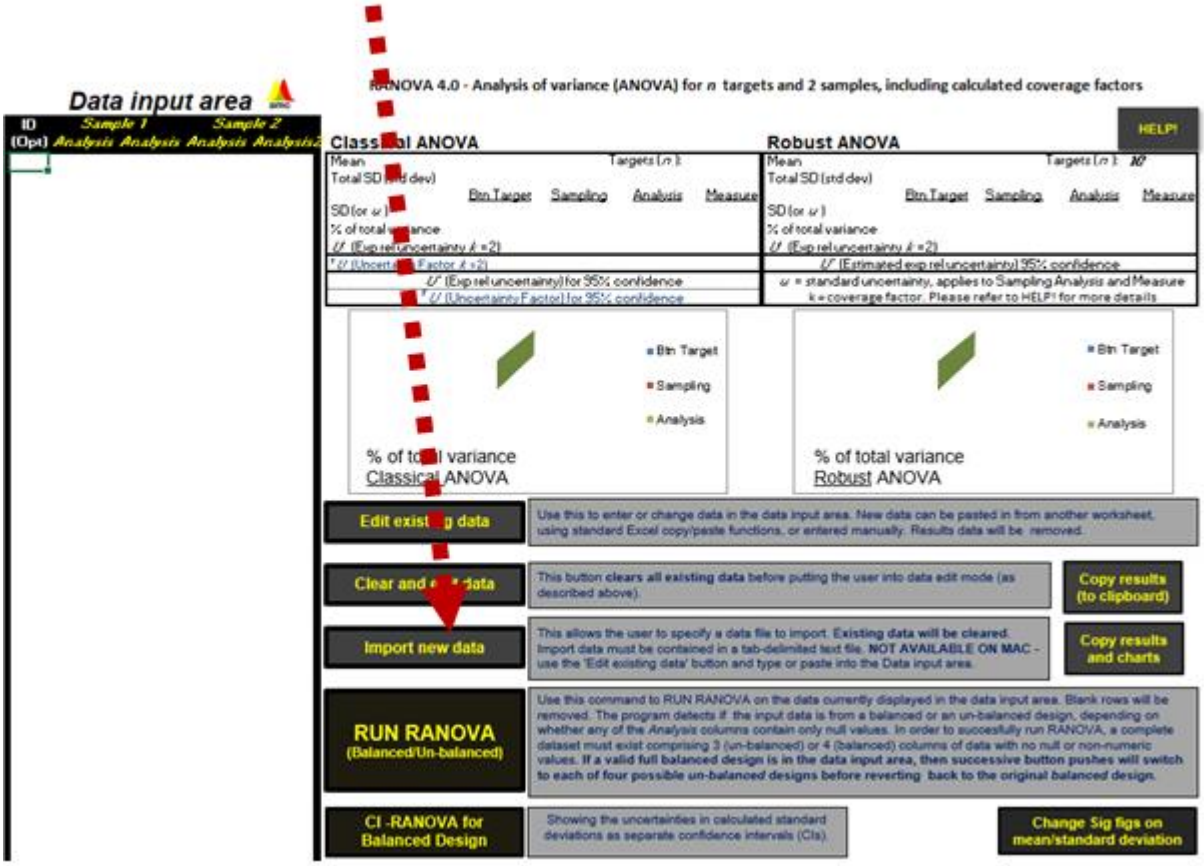
RANOVA 4.0 set up for an **unbalanced design** with three columns of measurements only. It will typically be the case that the fourth measurement column is cleared (*Sample 2, Analysis 2*), however any one of the four *Analysis* columns may be cleared for the **unbalanced design**.



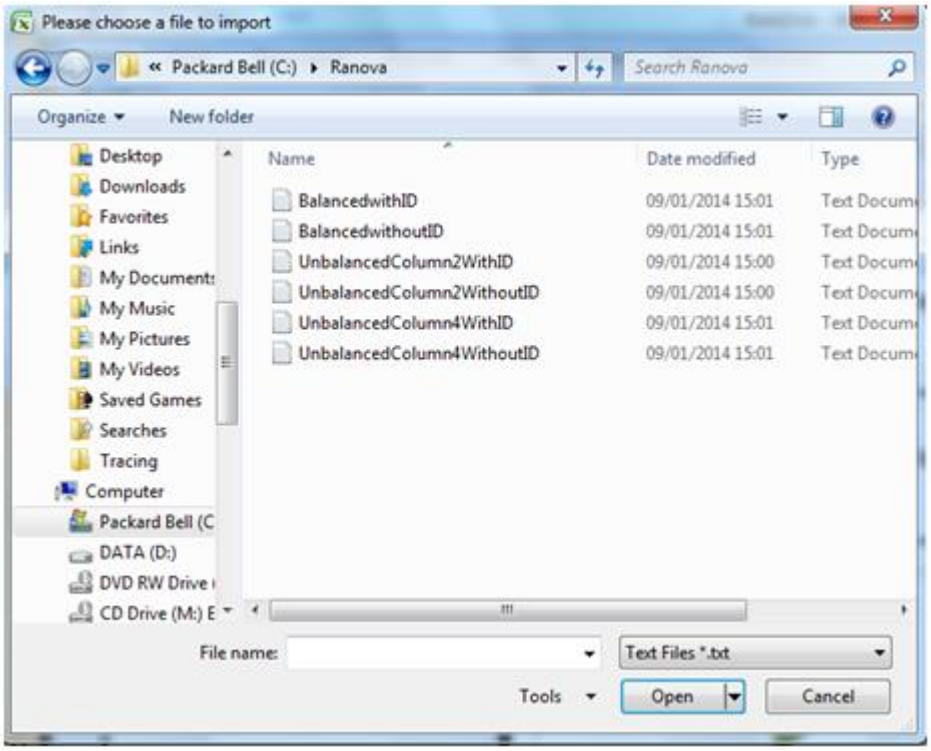
RANOVA 4.0 automatically detects an **unbalanced design**, but in order for this to work one of the four measurement columns must be COMPLETELY EMPTY, i.e. no zeros or spaces. The easiest way to ensure this is to select the whole area of the column to be cleared in the usual way (i.e. using the shift and arrow keys, or the mouse while holding the left button down) and then pressing the delete key.

3.3 Importing Data

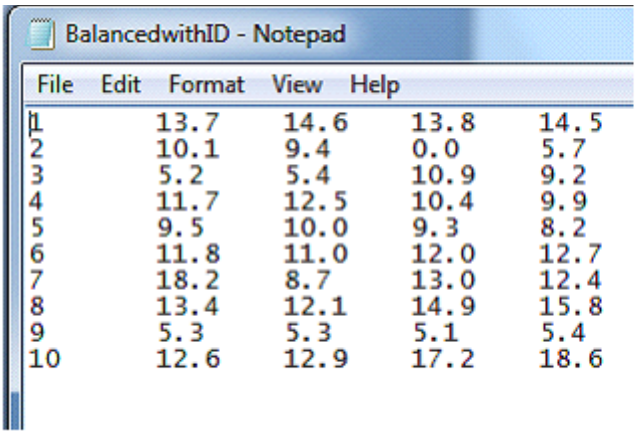
An alternative method of getting measurement data into RANOVA 4.0 is to use the *Import new data* button (Note this option is not available in Mac versions of Excel).



This command clears all data from the *data input area* (a warning message is displayed) and then shows a dialog box allowing the user to navigate to the relevant folder, e.g:

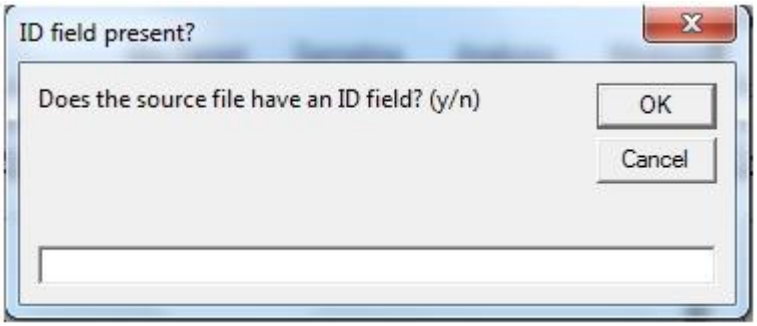


Navigate to the fie that you want to import. This must be a tab delimited text file with a .txt extension, e.g:



A tab-delimited text file opened in Windows Notepad, containing a **balanced experimental design** and measurement IDs in the left column.

The following message is then displayed:



If the first column of the tab-delimited text file contains an ID field (as shown in the example above) then enter a "y" into this box. If no ID column exists then enter a "n".

