

Fit Prob[™] offers an *effective* alternative to the ineffective R² metric for nonlinear curve fitting

Fit Probability:

- Designed for nonlinear curve fitting
- Measures how closely the curve fit matches the true curve
- Provides reliable metric for curve fit quality

R² can be a lousy measure of goodness-of-fit, especially when it is misused...
Charles Annis, P.E., Statistical Engineering

- Bioassays
- Immunoassays
- Quantitative Tests
- Qualitative Tests
- Multiplexed assays
- Parallelism
- Immunogenicity

A principal requirement for an effective metric to analyze nonlinear curve fits is the ability to distinguish a good curve fit from a poor one. With results usually > 0.9 for even obviously poor fitting curves (see Figure 3), the metric R² is unable to achieve this basic objective. R² is a measure of whether the response and concentration are related, which they are, and is not appropriate for assessing the quality of nonlinear fits.

In the literature^{1,2,3,4}, residual variance, which is the residual sum of squared errors (rSSE) per degree of freedom, is the metric used for this purpose. Residual variance, unlike R², measures how well the curve fits the individual data points. If the data is accurately weighted, the rSSE is χ^2 distributed and its probability makes an effective statistical metric, the Fit Prob[™]. The StatLIA[®] TrueFit[™] Data Re-

duction System pools the lab's previously run historical assays to determine the correct weighting.

An effective curve fit metric must be able to make the following determinations:

- Assess the quality of the entire curve in a manner that is able to discern even small, but significant, differences in curve fit quality (*rSSE, Residual Variance*).
- Measure the difference between the proposed curve fit dose/response relationship and the observed data (*rSSE*).
- Include the number of data points into the calculation to normalize the varying number of points in different curves. (*Degrees of Freedom*).
- Report how closely the observed dose/response relationship matches the true curve (*Fit Probability [Fit Prob[™]]*).

Figure 1

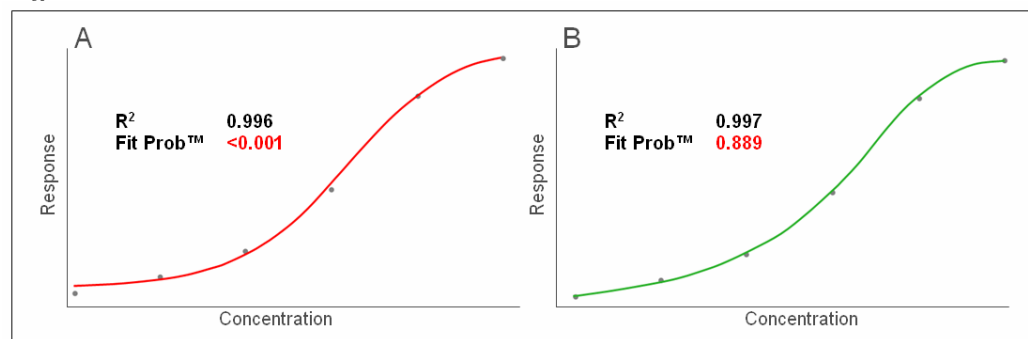
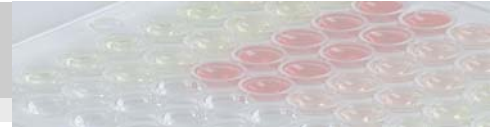


Table 1

Statistic	StatLIA [®] TrueFit [™] 5PL	4PL
rSSE	10.2	28,568
Degrees of freedom	1	2
Residual variance	10.2	14,284
Fit Prob [™]	0.889	<0.001
R ²	0.997	0.996

Figure 1 shows two curve fits with visibly different quality, yet R² failed to detect it. The reason is that R² was designed to measure the quality of fit for linear regression methods and it is not sufficiently sensitive to measure residual error in nonlinear regressions.

Table 1 summarizes the curve fit statistics for the two curves illustrated in Figure 1.



What is the biological question?

The biological question being addressed is how well the computed dose-response curve describes the biological reaction represented by the data points. By looking at how R^2 and Fit Prob™ are calculated, it clarifies why Fit Prob™ measures the accuracy of nonlinear curve fits and R^2 does not.

Residual Variance = Weighted Sum of Squared Residual Errors (rSSE) / Degrees of Freedom

Regression theory states that the fitting curve that best represents the observed data must be the closest to the true curve. The StatLIA® TrueFit™ Data Reduction system uses a weighted rSSE, where the residual error is multiplied by the weight (the reciprocal of the ex-

