*lancer*: an R package for linearity assessment and visualisation of multiple curves

## Manuscript Type

Application Note

# Abstract

## Summary

Linearity assessment plays a significant role in the validation of instrumentation and experimental procedures. Linearity can be tested by applying several graphical and numerical approaches. Summary statistics of these curves are mostly limited to the Pearson Correlation Coefficient, which is insufficient to fully test the for linearity. In addition, spreadsheet software, e.g. Microsoft Excel, only allow the analyst to repetitively plot, view and analyse the linearity of curves one at a time, a tedious and time-consuming process. While plotting of many curves at once and calculation of additional summary statistics for assessing linearity of curves can be done using programming languages like R, implementing this from scratch can be arduous task for the analyst. As such, we created the R package *lancer* so that curves can be analysed efficiently with a few functions. To our knowledge, this is currently not implemented in other software or programming languages. *lancer* furthermore reports the statistical results in tables/spreadsheets and records the plots in a pdf file. In addition, *lancer* can also create interactive trellis plots, displayed as a HTML folder, for exploratory analysis, helping analysts when dealing with large datasets.

## Availability and implementation

*lancer* is available on GitHub <https://github.com/SLINGhub/lancer>. The documentation and tutorials can be accessed from <https://slinghub.github.io/lancer/>

## Supplementary information

Supplementary data are available at *Bioinformatics* online.

## Issue Section

Data and text mining

# Introduction

Linearity assessment is an important performance test of an analytical instrument or method when a linear response is assumed. It is applied in many fields in analytical sciences, such as assay development (Ross and Sweep (2003) and Hsieh and Liu (2008)), calibration/dilution studies (Rodríguez *et al.* (1993) and Sands *et al.* (2021)) and laboratory tests. After analysis, curves representing response values versus concentration are plotted for each analyte of interest. As the accuracy of an analytical method is linked to linearity, a non-linear behaviour must be recognized and addressed accordingly.

Visual inspection of curves is useful but it must be accompanied by statistical tests as decision parameters. A commonly used test is the Pearson Correlation Coefficient. However, Sonnergaard (2006) suggests that it is an ineffective standalone numeric parameter to estimate linearity. While researchers like Van Loco *et al.* (2002), Sanchez (2021) and Logue and Manandhar (2018) have indicated other metrics for linearity evaluation, these metrics are generally not implemented in most software. Furthermore, using spreadsheet software, e.g. Microsoft Excel, to individually plot numerous curves is time-consuming. While programming languages like R can plot numerous curve easily, it is hard to implement for a novice analyst.

The R package *lancer* addresses these issues by assisting analysts useful functions to plot many curves with additional metrics that better describe the curves’ characteristics. It also provides analysts with an interactive viewer to group, filter and sort the curves, for examination of problematic cases, such as curves generated by saturated signals.

# Approach

Using response curves in metabolomic/lipidomic studies from Croixmarie *et al.* (2009) and Sands *et al.* (2021) as an example, Supplementary Figure 1 depicts the workflow of *lancer*. The workflow starts with two tables:

* Curve Batch Annotation, describing the curve(s), such as concentration (x-axis) and response curve batches, if any.
* Curve Signal Data, containing response values (y-axis) for each sample and curve.

Using a common column Sample Name, the two tables can be merged into one table (Curve Table) via create\_curve\_table.

Next, summary statistics are calculated via summarise\_curve\_table for each curve. Besides the Pearson Correlation Coefficient, an additional statistical test is the Mandel’s Fitting Test ( in [Equation 1](#eq-mandel-test)) as described by Andrade and Gómez-Carracedo (2013). The test can give evidence that a quadratic model fits better than a linear model, indicating a non-linear curve.

Another statistical test is Percent Residual Accuracy ( in [Equation 2](#eq-pra)) from Logue and Manandhar (2018). Ranging from to , according to which a perfectly linear curve gives a value of .

The software also calculates the concavity of the fitted quadratic model, to identify if the curve is dominantly non-linear at high (concavity ) or low (concavity ) concentrations.

Supplementary Figure 2 gives the summary statistics of three simulated curves (as examples), where one curve is linear and two curves show a plateau at high concentrations (denoted as saturation regime curve) and low concentrations (denoted as noise regime curve), respectively. The corresponding Pearson Correlation Coefficient values (r\_corr) are (, and respectively) even for the non-linear curves. However, non-linear curves are detected by much lower Mandel’s Fitting Test values (mandel\_p\_val) ( and respectively vs for the linear curve) and Percent Residual Accuracy values (pra\_linear) ( and respectively vs for the linear curve).

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| |  | | --- | | (A) Curve Grouping Workflow | |  |

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| |  | | --- | | (B) Interactive Visualisation | |  |

Fig. 1. *lancer*’s curve grouping workflow in [Fig. 1 (A)](#fig-linearity) and interactive visualisation of curves in [Fig. 1 (B)](#fig-visualisation).

After calculating the summary statistics for each curve, *lancer* uses the function evaluate\_linearity to group the curves according to the workflows proposed in [Fig. 1 (A)](#fig-linearity). Workflow 1 uses the Pearson Correlation Coefficient and Percent Residual Accuracy to determine if the curve is linear (labelled as Good Linearity) or not (labelled as Poor Linearity). Workflow 2 goes one step further, using the Mandel’s Fitting Test and the fitted quadratic model’s concavity to check if a non-linear curve plateaus at low (labeled as Noise Regime) or high (labelled as Saturation Regime) concentrations. Non-linear curves that do not follow these trends are labelled as Poor Linearity.

A benchmark workflow using only Pearson Correlation Coefficient value of is compared with Workflow 2 on simulated data sets of 200 linear curves (labelled as Linear), curves that plateau at low (labelled as Noise Regime) or high (labelled as Saturation Regime) concentrations. Supplementary Figure 3 shows that Workflow 2 better identifies the saturation and noise regime curves than the benchmark workflow. While Workflow 2 correctly classifies a lower number of linear curves than the benchmark workflow, its percentage of correctly classified linear curves, 181/200 (90.5%), is high. See <https://lancer-simulation.netlify.app> for report details. While the threshold values of Pearson Correlation Coefficient and Percent Residual Accuracy are based on the interpretation of Y. H. Chan (2003) and Logue and Manandhar (2018), they remain subjective and arbitrary. Nevertheless, *lancer* allows optimization of these threshold values according to the analyst’s preference.

Although *lancer* can export the results in Excel or pdf, an interactive user interface can generate a better overview of the data. [Fig. 1 (B)](#fig-visualisation) shows a HTML folder, exported by *lancer*, such that clicking on the index.html file inside the folder will open an interactive trellis plot that can group, filter and sort curves. This allows room for exploratory data analysis, such as identifying curves with linearity issues or understanding the effects of changing the Pearson Correlation Coefficient threshold to another value. Such information is hard to achieve with other common software. An example of an interactive viewer created by *lancer* can be found at <https://lancer-interactive-example.netlify.app>

# Conclusion

Linearity is one of the most important parameters of an analytical method to be evaluated. Our R package, *lancer*, can estimate linearity efficiently in high-throughput settings and with functions that plot many curves quickly, reporting curve summary statistics to better describe the shape of the datasets. It also provides an interactive trellis plot for exploratory data analysis. It is available on GitHub <https://github.com/SLINGhub/lancer> while the documentation and tutorials can be accessed from <https://slinghub.github.io/lancer>.

# Acknowledgements

These should be included at the end of the text and not in footnotes. Please ensure you acknowledge all sources of funding, see funding section below.

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