Press *OK* and data will be imported into the *data input area*. Note that no validation is performed at this stage, so data will be imported as it appears in the text file.

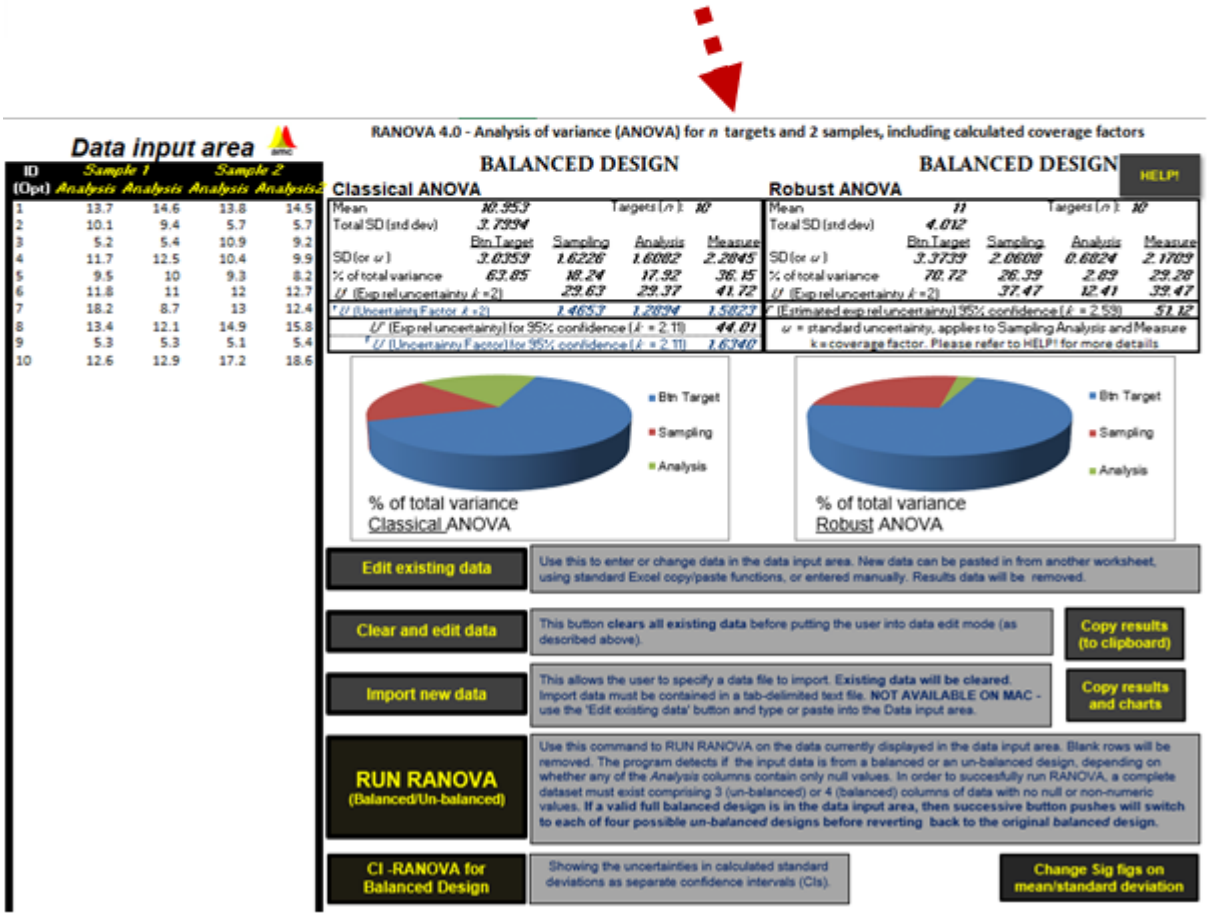
3.4 Running RANOVA4.0

*Running RANOVA 4.0 with a **balanced design** in the data input area*

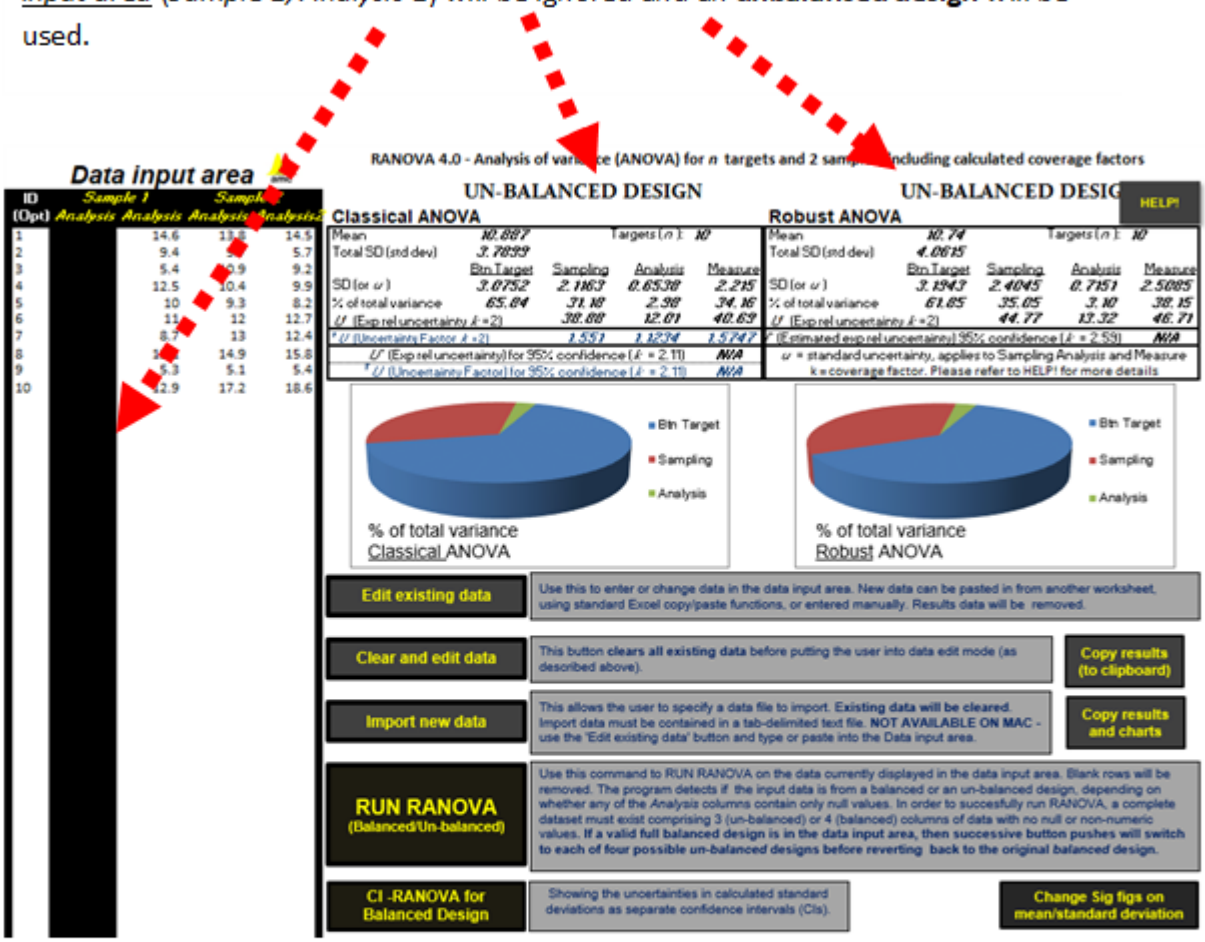
Pressing the *RUN RANOVA* button will instruct the program to attempt to perform analysis of variance on the measurements in the *data input area*. If a valid balanced or unbalanced experimental design has not been entered, then RANOVA 4.0 will report an error.

IMPORTANT! If any blank rows of measurements are embedded in the data, then these will be removed and a warning message given. It is important to take heed of this warning, especially if there is the possibility that data from a previous session could have accidentally been left further down in the Data input area, and so is not visible without scrolling down. This could lead to results that are not consistent with the intended dataset.

Here is an example of a full **balanced design**:



If the *RUN RANOVA* button is pressed again, then RANOVA 4.0 will repeat the ANOVA calculations, but this time the first column of measurements in the *data input area* (*Sample 1, Analysis 1*) will be ignored and an **unbalanced design** will be used.

