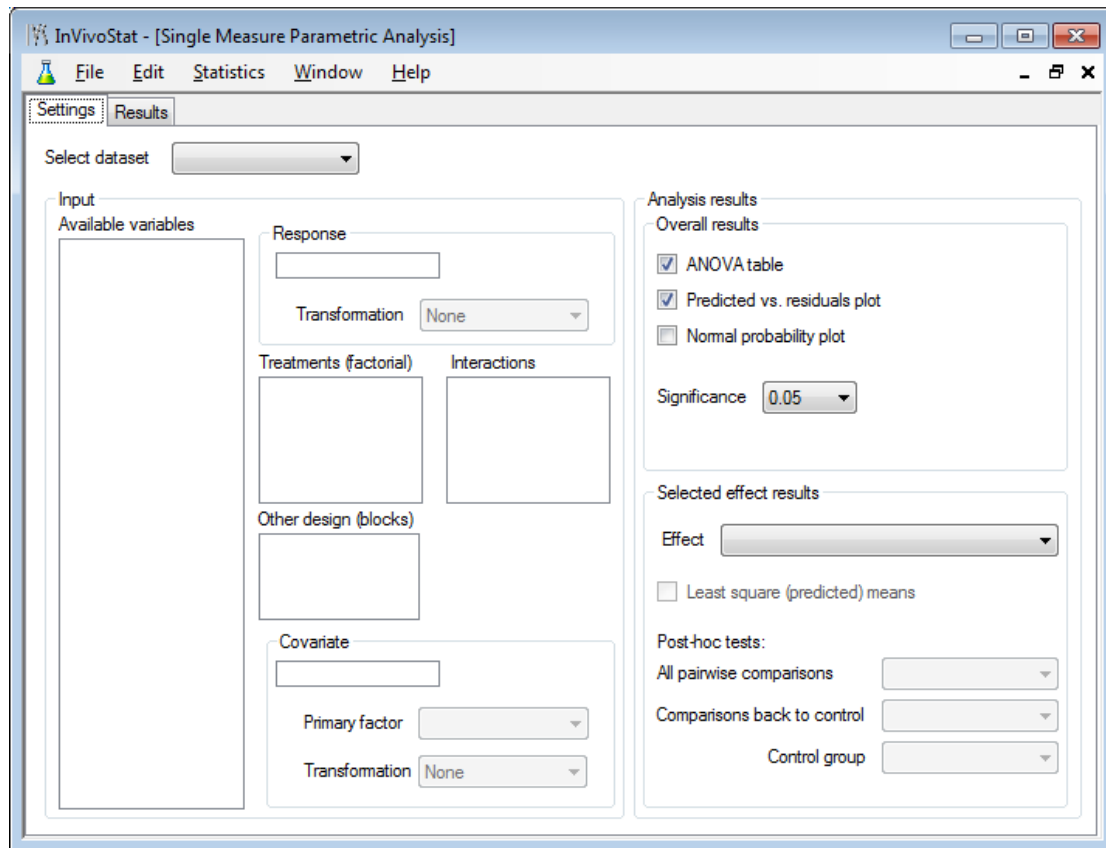The Single Measures Parametric Analysis module in SilveR is available from the Statistics drop-down menu entitled "Single Measures Parametric Analysis". The user interface is:

The Single Measures Parametric Analysis module performs a General Linear Model analysis. This allows the user to fit multiple treatment (factorial) factors, other design (blocks) factors and a single covariate. All interactions involving the treatment factors are included in the statistical model but none of the interactions involving the blocking factors are included.

1. Setting up the model

Once the dataset has been opened, the user can select the variables for the analysis by dragging and dropping them from the "Available variables" list into the "Response", "Treatments (factorial)", "Other design (blocks)" and "Covariate" boxes.

Once selected, the user has the option of applying a transformation to the response variable, either $\log_{10}$, $\log_e$, square root, arcsine or rank. If selected the covariate will be transformed using the same transformation, unless otherwise specified by the user.

If a covariate is selected, then the user has the option of selecting the "Primary factor". This factor is used to categorise the scatterplot (produced in the output). The Primary factor should be one of the factors of interest to the experimenter.

2. Selecting the analysis options

There are several results from the General Linear Model analysis that are available to the user. These are selected before running the analysis. These include the overall tests

1) ANOVA table
Produces overall tests of the effects of the factors.

2) Predicted vs. residuals plot
Allows the user to check the variance assumption of the parametric analysis.

3) Normal probability plot
Allows the user to check the normality assumption of the parametric analysis.

4) Significance level
The default is 5%, although this can be changed.