Figure 2

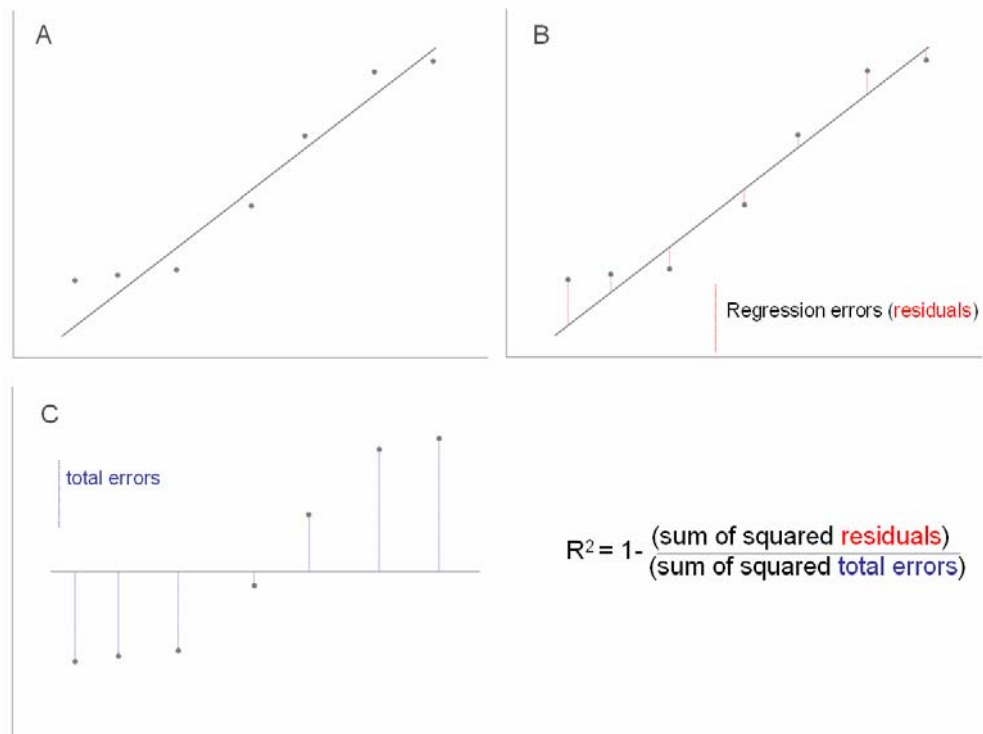


Figure 2 explains the lack of sensitivity of R^2 by how the metric is calculated. The calculation depends on a fraction of the sum of squared residual errors (rSSE) over the sum of squared total errors. As shown in Figure 2C, the problem lies with the dominating influence of the sum of squared total errors, which measures the difference in magnitude between the data points and a horizontal line drawn through the mean of the data points. The sum of squared residual errors (deviations from the regression line) is small versus the sum of the squared total errors, thus resulting in poor sensitivity to residual error.

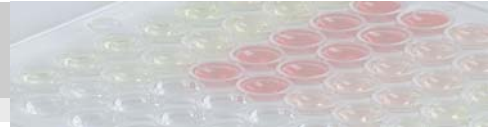
The squared total errors value dwarfs the rSSE value, which pushes the R^2 value to 1.

$R^2 = 1 - \text{Sum of Squared Residual Errors (rSSE) / Squared Total Errors}$

The problem with R^2 lies with the calculation of the sum of squared total errors, which measures the difference in magnitude between the data points and a horizontal line drawn through the mean of the data points (Figure 2C). The sum of squared residual errors from the regression is small versus the sum of the squared total errors, thus resulting in poor sensitivity to residual error. This explains why the R^2 values of even bad fits are usually >0.9 . The large squared total errors value dwarfs the rSSE value, which pushes the R^2 value to 1.

pected variance). Weighting is performed to normalize the magnitude of the observed variance to the magnitude of the expected variance along the curve. (*Residual variance and Fit Prob™ require accurate weighting to be effective.*) The rSSE provides a quantifiable metric that can be monitored during curve fitting. The curve fit that yields the lowest rSSE (and therefore the highest Fit Prob™) must be the one that most resembles the true curve.

While the rSSE is a good measure of fit, it does not by itself allow one to compare between curve fits that use different numbers of data points. Thus, one