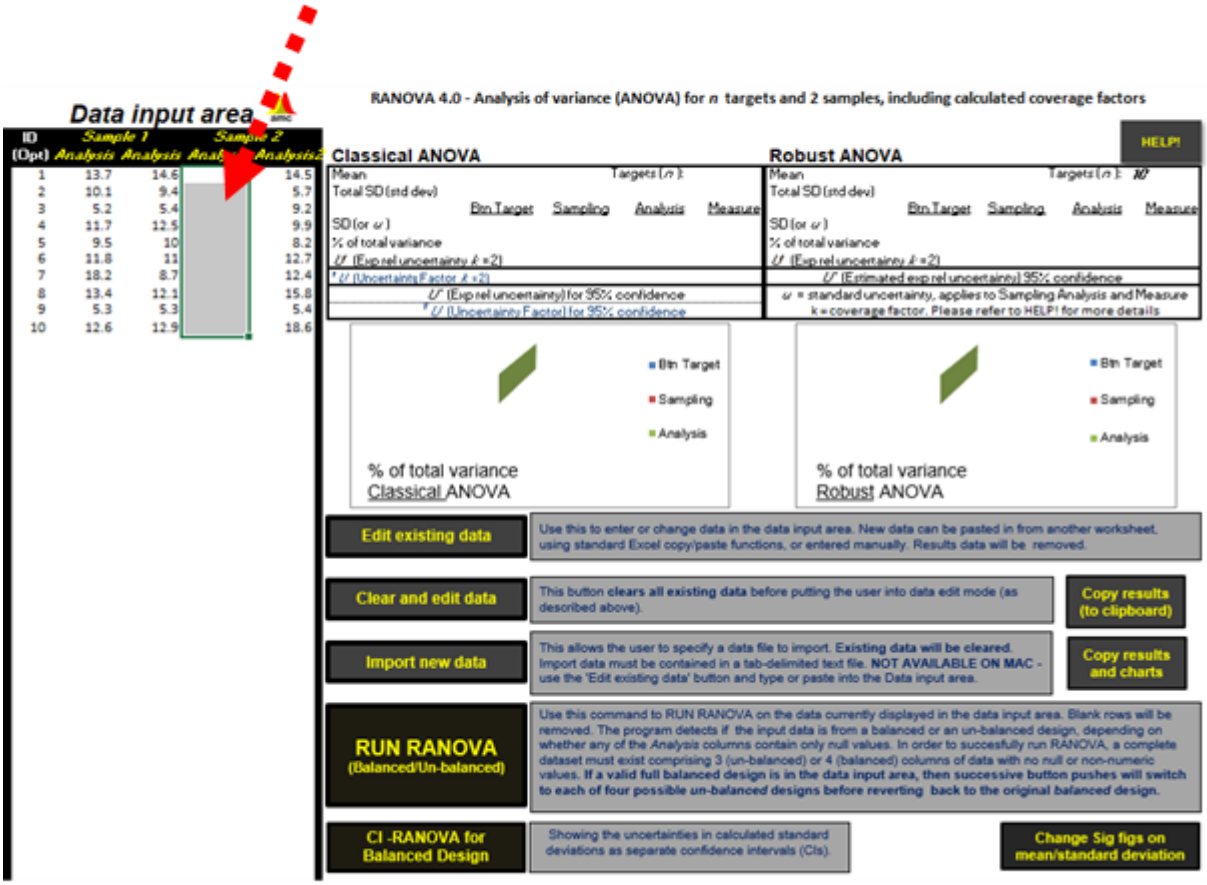


Successive presses of the *RUN RANOVA* button will sequentially suppress each column of measurements. So on the third press, *Sample 1 Analysis 2* will be ignored. On the fourth press, *Sample 2 Analysis 1* will be ignored, and on the fifth press *Sample 2 Analysis 4* will be ignored. Pressing the button a sixth time will revert to the full balanced design.

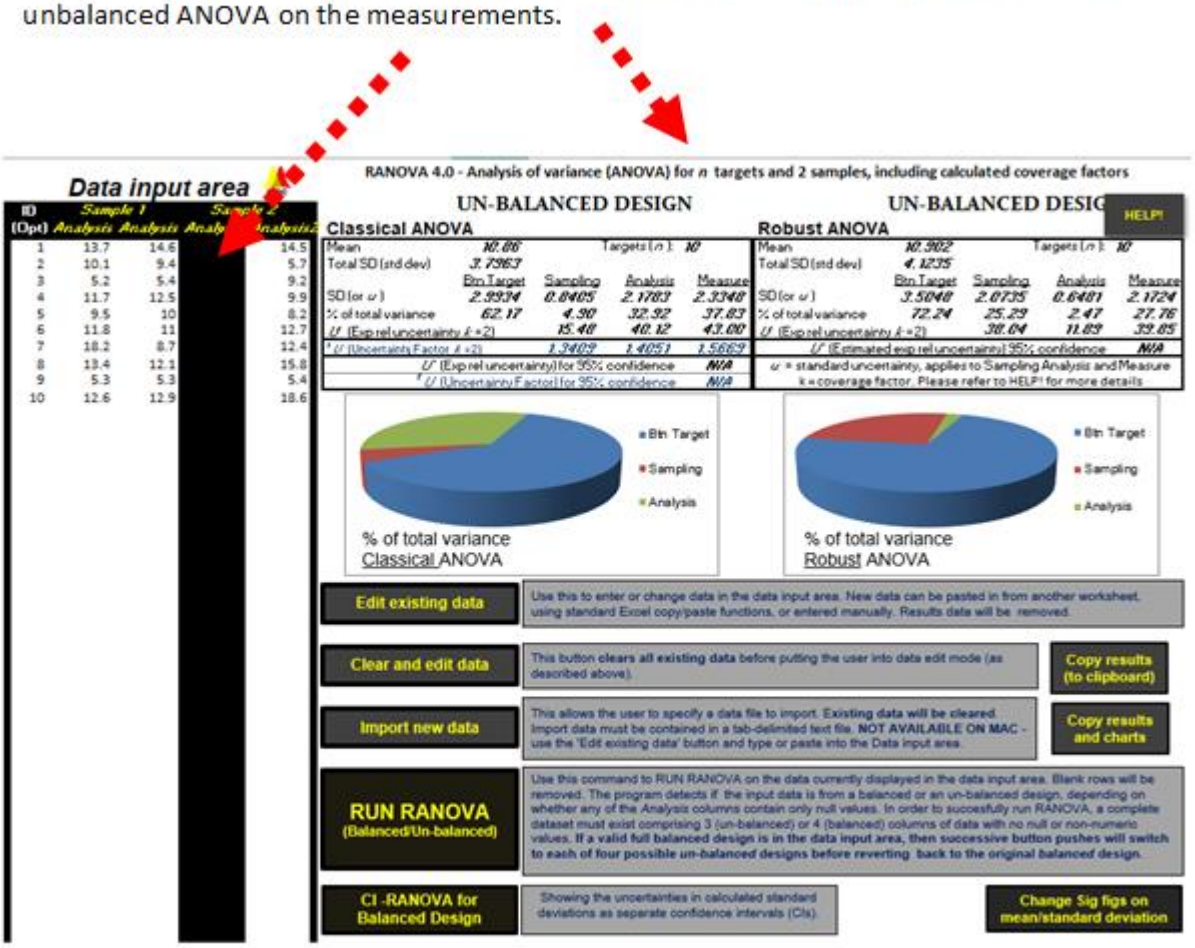
Note that the improved (or calculated) k-factors for expanded relative uncertainty at 95% confidence are not available for unbalanced experimental designs.

*Running RANOVA 4.0 with an **unbalanced design** in the data input area*

If one of the four columns in the *data input area* is empty, then the program will detect that an **unbalanced design** must be used. It would usually be that case that the fourth measurement column, corresponding to *Sample 2, Analysis 2* would be left empty, however RANOVA 4.0 allows any one of the four measurement columns to be empty.



When *RUN RANOVA* is pressed, RANOVA 4.0 blanks out the empty column and performs unbalanced ANOVA on the measurements.