The user then has various other options available:

5) Selected effect
This is the effect that the user is interested in comparing the levels of.

6) Least square means
These are the predicted means from the statistical analysis and take into account all effects in the statistical model, such as the covariate.

7) All pairwise tests
Produces all pairwise comparisons between the levels of the Selected effect. The user has the option of adjusting the p-values for multiple comparisons. Options include Holm, Hochberg, Hommel, Benjamini-Hochberg, Tukey or Bonferonni.

8) Comparisons back to control
Produces all to one comparisons between the levels of the Selected effect. The user should select the control group from the drop down list. This option is only available if the user selects a main effect as the Selected effect. The user has the option of adjusting the p-values for multiple comparisons. Options include Holm, Hochberg, Hommel, Benjamini-Hochberg, Dunnett or Bonferonni.

3. Output Results

Response and covariate
SilveR identifies the response being analysed and also the covariate (if one is selected). This section also describes any transformations that have been applied.

Scatterplot of the raw data
SilveR produces a scatterplot of the raw data. This should be used to identify possible outliers. On the plot the X-axis corresponds to the levels of the highest order interaction between the treatment factors and the Y-axis corresponds to the response.

Categorised scatterplot of the raw data (ANCOVA only)
When fitting a covariate in a statistical analysis, certain assumptions are made. This plot allows the user to test these assumptions. Underneath the plot is a list of the assumptions and also advice on how the plot can be used to assess them.

ANOVA table
The ANOVA table gives tests of the overall effects of the model effects. SilveR presents the Type II model fit as this was felt to be a reasonably robust approach. Below the table any statistically significant effects are listed.

Diagnostic plots
If requested SilveR produces the predicted vs. residual plots and the normal probability plot. The residuals plotted on the predicted vs. residuals plot are the standardized residuals as these can provide a test for outliers. Any observation with a residual greater (or less than) 3 could be considered an outlier.

Plot of the predicted means
SilveR produces a plot and table of the least square means from the model, with confidence intervals.

All pairwise tests
SilveR produces a table of all pairwise comparisons between the levels of the Selected effect (either a main effect or an interaction). As well as the size of the difference between the means, SilveR also provides p-values to test these differences either unadjusted for multiplicity or with a multiple comparison adjustment. A list of statistically significant comparisons is given below the table.

All to one comparisons
SilveR produces a table of all to one pairwise comparisons between the levels of the Selected effect. As well as the size of the difference between the means, along with confidence limits, SilveR provides p-values either unadjusted for multiplicity or with a multiple comparison adjustment.

References
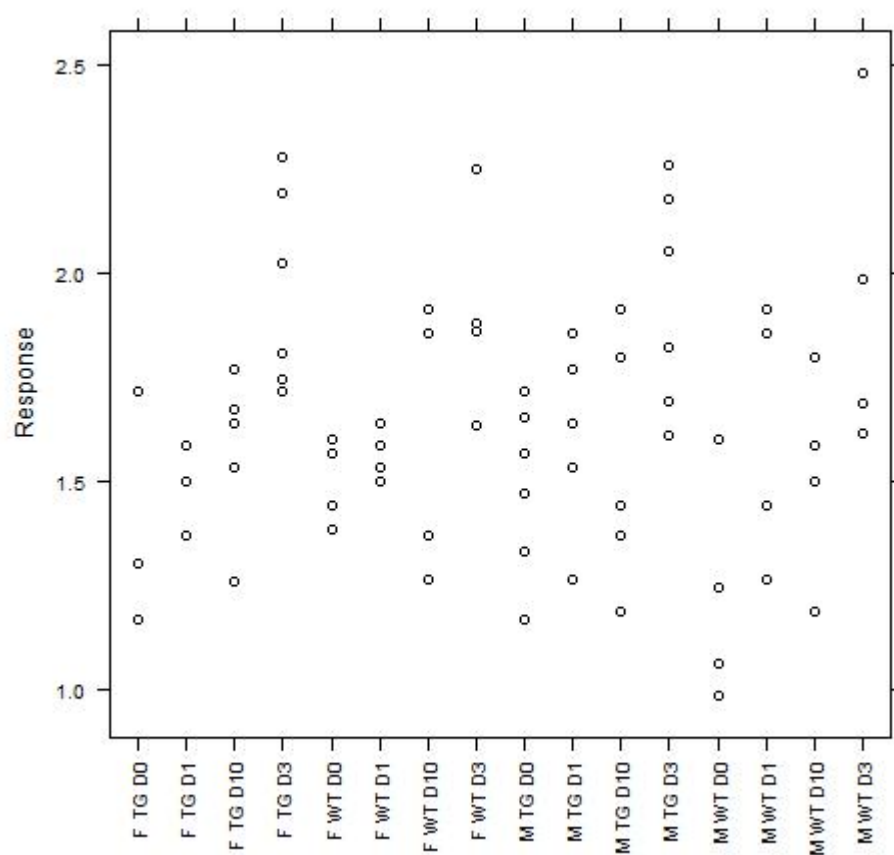Finally a list of references for the methods applied in the analysis is given.

