Simple Line

The objective of this case study is to investigate how measured absorbance changes in relation to protein concentration and to quantify that relationship using linear curve fitting in **Isalos Analytics Platform.** In this case study, the **Simple Line** model is used to estimate the slope and intercept of a standard calibration relationship using published absorbance and concentration data.

The dataset used in this case study contains **OD562 absorbance** values and corresponding **protein concentration** values published by *Wang et al.,* at *International Journal of Molecular Sciences(2023).* In this model, the independent variable (X) is the **average absorbance at 562 nm (OD562)**, and the dependent variable (Y) is the **true protein concentration (µg/µL)**.

The **Simple Line** model describes a straight-line relationship in which the response changes proportionally with the predictor variable. The equation used in this analysis is:

$$\boldsymbol{Y = (\text{Slope} \times \text{X}) + \text{Y Intercept}}$$

In this model, the **slope** represents the change in protein concentration for each unit increase in absorbance, and the **intercept** represents the predicted concentration when the absorbance is zero.

The purpose of this analysis is to determine whether the absorbance-concentration data are consistent with a linear calibration relationship and to convert the measured optical signal into a biologically meaningful quantitative output. The main results obtained from the fit are the **slope** and **intercept**, where the slope reflects the sensitivity of the assay and the intercept reflects the baseline offset of the fitted line. These results are useful because they allow direct interpretation of how absorbance relates to protein concentration.