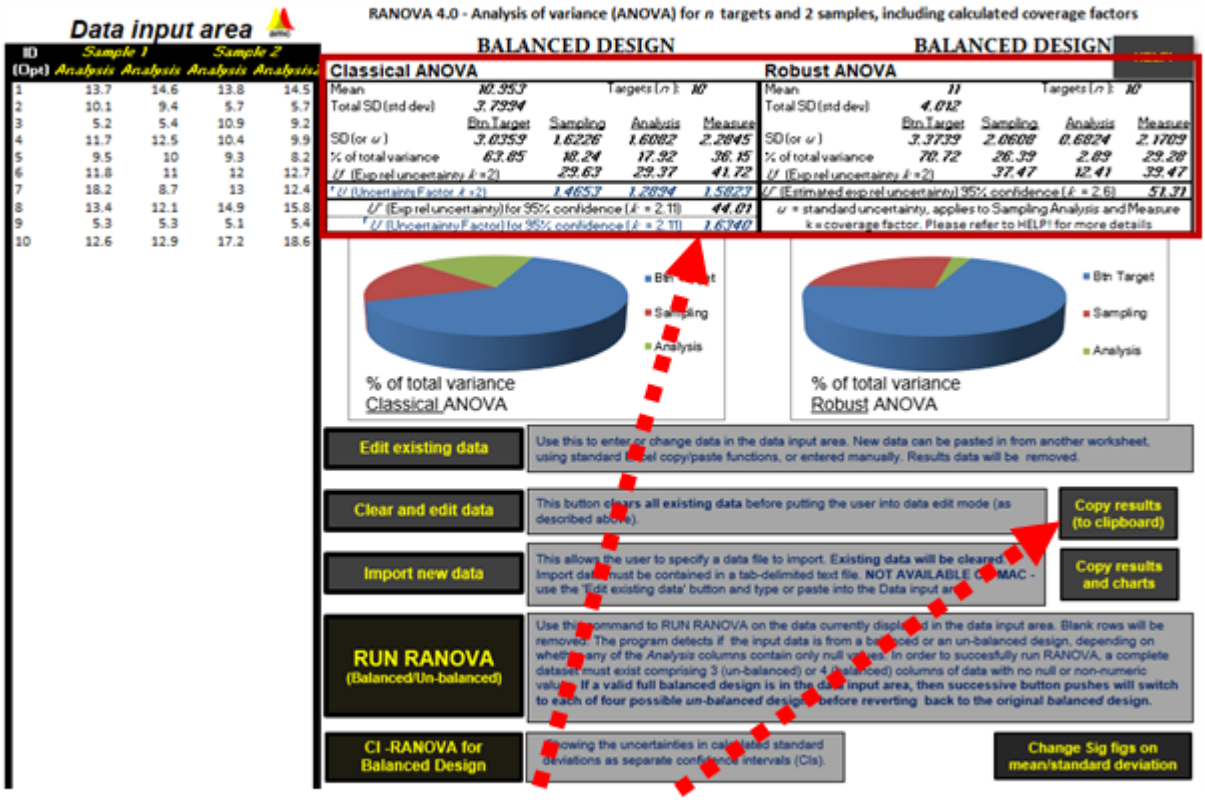


In this case, successive presses of the *RUN RANOVA* button will not have any effect, as there is only one valid experimental design in the *data input area*.

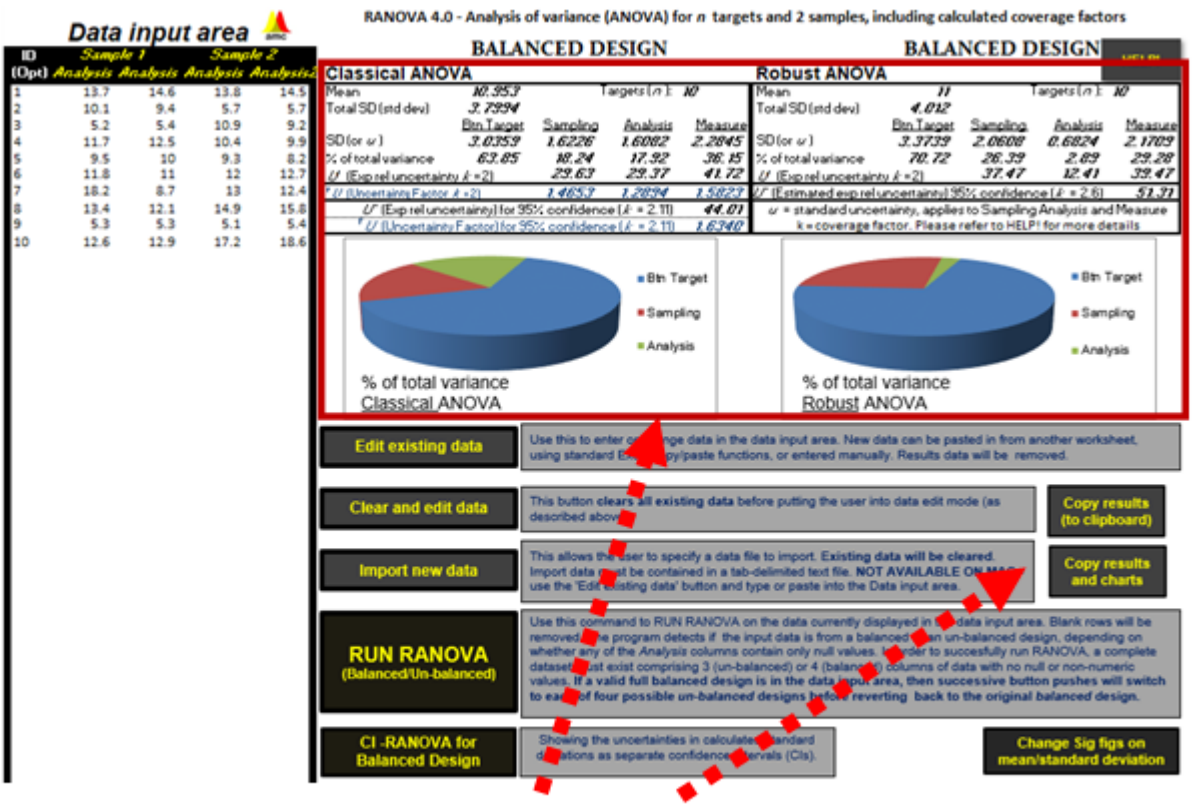
3.5 Copying Results

Copying RANOVA 4.0 Results to other applications

It may be desired to export the results to another application (e.g. another Excel spreadsheet). This can be achieved by pressing either of the buttons on the right hand side of the *control area*.



Pressing the top button copies the numerical results to the clipboard. They can then be immediately pasted into another spreadsheet or application.

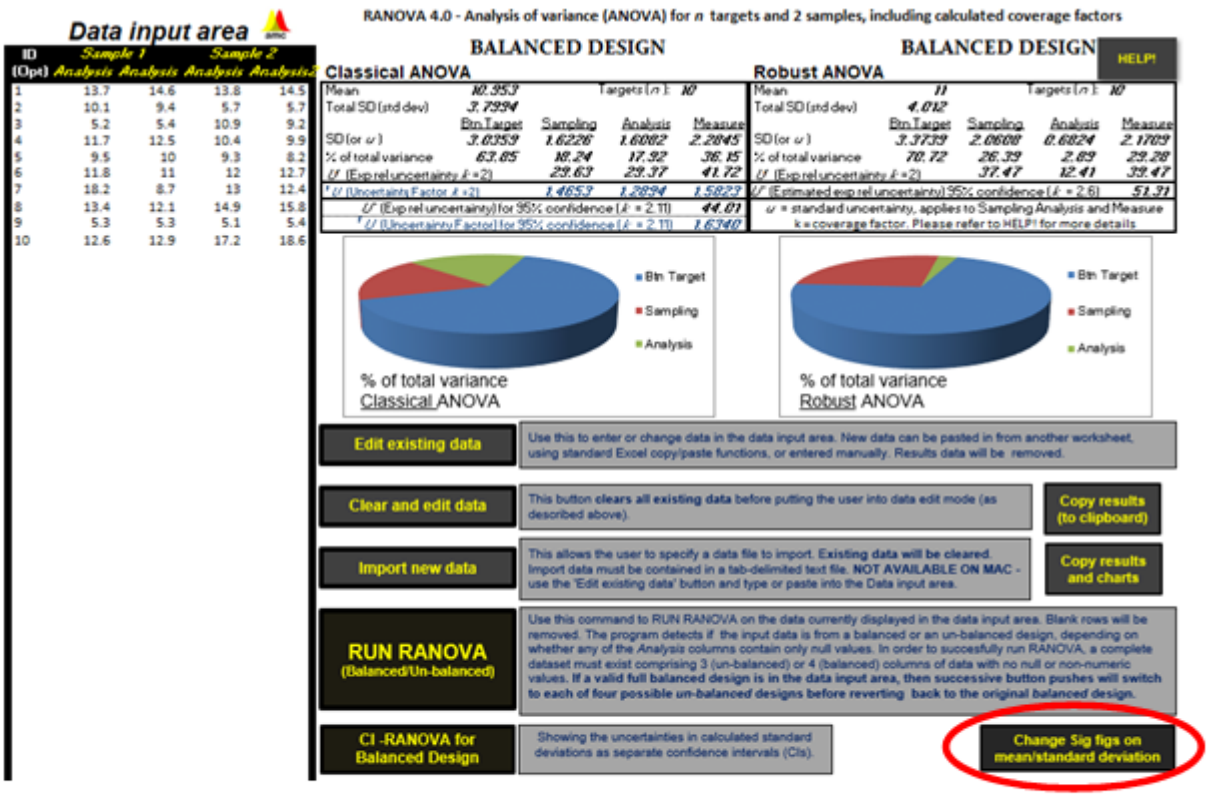


Pressing the lower button copies the numerical results and charts to the clipboard.