4. Sample output

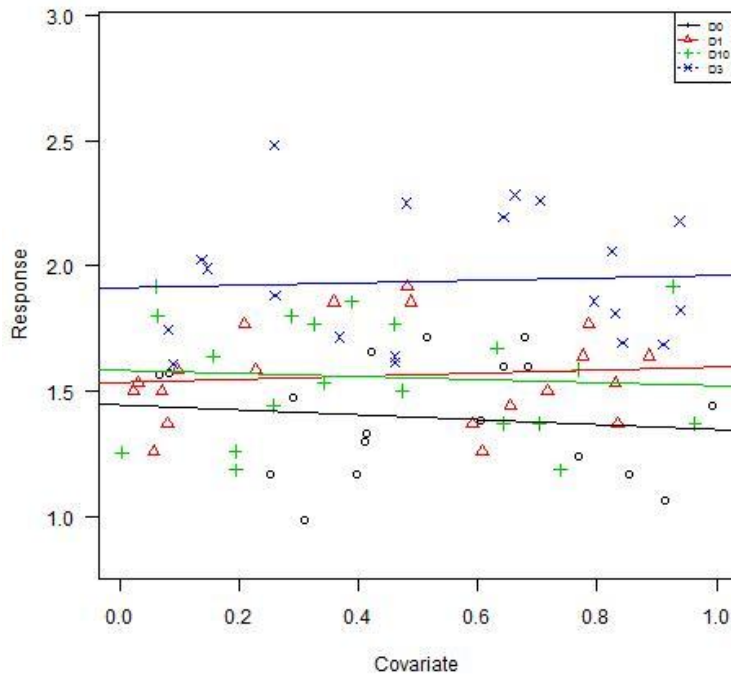## SilveR Single Measure Parametric Analysis

Response and covariate

The Response response is currently being analysed by the Single Measures Parametric Analysis module, with Covariate fitted as a covariate.

Scatterplot of the raw data

Tip: Use this plot to identify possible outliers.

Covariate plot of the raw data

Tip: Is it worth fitting the covariate? You should consider the following:

a) Is there a relationship between the response and the covariate?... It is only worth fitting the covariate if there is a strong positive (or negative) relationship between them. The lines on the plot should not be horizontal.

b) Is the relationship similar for all treatments?... The lines on the plot should be approximately parallel.

c) Is the covariate influenced by the treatment?... We assume the covariate is not influenced by the treatment so there should be no separation of the treatment groups along the x-axis on the plot.

These issues are discussed in more detail in Morris (1999).

Analysis of Covariance (ANCOVA) table

	Sums of Squares	Degrees of Freedom	Mean Square	F-value	p-value
Covariate	0.00	1	0.00	0.02	0.8834
Sex	0.00	1	0.00	0.00	0.9803
Strain	0.00	1	0.00	0.04	0.8519
Treatment	3.07	3	1.02	16.99	< 0.001
Sex:Strain	0.06	1	0.06	1.03	0.3149
Sex:Treatment	0.14	3	0.05	0.76	0.5193
Strain:Treatment	0.03	3	0.01	0.17	0.9157
Sex:Strain:Treatment	0.16	3	0.05	0.89	0.4522
Residuals	3.67	61	0.06		

Comment: ANCOVA table calculated using a Type II model fit, see Armitage et al. (2001).
Conclusion: There is a statistically significant overall difference between the levels of Treatment.
Tip: While it is a good idea to consider the overall tests in the ANCOVA table, we should not rely on them when deciding whether or not to make pairwise comparisons between the factor levels.