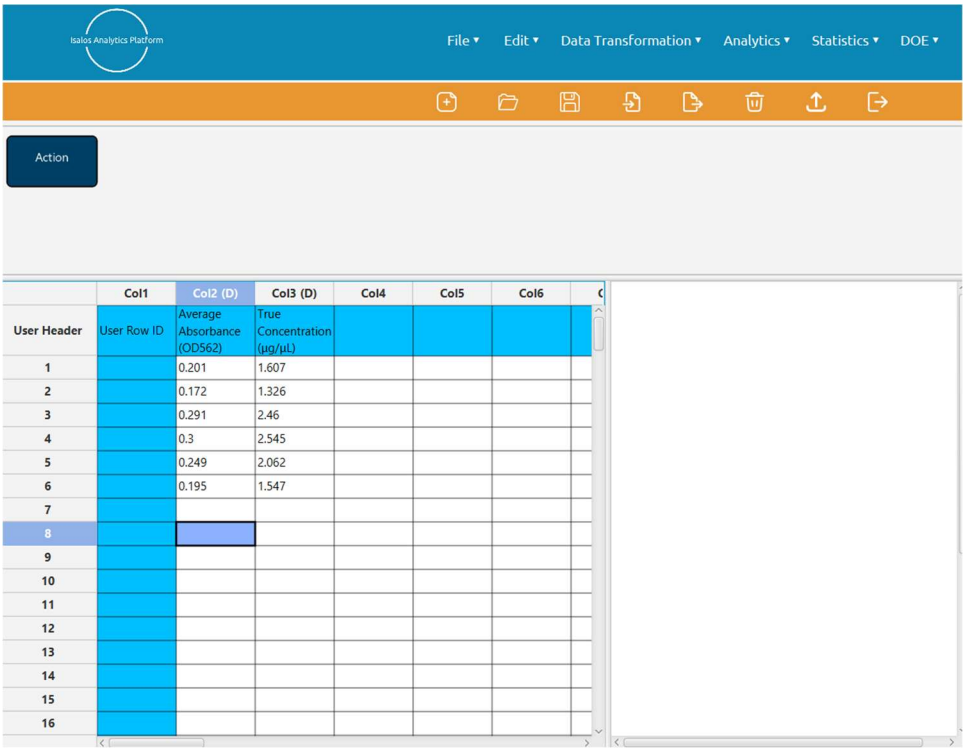
Isalos version used: 2.0.2

Scientific Article: https://www.mdpi.com/1422-0067/24/17/13236

Step 1: Import data from file

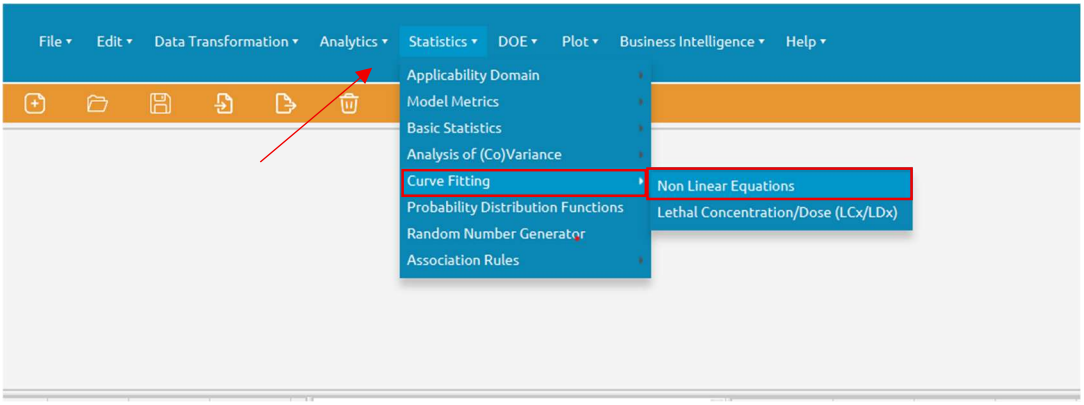
Right-click on the input spreadsheet panel on the left and choose “**Import from File”**. Then browse to the file containing the XY dataset for this case study and load the sheet in which each row corresponds to one absorbance value and one protein concentration value.

The data will appear on the left spreadsheet.