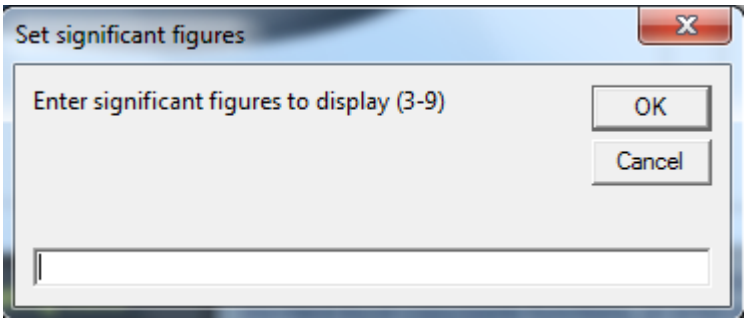
3.6 Changing Significant Figures

Changing the significant figures displayed in the Results section

It may be desirable to change the number of significant figures shown (the default is 5). This can be acheived by pressing the button labelled "Change Sig figs on mean/standard deviation" which is located at the bottom of the sheet.



On pressing this button, the following input box will be displayed:



A number between 3 and 9 must be entered. The new number of significant figures will be applied to the results section the next time the RANOVA button is pressed.

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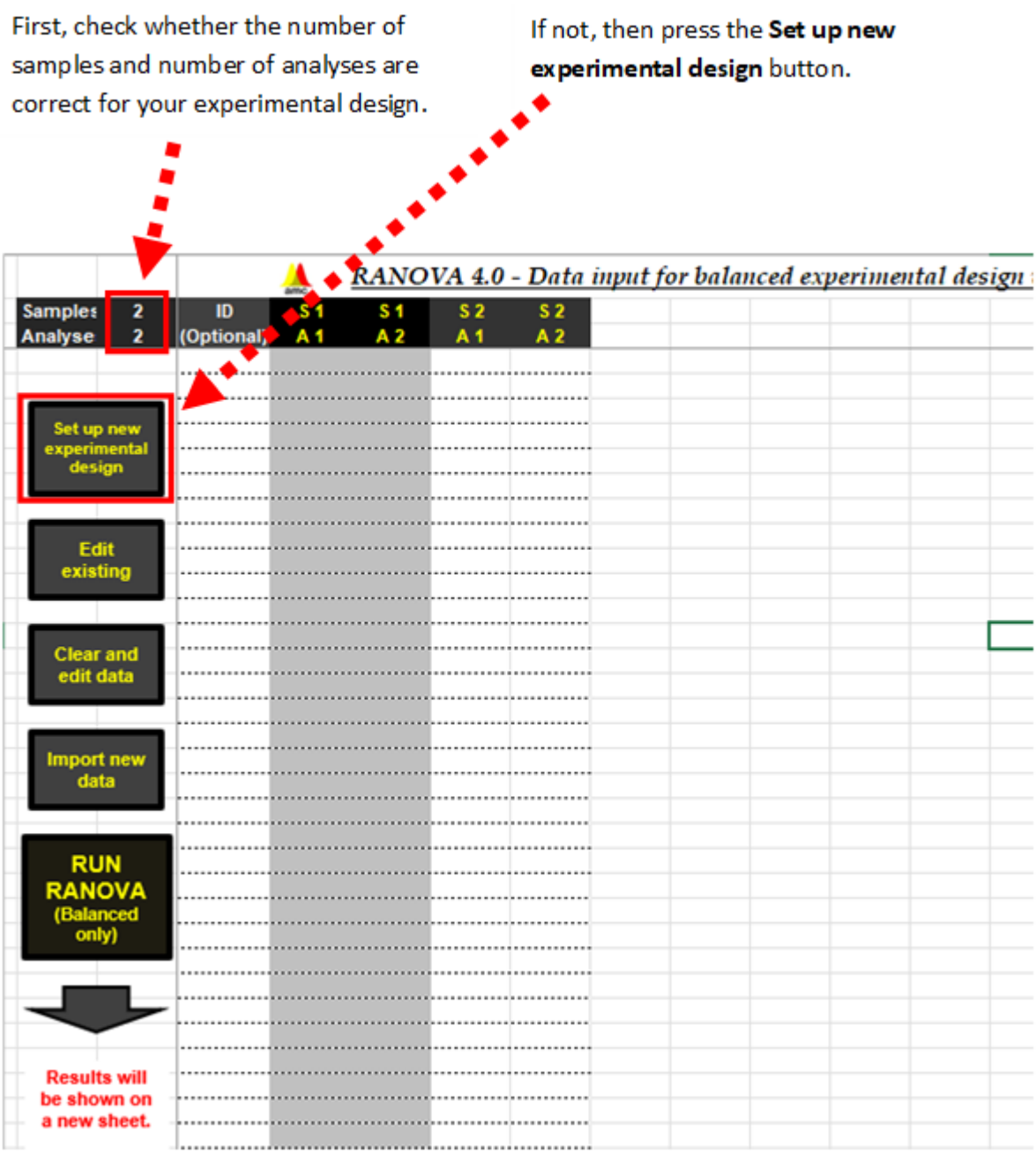
3.7 Extended Balanced Designs

Balanced *experimental design with* ***more than 2 samples, 2 analyses***

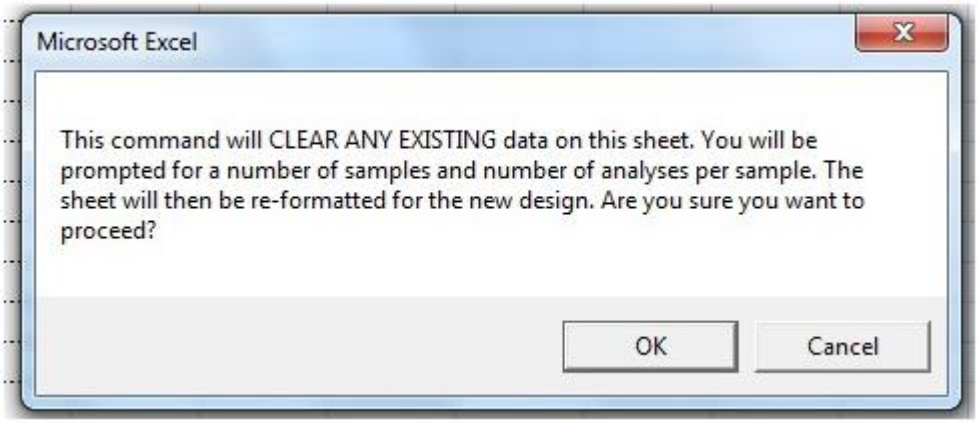
RANOVA 4.0 includes an additional sheet (Extended Balanced Design) to allow more than 2 samples and/or analyses to be specified in a **balanced** experimental design. Extended **unbalanced** designs are not currently allowed, although the unbalanced functions on the RANOVA sheet for a 2 sample, 2 analyses design remain fully operational.

In order to use the **Extended Balanced Design** sheet it is first necessary to specify the number of samples per target, and the number of analyses per sample. A minimum of two and a maximum of 8 are allowed for each, allowing for the possibility of 7 x 7 = 49 different experimental designs, including the simple 2 x 2 design already provided for on the **RANOVA** sheet.

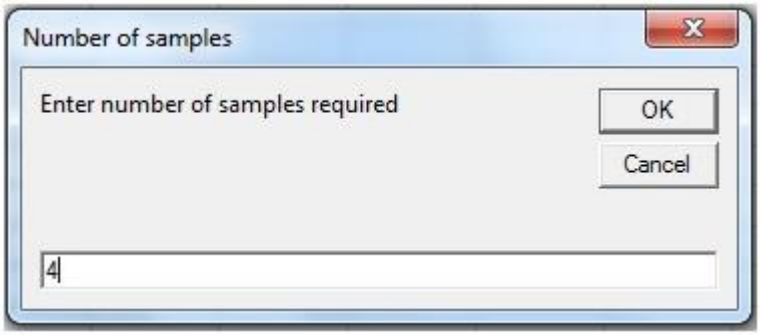
The following example explains how to set up and use this sheet with the data contained in the sample file ExtendedwithID.txt, which contains randomly generated simulated measurements for a 4 sample, 2 analyses experimental design with 9 targets.