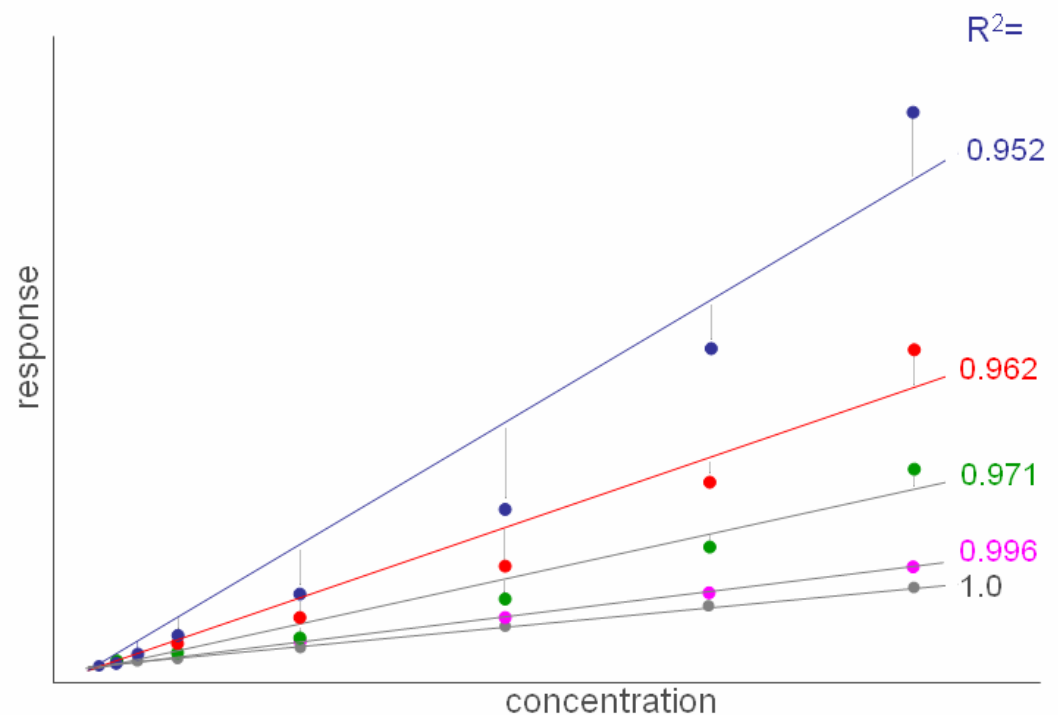
way to normalize the rSSE value is to divide it by the degrees of freedom in the data set. The degrees of freedom are the number of data points in the data set minus the number of parameters used in the curve fit model. For instance, if a data set with nine points is fitted with a 5PL, the degrees of freedom would be four (i.e. nine minus five). Residual variance equals the

The χ^2 test of the rSSE tells the percentage of assays that, if performed under the same conditions, would have a higher rSSE, or lower quality curve fit⁵. As a percentage, this number is referred to as the *Fit Probability*, and ranges from 0 (no fit) to 1 (perfect fit). See Table 1 and Figure 1.

$$\text{Fit Probability} = \chi^2 \text{ Dist (rSSE, df)}$$

Figure 3

Figure 3 shows a range of plotted data sets that increasingly drift from linear to nonlinear (logarithmic). As the data sets become more nonlinear, the regression errors increase markedly, yet the R^2 values remain greater than 0.95 for all the data sets.



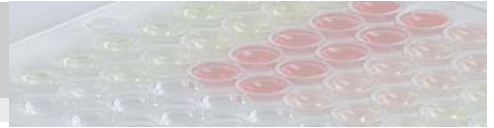
rSSE divided by the degrees of freedom.

How good is good enough? (Fit Prob™)

The residual variance is a sensitive metric for curve fit comparisons, but does not tell us the likelihood that it is a good fit. The biological question that needs to be answered now is whether the residual variance difference between the candidate standard curve and the true curve is statistically significant. The hypothesis is that the candidate standard curve is the true curve (null hypothesis). To test this hypothesis, a quantitative statistical test is needed that can measure the differences between populations. Since the residual variance is a χ^2 distributed value, the χ^2 test is the appropriate metric to test this hypothesis.

References

- (1) Bates, D.M.; Watts, D.G. Comparing Models. Nonlinear Regression Analysis and Its Applications; Wiley: New York 1988; 103-108.
- (2) Dudley, R.A.; Edwards, P.; Ekins, R.P.; Finney, D.J.; McKenzie, I.G.M.; Raab, G.M.; Rodbard, D.; Rodgers, R.P.C. Guidelines for Immunoassay Data Processing. *Clinical Chemistry* 1985, 31 (8), 1264-1271.
- (3) Finney, D.J.; Phillips, P. The Form and Estimation of a Variance Function, with Particular Reference to Immunoassay. *Applied Statistics* 1977, 26, 312-320.
- (4) Rodbard, D.; Munson, P.J.; DeLean, A. Improved Curve Fitting, Parallelism Testing, Characterization of Sensitivity and Specificity, Validation, and Optimization for Radioimmunoassays. In *Radioimmunoassay and Related Procedures in Medicine 1*, Vienna, Italy 1977; Int. Atomic Energy Agency 1978; 469-504.
- (5) Gottschalk, P.G.; Dunn, J.R. The Five Parameter Logistic: A Characterization and Comparison with the Four-Parameter Logistic. *Analytical Biochemistry*, 2005; 343:54-65.



Visit brendan.com for these related technical reports from Brendan Technologies:

- “Improve assay accuracy with StatLIA® TrueFit™ Data Reduction System...
Where the biology defines the curve fit.”
- “Measuring Dose Estimates with Quantifiable Confidence: Confidence Limits and Maximal/Minimal Acceptable Concentrations”
- “Bioassay parallelism is a quantifiable measure of similitude in biological activity.”

Visit brendan.com/demo to download a demo version of StatLIA®.

Copyright © 2007 Brendan Technologies, Inc. All rights reserved.

StatLIA is a registered trademark and TrueFit and Fit Prob are trademarks of Brendan Technologies, Inc., Research Center Plaza, 2236 Rutherford Road, Suite 107, Carlsbad, California, 92008, USA, 760-929-7500 (v), 760-929-7504 (f), sales@brendan.com.