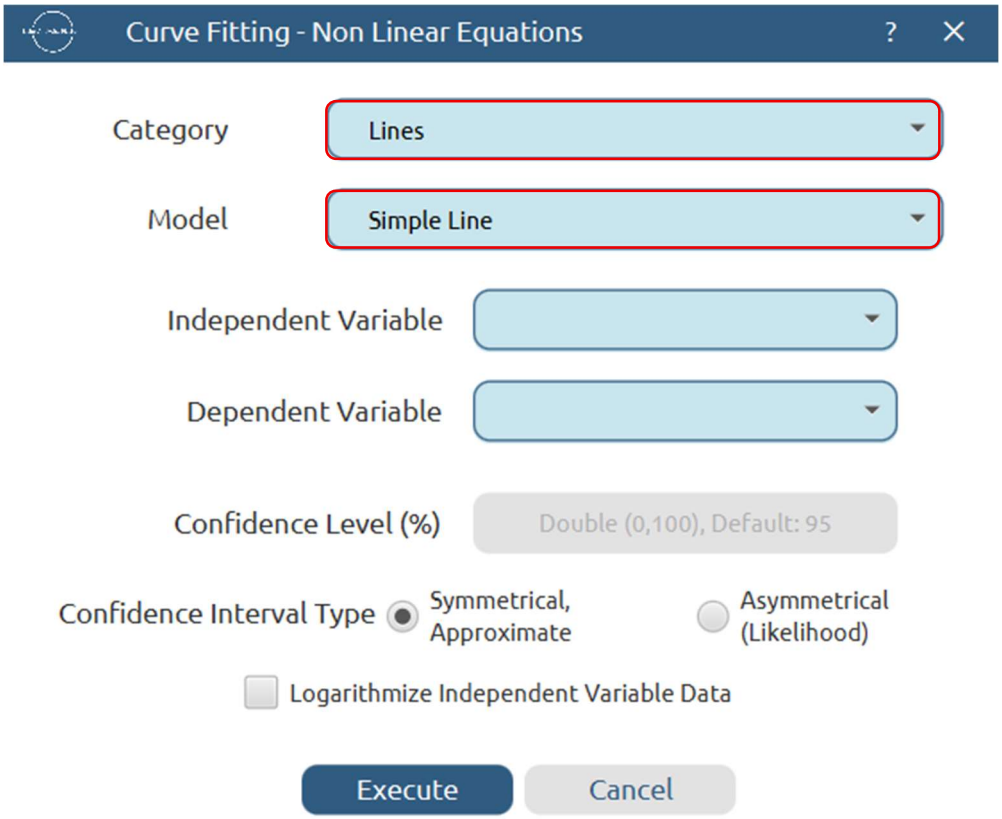


Step 2: Select the desired statistical analysis model

From the toolbar, open the **Statistics** drop-down list and navigate through **Statistics > Curve Fitting > Non Linear Equations**.

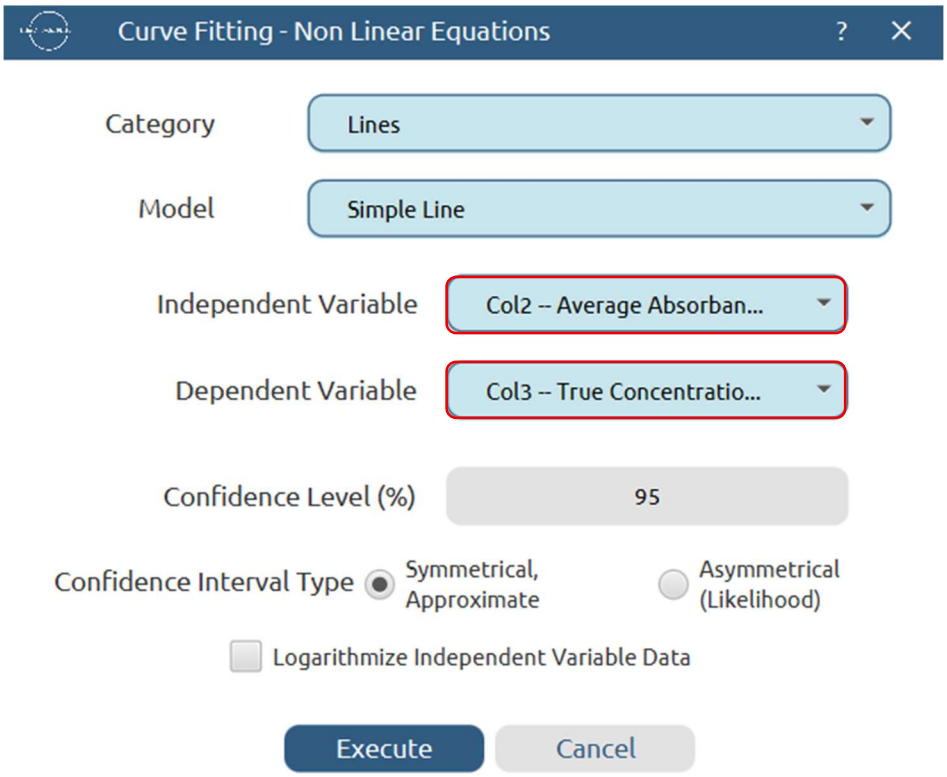


In the category list, select the **Lines** category and then choose **Simple Line** from the model menu.