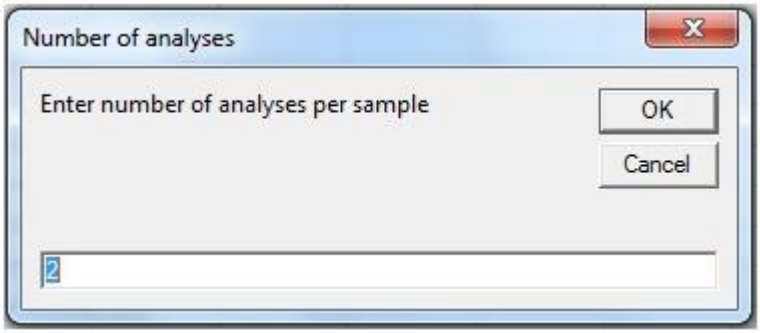
A message will be displayed warning that any existing measurement data will be cleared from the sheet.



If you select OK, you will then be prompted for the number of samples in the new experimental design (defaults to the last used). Enter the number 4 and press OK.



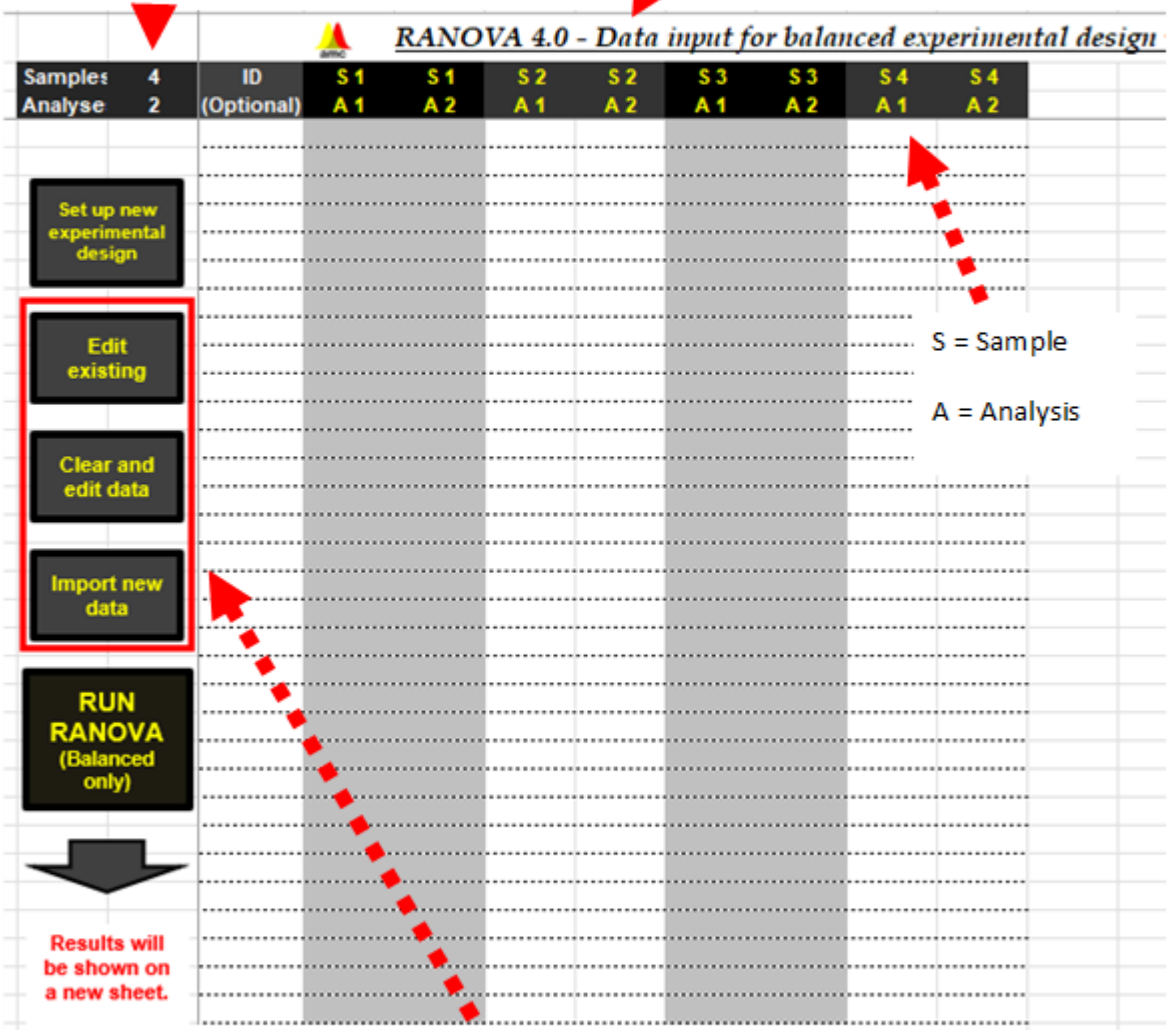
You will then be prompted for the number of analyses in the new experimental design. Enter the number 2 and press OK.



The Extended Balanced Design sheet will be set up for your chosen parameters. Note that it is **not** necessary to tell the program how many targets are to be used.

The numbers of samples and analyses are displayed here. Note these are for info only and cannot be directly changed.

The worksheet is formatted for the new experimental design. If a large number of samples and/or analyses is chosen, then the formatted area may extend beyond the visible part of the sheet. Use the slider at the bottom (not shown) to view to the right).



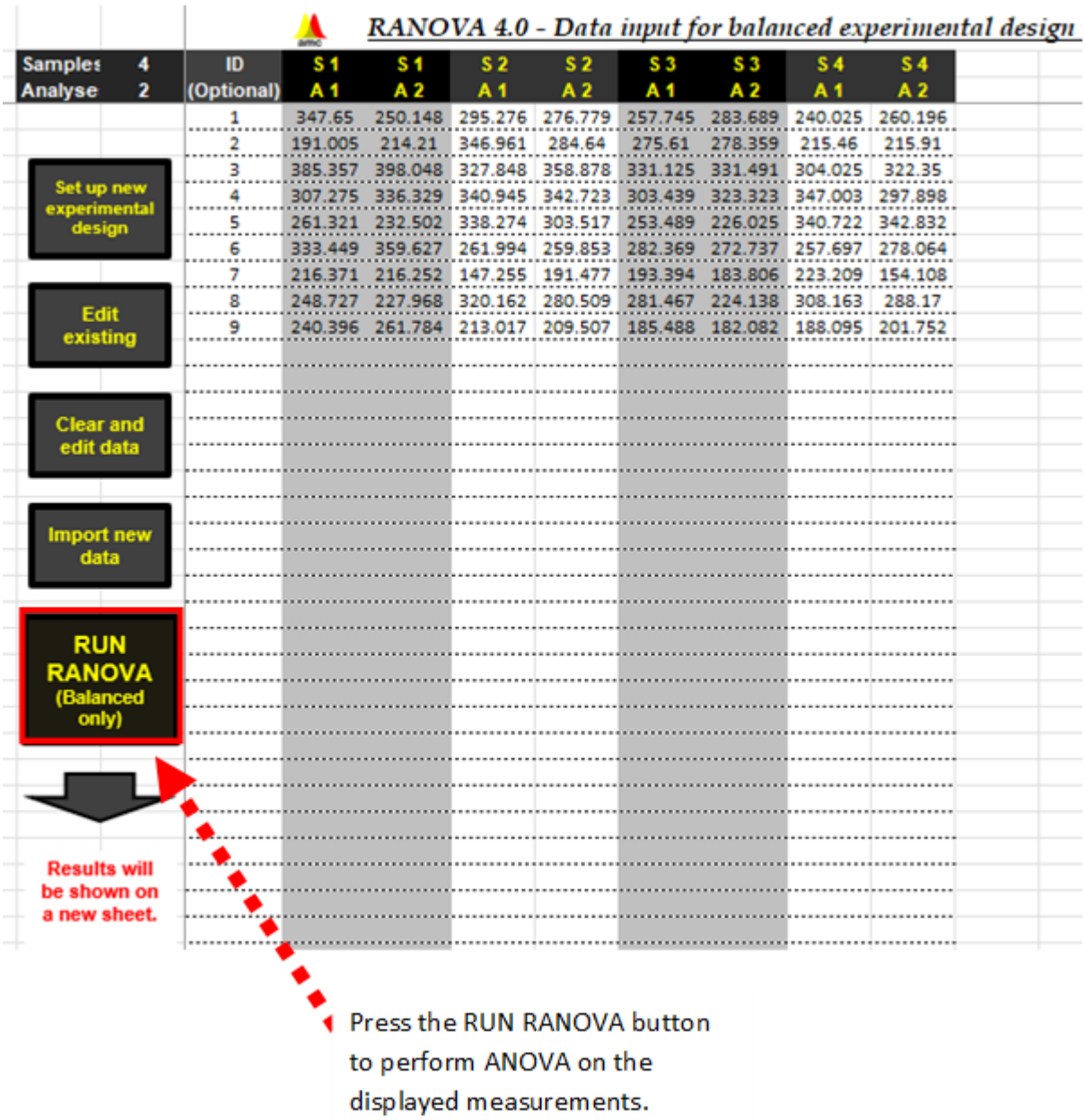
These three buttons work in a similar way to the buttons on the **RANOVA** sheet. See Sections 3.2 and 3.3 for full descriptions of their functions. Note that the **Import new data** button requires a tab-delimited text file in the correct format for the chosen experimental design. If this file contains an incorrect number of columns or an incomplete dataset then an error message will be displayed.