Diagnostic plots

Predicted vs. residuals plot
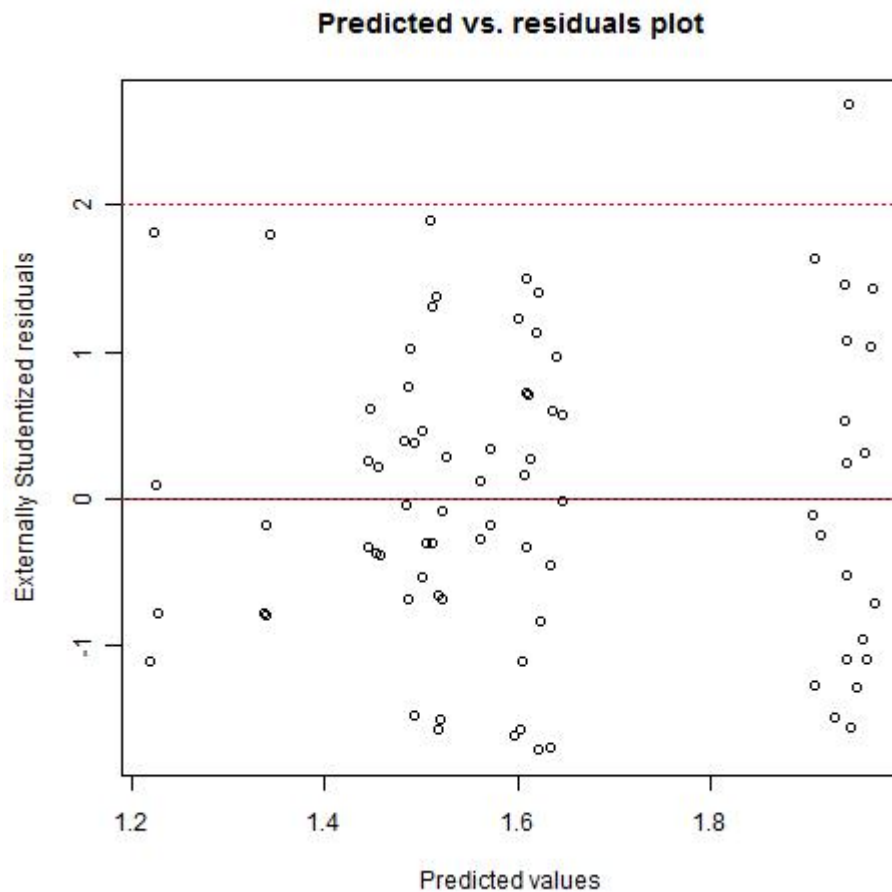
Tip: On this plot look to see if the spread of the points increases as the predicted values increase. If so the response may need transforming.
Tip: Any observation with a residual less than -3 or greater than 3 (SD) should be investigated as a possible outlier.

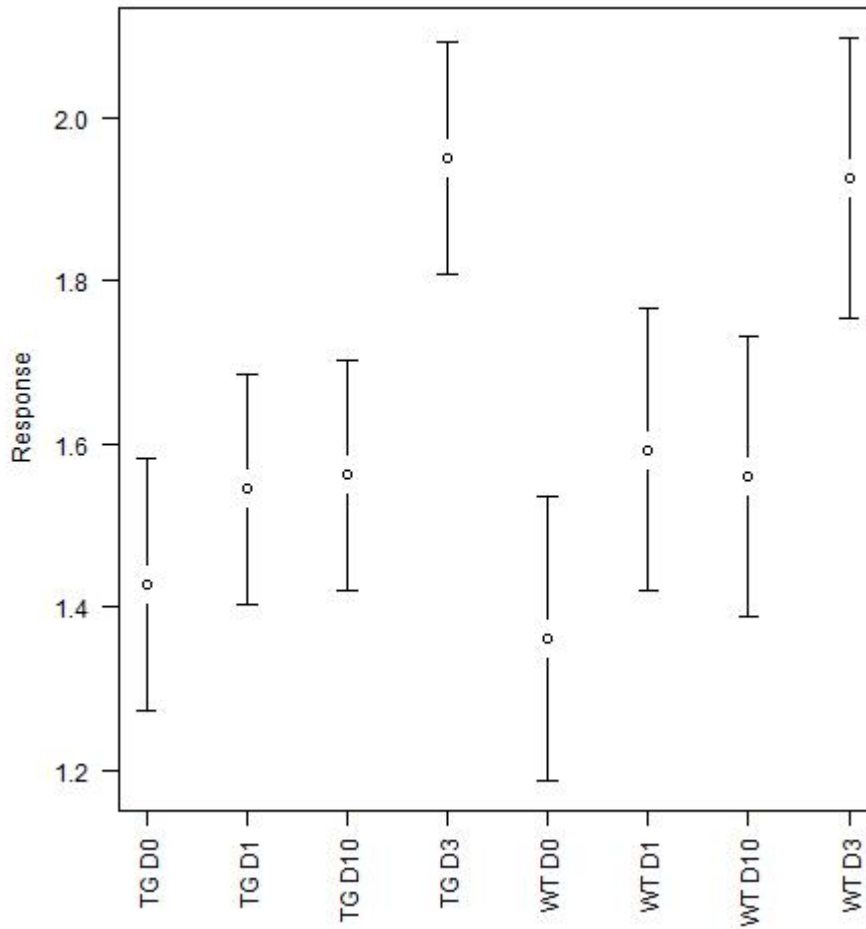### Plot of the predicted means with 95% confidence intervals

