# Analysing growth curves and other user-defined data in opm

Lea A.I. Vaas Leibniz Institute DSMZ Markus Göker Leibniz Institute DSMZ

#### Abstract

This is tutorial about the analysis of growth curves and other user defined kinetics with the **opm** package in the version of January 7, 2014. It is explained how any kinds of growth or respiration measurements can be input into **opm**. Data without a real structuring into plates and wells can nevertheless be studied with **opm** by using a *virtual* arrangement into plates and wells. This convention is not an oddity but rather the appropriate means to enable the visual and statistical comparisons of interest and to disable those that make no sense for the data. We also show how Phenotype Microarray (PM) data with user-defined plate types can be analysed. Analysing such data visually and statistically requires in some cases adaptations of function arguments whose defaults are targeting PM data. All these practically relevant issues are explained in detail.

Keywords: Growth Kinetics.

## 1. Introduction

A detailed description of the OmniLog® Phenotype Microarray (PM) system, its measuring procedure and data characteristics are found in the vignette "opm: An R Package for Analysing Phenotype Microarray and Growth Curve Data" (called "main tutorial" in the following). How substrate information stored within opm can be accessed and used for advanced visual and statistical analyses is explained in the vignette "Working with substrate information in opm" (called "substrate tutorial" in the following). The description of the methods below do not presuppose that the user is already familiar with the usage of opm. But for details on its approaches to visualisation and statistical analysis we will refer to the main tutorial, the substrate tutorial as well as the entries of the opm manual. Especially the concepts behind the different classes of opm objects are only explained in the main tutorial, and the methods available for these classes are only explained in the main and substrate tutorial.

In addition to visual inspection or statistical comparative analyses of PM data, as described in the main tutorial and the substrate tutorial, users might be interested in analysing data other than PM data, or analysing PM with user-defined plate types. To work with user-defined PM plates only requires registering these plates, i.e. storing a mapping from well coordinates to substrate names, and optionally also a full, descriptive name for the plate. The analysis of data other than PM data, such as growth curves, additionally requires inputting these data and converting them to OPMX objects. If these data are not really structured into plates and wells, a *virtual* arrangement into plates and wells must be established, as well as a virtual positioning

of the plates in a reader, which is used for identifying each plate. This nomenclature may be unusual for data that have not been measured in plate readers, but presents no problems in practice. Users should be aware, however, which kinds of comparisons can be made within and between plates of the same plate type. Indeed, the arrangement into virtual plates and wells is the appropriate means to ensure that the visual and statistical comparisons of interest for the data are possible and that those that make no sense are disabled. Note that some defaults of the plotting functions are only suitable for PM data. Hence, the functions should be called slightly distinctly.

Besides these slight restrictions, which are illustrated with examples below, non-PM data can be analysed with **opm** almost like PM data.

## 2. Preparation

As usual, **opm** must be loaded before any analysis can begin:

```
R> if ("package:opm" %in% search())
         detach("package:opm", unload = TRUE)
R> library("opm")
```

## 3. Growth-curve data input

#### 3.1. User-entered data frames

R> data("potato")
R> head(potato)

07-08-1 0.16M NaCl

07-08-1 0.16M NaCl

In the following we will use the growth-measurements data set from Vaas, Marheine, Sikorski, Göker, and Schumacher (2013) as exemplar. These data have been entered by hand and then input into R with one of the functions for reading Comma-Separated Values (CSV), yielding a data frame that comes with **opm**:

```
Genotype Treatment Replicate Time FM DM
1 07-08-1 0.16M NaCl 1 2 597 44
2 07-08-1 0.16M NaCl 2 2 550 40
3 07-08-1 0.16M NaCl 3 2 633 48
4 07-08-1 0.16M NaCl 4 2 490 31
```

5

For details on this data set, enter ?potato at the R prompt. The measurements are in "long" format and must be reshaped using the eponymous function into "wide" format. The "long" format was deliberately chosen for demonstrating the use of the reshape function. We reshape separately for the Dry Mass (DM) and Fresh Mass (FM) measurements within the data set:

2 617 47

4 585 55

For reshape, "long" format means that each measurement is stored in a separate record with one entry per row (see above). Thus here for each data point the "Genotype", "Treatment" and "Time" entries have to be repeated, resulting in a data frame with 540 rows in 6 columns. A call to reshape can rearrange the data set into a form where the columns "Genotype", "Treatment" and "Replicate" are kept and the columns "Time" and either "FM" or "DM", respectively, are merged, resulting in 9 columns representing the measurement times:

#### R> head(potato.fm)

```
Genotype Treatment Replicate FM.2 FM.4 FM.6 FM.8 FM.10 FM.12 FM.14 FM.16 FM.18
1
   07-08-1 0.16M NaCl
                               1 597 585 882 844
                                                      1291
                                                            1847
                                                                  2232 2560
2
    07-08-1 0.16M NaCl
                               2 550
                                       614
                                            908 1103
                                                      1240
                                                            1798
                                                                  2184
                                                                         2832
                                                                               2501
                                       570
3
    07-08-1 0.16M NaCl
                               3 633
                                            855 1200
                                                      1392
                                                                  2360
                                                                         2522
                                                            1827
                                                                              3113
    07-08-1 0.16M NaCl
                                 490
                                                       1478
                               4
                                       681 1087
                                                 994
                                                            1921
                                                                  2315
                                                                         2317
                                                                               2761
    07-08-1 0.16M NaCl
                               5
                                  617
                                       707
                                            962
                                                 849
                                                       1446
                                                             1853
                                                                  2335
                                                                         2564
                                                                               2426
   07-08-1 0.32M NaCl
                                  395
                                       551
                                            392
                                                 342
                                                       322
                                                              322
                                                                    368
```

Thus the dimensions of the data dwindled to 60 rows in 12 columns. Now the data are in the right arrangement for the next step, the conversion into OPMX or MOPMX objects. (When entering data manually, users who directly choose the "wide" format can, of course, skip the conversion with reshape.)

The main function for converting user-defined data frames to OPMX or MOPMX objects is opmx, which can directly be applied to the objects created in the last step. This works because the "horizontal" input format of opmx corresponds to the "wide" format of reshape.

```
R> potato.fm <- opmx(potato.fm, position = c("Genotype", "Replicate"),
    well = "Treatment", prefix = "FM.",
    full.name = c(fm = "Growth experiment, fresh mass"))
R> potato.dm <- opmx(potato.dm, position = c("Genotype", "Replicate"),
    well = "Treatment", prefix = "DM.",
    full.name = c(dm = "Growth experiment, dry mass"))</pre>
```

The data frames passed to opmx contain all substrate information in their "Treatment" column. Its content will be interpreted as substrate names for wells, which are virtual in our case. Hence, opm registers the mapping from well coordinates to substrate names on the fly. The substrate names are taken directly from the data frame in "horizontal" format and registered after sorting. The plate type must be provided, however. As it is not within the data frame, the short name of the plate type is taken from the full.name argument, whose main purpose is to enter the full, descriptive name of the plate type. That is, a virtual plate with virtual wells, yielding a user-defined plate type, will be registered. The prefix argument helps identifying the columns with measurements over time.

"Genotype" and "Replicate" go to the metadata of the resulting object and together identify each plate. In the case of PM data, this is done using the position of the plate within the OmniLog® reader. Thus the relevant argument here is position, which must be supplied unless there is a column of that name. If so, its content is used literally, otherwise it is

newly constructed from the columns explicitly given in the position argument, yielding a grouping of plates equivalent to the combination of factor levels in these columns. So the "plate position" is usually also virtual, but just acts as an identifier of the plate.

The registered plate type can be queried as follows:

Note the prefix "CUSTOM:", which is used to distinguish user-defined plate types from those that come with **opm**. The object resulting from listing can be output with to\_yaml or saveRDS for externally storing plate types in files. Indeed, please keep in mind that the definition of plate types is only available in the current R session. The definitions will be lost once the session is terminated.

With the resulting potato.dm and potato.fm objects the user can now follow the **opm** work flow for processing PM data. Please continue in Section 4 and the following sections for plotting and statistical analysis of the estimated curve parameters.

It is possible to first register the plate, as shown in Section 3.2, and then convert the data *via* opmx. This makes most sense if another ordering of wells should be enforced. Otherwise opmx takes the substrate names directly from the data frame in "horizontal" format and registers them after sorting.

#### 3.2. Direct registration of plate types

An example input file comes with **opm** containing growth-curve data derived from an experiment with two *Escherichia coli* strains (Deutsche Sammlung von Mikroorganismen (DSM) 18039 = K12 and the type strain DSM  $30083^{T}$ ) on increasing Glucose concentrations. Here we are dealing with a real plate with real wells, but the registering procedure would be the same for virtual plates with virtual wells. Thus, it will here be shown how to prepare a plate map and register it as a new plate type. Section 3.2 then shows how to import the data and subsequently convert them to an OPMX or MOPMX object. Each combination of strain and Glucose concentration was repeated twice on the plate. It will thus be shown how to define

a numbering of these repetitions suitable for later on using the split function to split the object into one object per repetition.

The **opm** package offers several ways to set up a user-defined plate layout. The function **register\_plate** is useful for both customised PM plates and measurements from quite different experiments such as growth curves and other kinds of kinetics.