You may directly input or copy/paste data into the formatted area, however in this example we will import data from the file ExtendedwithID.txt. For details of the use of the import function refer to Section 3.3. Click the **Import new data button** and navigate to the RANOVA folder that was set up during installation. Select the file ExtendedwithID.txt. This file does contain an ID column so when the prompt "Does the source file have an ID

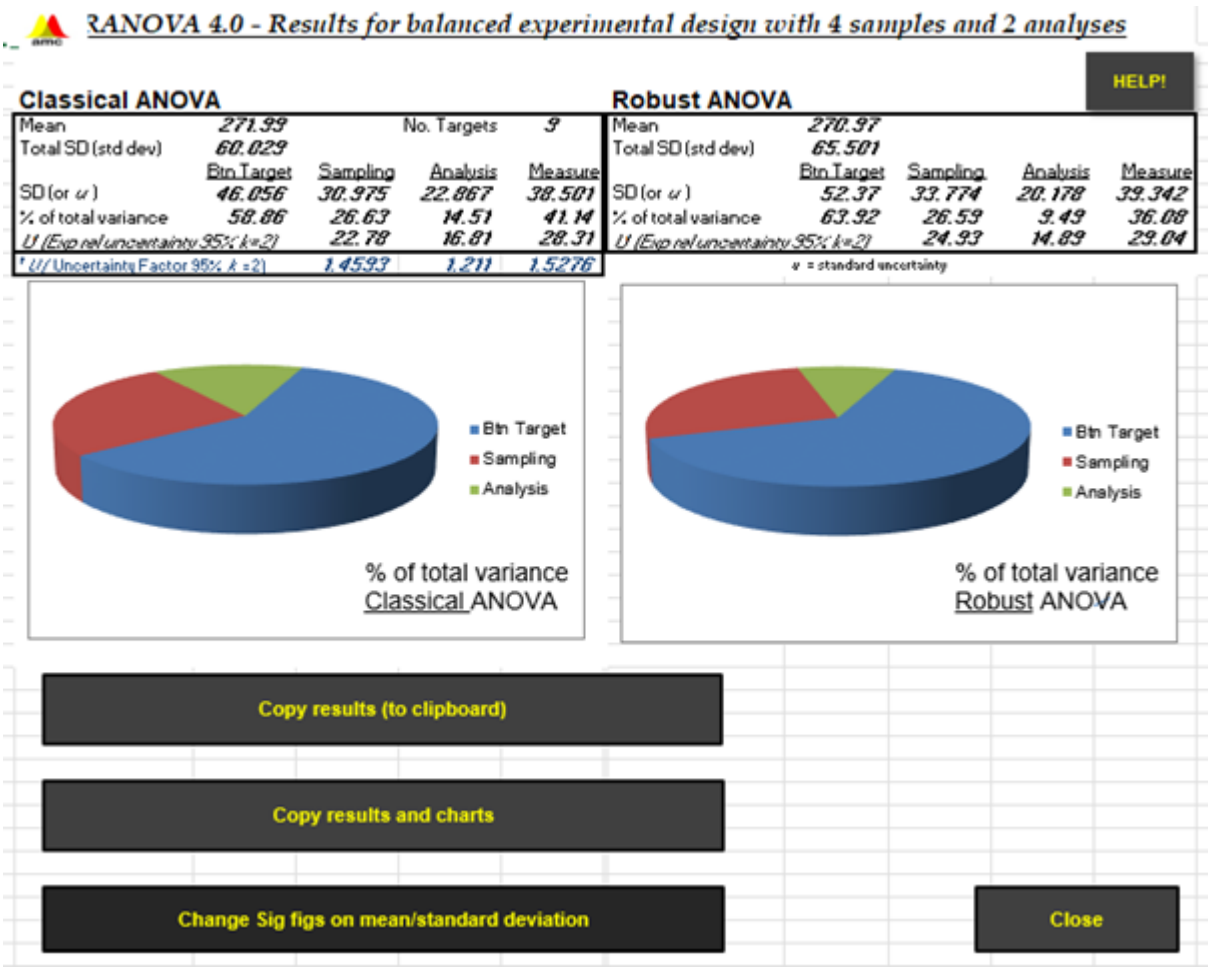
field? (y/n)" appears, enter a "y". If you enter "n" at this point then an error message will appear, because the expected number of columns will not match the number of columns in the source file.

Note that when using the Import new data function, or when copying measurements from another source, it is essential to be sure that the correct experimental design has been set up first. The example above uses an experimental design with 4 samples per target and 2 analyses per sample, resulting in (4 x 2 measurements per target) + the ID field = 9 columns of data. As far as the RANOVA program is concerned, a 9 column dataset would be equally valid for an experimental design comprising just 2 samples per target with 4 analyses per sample (plus ID), but this would produce different results when the ANOVA is run. The experimental design in use can easily be checked at any time by viewing the numbers of Samples and Analyses on the left of the grey header bar.

The measurements in the imported data file will be displayed in the formatted area. Note there are 9 targets in this example.



Results of the ANOVA will be displayed on a new sheet.



This is essentially the same information as provided by the Results area on the **RANOVA** sheet, and the buttons perform the same actions as described in the sections "Running RANOVA 4.0" and "Copying Results".