Step 3: Configure variables and confidence intervals

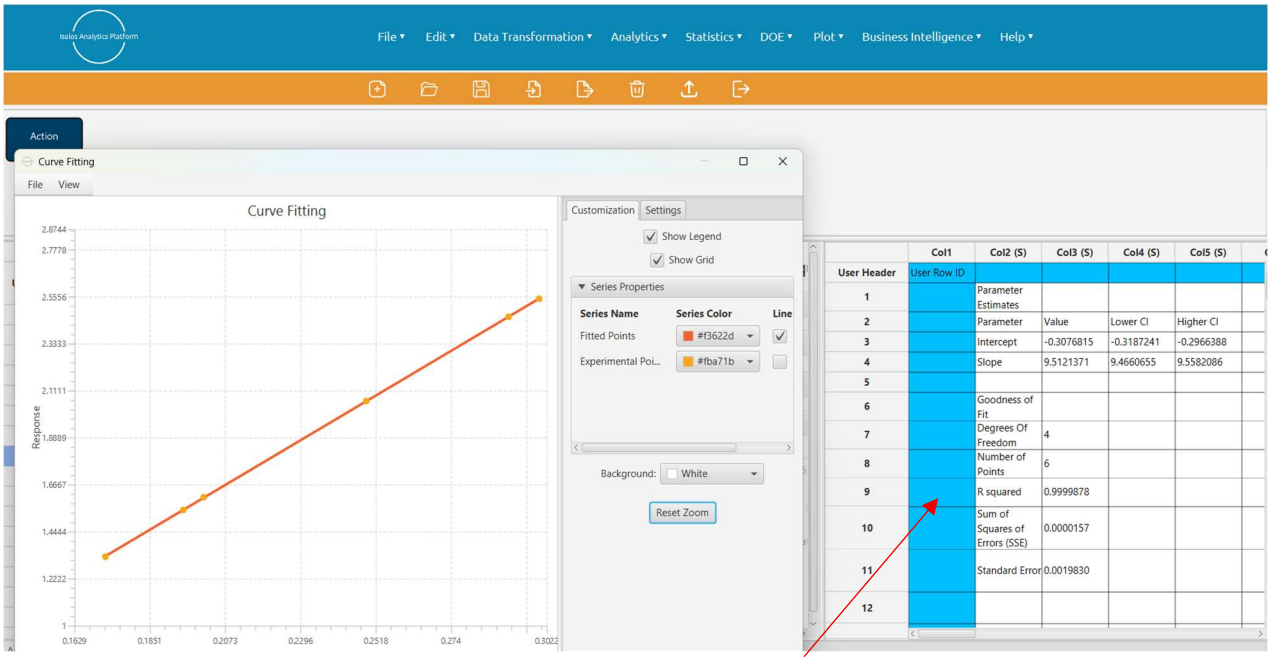
Set the column containing the "**average OD562 absorbance"** values as the independent variable (X), and set the column containing the "**true protein concentration"** values as the dependent variable (Y). Set the confidence level to **95%** and choose **Symmetrical Approximate** as the confidence interval type.



Step 4: Analyze the output and fitted line

Once the analysis is completed, Isalos presents the fitted straight line together with the experimental data points, allowing direct visual assessment of how well the model captures the absorbance-concentration relationship. The results page presents the estimated model parameters with their confidence limits, together with goodness-of-fit statistics and the corresponding fitted plot. In the **Simple Line** model, the main fitted parameters are the **slope** and **intercept**, and the graphical output displays an increasing linear relationship if absorbance and concentration are positively associated.

The **Goodness of Fit** section of the table summarizes key fitting statistics such as the **number of data points used**, **degrees of freedom**, **residual sum of squares**, and the **standard error** of the regression. These outputs should be interpreted together with the fitted plot in order to evaluate how well the model describes the data and how reliable the estimated parameters are.



The fitted results indicate that the OD562 absorbance data are extremely well described by a **Simple Line** model, showing a strong linear relationship between optical signal and true protein concentration in the assay. The estimated **slope** of **9.512** indicates that protein concentration increases by about **9.51 µg/µL** for each one-unit increase in average OD562 absorbance, while the estimated **intercept** of **-0.308** represents the extrapolated concentration at zero absorbance and likely reflects baseline offset.

The narrow confidence intervals, together with the extremely high $\boldsymbol{R^2}$ value of **0.99999** and the very low residual error, indicate that the linear model provides an excellent description of the data and that the fitted slope and intercept are highly reliable for this case study.

References:

(1) Zhang, G., Li, L., Yang, Z., Zhang, C. and Kang, X., 2023. TMT-based proteomics analysis of senescent nucleus pulposus from patients with intervertebral disc degeneration. *International Journal of Molecular Sciences*, *24*(17), p.13236.