Table of the predicted means with 95% confidence intervals

Level	Mean	Lower 95% CI	Upper 95% CI
TG D0	1.43	1.27	1.58
TG D1	1.54	1.40	1.69
TG D10	1.56	1.42	1.70
TG D3	1.95	1.81	2.09
WT D0	1.36	1.19	1.54
WT D1	1.59	1.42	1.76
WT D10	1.56	1.39	1.73
WT D3	1.93	1.75	2.10

All pairwise comparisons without adjustment for multiplicity (LSD tests)

Comparison	Difference	Lower 95% CI	Upper 95% CI	SEM	p-value
TG D1 vs. TG D0	0.12	-0.09	0.33	0.104	0.268
TG D10 vs. TG D0	0.13	-0.07	0.34	0.104	0.206
TG D3 vs. TG D0	0.52	0.31	0.73	0.106	< 0.001
WT D0 vs. TG D0	-0.07	-0.30	0.17	0.117	0.572
WT D1 vs. TG D0	0.16	-0.07	0.40	0.116	0.159
WT D10 vs. TG D0	0.13	-0.10	0.36	0.116	0.257
WT D3 vs. TG D0	0.50	0.27	0.73	0.116	< 0.001
TG D10 vs. TG D1	0.02	-0.18	0.22	0.099	0.866
TG D3 vs. TG D1	0.41	0.20	0.61	0.101	< 0.001
WT D0 vs. TG D1	-0.18	-0.41	0.04	0.113	0.110
WT D1 vs. TG D1	0.05	-0.17	0.27	0.111	0.667
WT D10 vs. TG D1	0.02	-0.21	0.24	0.111	0.888
WT D3 vs. TG D1	0.38	0.16	0.60	0.111	0.001
TG D3 vs. TG D10	0.39	0.19	0.59	0.101	< 0.001
WT D0 vs. TG D10	-0.20	-0.43	0.03	0.113	0.082
WT D1 vs. TG D10	0.03	-0.19	0.25	0.111	0.780
WT D10 vs. TG D10	0.00	-0.22	0.22	0.111	0.992
WT D3 vs. TG D10	0.36	0.14	0.59	0.111	0.002
WT D0 vs. TG D3	-0.59	-0.81	-0.37	0.111	< 0.001
WT D1 vs. TG D3	-0.36	-0.58	-0.13	0.112	0.002
WT D10 vs. TG D3	-0.39	-0.61	-0.17	0.112	< 0.001
WT D3 vs. TG D3	-0.02	-0.25	0.20	0.112	0.830
WT D1 vs. WT D0	0.23	-0.01	0.48	0.123	0.063
WT D10 vs. WT D0	0.20	-0.05	0.44	0.123	0.110
WT D3 vs. WT D0	0.56	0.32	0.81	0.123	< 0.001
WT D10 vs. WT D1	-0.03	-0.28	0.21	0.122	0.791
WT D3 vs. WT D1	0.33	0.09	0.58	0.122	0.008
WT D3 vs. WT D10	0.37	0.12	0.61	0.122	0.004

Conclusion: The following pairwise tests are statistically significantly different at the 5% level: TG D3 vs. TG D0, WT D3 vs. TG D0, TG D3 vs. TG D1, WT D3 vs. TG D1, TG D3 vs. TG D10, WT D3 vs. TG D10, WT D0 vs. TG D3, WT D1 vs. TG D3, WT D10 vs. TG D3, WT D3 vs. WT D0, WT D3 vs. WT D1, WT D3 vs. WT D10.

Warning: It is not advisable to draw statistical inferences about an effect if there is a significant higher-order interaction involving that effect. In the above table we have assumed that certain higher order interactions are not significant and have removed them from the statistical model, see log for more details.

Warning: As these tests are not adjusted for multiplicity there is a risk of false positive results. Only use the pairwise tests you planned to make a-priori, these are the so called Planned Comparisons, see Snedecor and Cochran (1989).

Analysis description

The data were analysed using a 3-way ANCOVA approach, with Sex, Strain and Treatment as treatment factors and Covariate as the covariate. This was followed by Planned Comparisons on the predicted means to compare the levels of the selected effect.

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