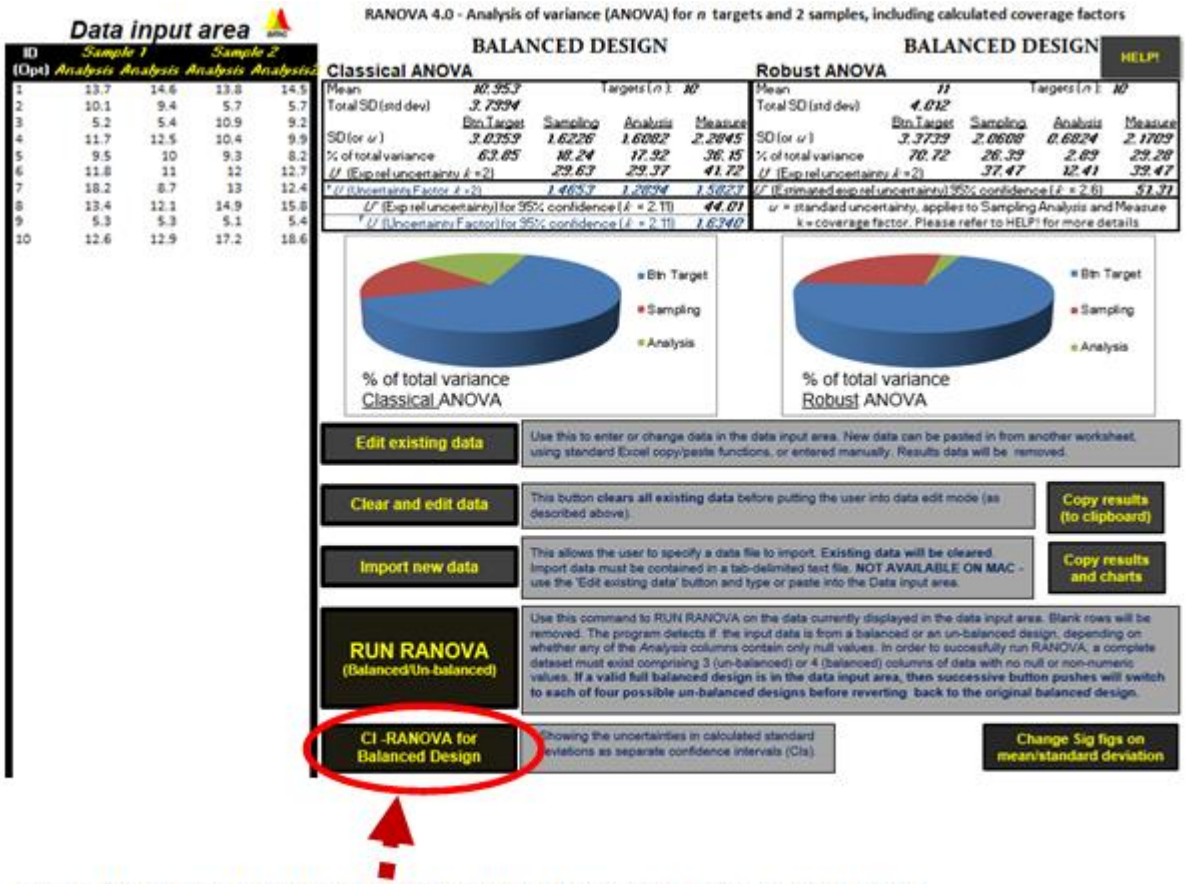
Note that this sheet is normally hidden and will only be visible when the RUN RANOVA button is pressed on the Extended Balanced Design Sheet. It will be hidden again once the user clicks on either of the other two sheets. This method ensures that there can never be any discrepancy between inputs and outputs, i.e. there can be no confusion about the source of the data displayed on the **Extended RANOVA** sheet. If you return to the **Extended Balanced Design** sheet to view the input data, then this sheet will temporarily disappear, but it can easily be viewed again by pressing the **RUN RANOVA** button.

Viewing inputs and outputs together could be achieved by copying the inputs and the outputs from the two sheets into another Excel sheet or other application.

3.8 Balanced design with Confidence Intervals

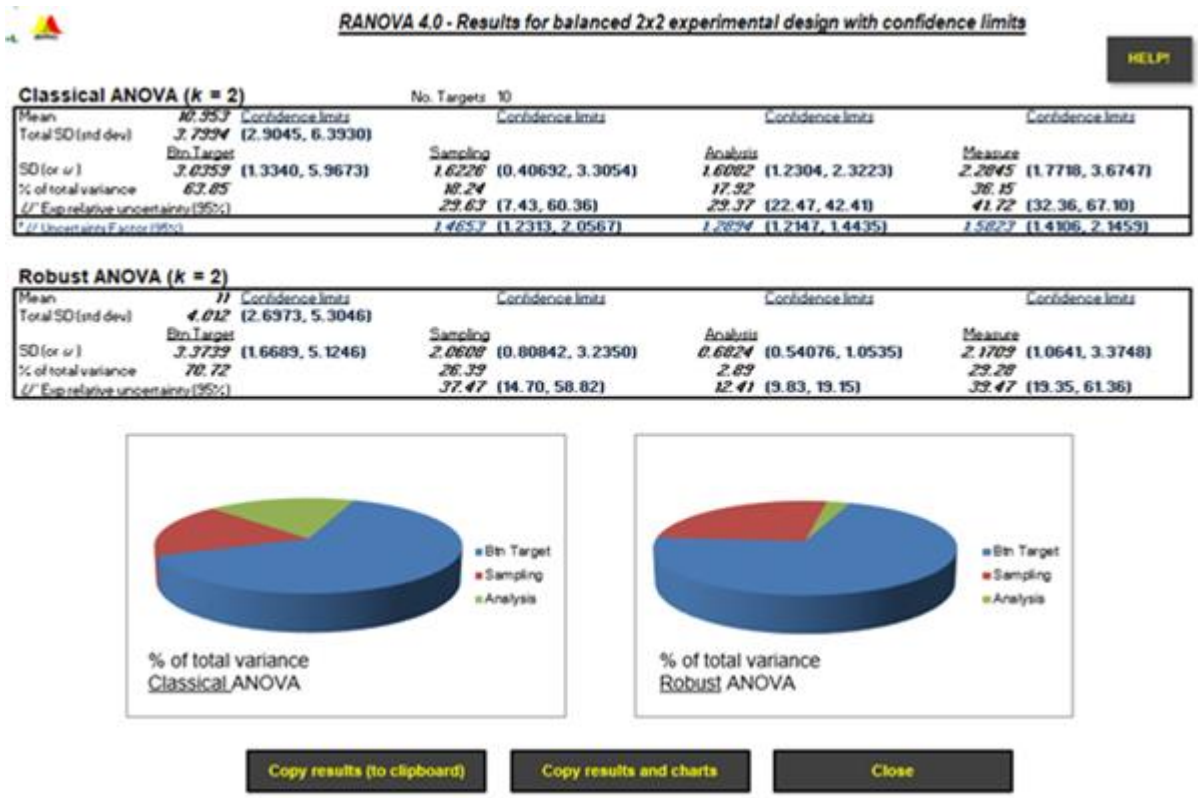
Confidence Intervals *on Nx2x2 balanced experimental design*

RANOVA 4.0 includes an option *CI RANOVA* to include estimates of 95% confidence intervals on the calculated variances.



This option is only available with a Nx2x2 balanced design in the *Data Input Area*. It is not available for unbalanced or extended designs

Results of the ANOVA with confidence limits will be displayed on a new sheet. Note that a red "Please Wait" message will be displayed beneath the pi-charts, this is due to the lengthy bootstrapping process required to calculate confidence limits for Robust ANOVA.



RANOVA 4.0 - Results for balanced 2x2 experimental design with confidence limits

HELP!

Classical ANOVA (*k* = 2) No. Targets 10

		Confidence limits		Confidence limits		Confidence limits		Confidence limits
Mean	10.953							
Total SD (std dev)	3.7994	(2.9045, 6.3930)						
	Btn Target		Sampling		Analysis		Measure	
SD (or u)	3.0359	(1.3340, 5.9673)	1.6226	(0.40692, 3.3054)	1.6082	(1.2304, 2.3223)	2.2845	(1.7718, 3.6747)
% of total variance	63.85		18.24		17.92		36.15	
U/ Exp relative uncertainty (95%)			29.63	(7.43, 60.36)	29.37	(22.47, 42.41)	41.72	(32.36, 67.10)
* U/ Uncertainty Factor (95%)			1.4653	(1.2313, 2.0567)	1.2894	(1.2147, 1.4435)	1.5823	(1.4106, 2.1459)

Robust ANOVA (*k* = 2)

		Confidence limits		Confidence limits		Confidence limits		Confidence limits
Mean	11							
Total SD (std dev)	4.012	(2.6973, 5.3046)						
	Btn Target		Sampling		Analysis		Measure	
SD (or u)	3.3739	(1.6683, 5.1246)	2.0608	(0.80842, 3.2350)	0.6824	(0.54076, 1.0535)	2.1709	(1.0641, 3.3748)
% of total variance	70.72		26.39		2.89		29.28	
U/ Exp relative uncertainty (95%)			37.47	(14.70, 58.82)	12.41	(9.83, 19.15)	39.47	(19.35, 61.36)

The buttons perform the same actions as described in the sections "Running RANOVA 4.0" and "Copying Results"

As with the output for extended balanced designs, this sheet is normally hidden and will only be visible when the CI RANOVA button is pressed from the RANOVA sheet.

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Disclaimer

The RANOVA 4.0 Excel workbook with its embedded macros are freely available on the RSC AMC website and may be downloaded for personal use. It has been tested using MS-Excel 2021 running on the Windows 10 operating system, however it is not guaranteed to function correctly on any operating system or Excel release, and future support will not be provided. Validation of the calculations of variances was carried out by comparing the output of 50 simulated data sets with that produced by ROBAN. Neither the authors nor the AMC accept liability for any loss or damage arising from the use of this software however caused.

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calculated from two estimated variance components. *Accred Qual Assur*
https://doi.org/10.1007/s00769-024-01579-w

12. Satterthwaite FE (1946) An approximate Distribution of Estimates of Variance Components. Biometrics Bulletin, 2(6), pp110-114
https://doi.org/10.2307/3002019

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