For small data sets it might be feasible to type the substrate allocation manually into a character vector, as done in the following. The short name of the plate type will be "growth", as simply given by the named function argument. Here two argument of the same name are passed to the function for registering the full name and the well mapping in a single call:

```
R> register_plate(
     growth = c(
       A01 = "Negative Control #1", A02 = "10mM Glucose #1",
       A03 = "20mM Glucose #1", A04 = "50mM Glucose #1",
       A05 = "100mM Glucose #1", A06 = "200mM Glucose #1"
       B01 = "Negative Control #2", B02 = "10mM Glucose #2",
       B03 = "20mM Glucose #2", B04 = "50mM Glucose #2",
       B05 = "100mM Glucose #2", B06 = "200mM Glucose #2"
       CO1 = "Negative Control #3", CO2 = "10mM Glucose #3",
       CO3 = "20mM Glucose #3", CO4 = "50mM Glucose #3",
       CO5 = "100mM Glucose #3", CO6 = "200mM Glucose #3"
       D01 = "Negative Control #4", D02 = "10mM Glucose #4",
       D03 = "20mM Glucose #4", D04 = "50mM Glucose #4",
       D05 = "100mM Glucose #4", D06 = "200mM Glucose #4"
     growth = "Growth on Glucose"
R> listing(wells(plate = "custom:growth"))
```

However, manually entering the well mapping is error prone and not efficient when dealing with data sets containing more than a few wells. Alternatively, a user-designed plate can also be registered with a plate map given as matrix. The matrix then directly represents the allocation of the used substrates on the plate. Because of the repetitions in the substrate names (note the numbering, which is necessary here to generate unique substrate names, and later on important to split the plate), the texts can also be generated with fewer code:

Plates with other layouts can be put together in the same way but using other more or fewer row and/or column names. Plates with other repetition structure, or no repetitions of

substrates at all, can be put together in the same way, too, but modifying or omitting the way a substrate numbering is introduced. Instead of a matrix, a data frame could be used as well. We will try this here after showing how to delete a plate type again by providing a NULL argument:

```
R> register_plate(growth = NULL)
R> growth <- as.data.frame(growth)
R> register_plate(growth = growth, growth = "Growth on Glucose")
R> listing(wells(plate = "CUSTOM:GROWTH"))
```

### 3.3. Input of TECAN data

The data for which we have registered a full plate name and a mapping from well coordinates to substrate names in Section 3.2 are contained in an exemplar input file that comes with **opm**. It can be found, and input into R, as follows:

```
R> tecan.file <- opm_files("growth")</pre>
R> tecan.file <- grep("tecan", tecan.file, ignore.case = TRUE, value = TRUE)
R> tecan <- read.table(tecan.file)</pre>
R> head(tecan)
  V1
        V2
              V3
                    ۷4
                          V5
                                 V6
                                       ۷7
1 <> 1.000 2.000 3.000 4.000 5.000 6.000
2 A 0.087 0.088 0.087 0.088 0.085 0.084
3 B 0.087 0.088 0.087 0.086 0.087 0.085
4 C 0.083 0.082 0.081 0.083 0.079 0.077
5 D 0.083 0.083 0.081 0.082 0.080 0.079
6 <> 1.000 2.000 3.000 4.000 5.000 6.000
```

This file was output by a [Lea, please enter the name] instrument as distributed by the TECAN corporation. After recording the data, the [Lea, please enter the name] software provided by TECAN generated such as file via [Lea, please enter the procedure].

The resulting format is not particularly useful within R but can be converted using the "rectangular" mode of opmx:

```
R> tecan <- opmx(tecan, "rectangular", plate.type = "growth", position = "1A",
     interval = 1)
R> tecan
Class
                             OPM
From file
                            71
Hours measured
Number of wells
                             24
                            CUSTOM: GROWTH
Plate type
Position
                            1A
Setup time
                            Tue Jan 7 23:31:53 2014
{\tt Metadata}
                            FALSE
Aggregated
                            FALSE
Discretized
```

Note that we have to refer to the previously registered plate type, "growth". The position argument is important for identifying each plate if several plates of this plate type are to be dealt with. The format of the position entry can, in principle, be arbitrarily selected by the user, but the shown format is the recommended one, i.e. an integer followed by a single letter. The optional interval argument provides the time interval between two consecutive measurements. Ideally, it is provided in hours. Time series with irregular intervals can be entered with the same argument (by directly providing each time point).

[Lea, please check whether this is the correct interval argument for these data.]

The generated OPM object can now be split according to the repetition structure of the wells, and metadata can be added that describe each resulting plate:

[Lea, please enter the correct strain designations in the code snippet for assigning metadata.]

## 4. Visualisation of growth curves

Visualising raw measurements of growth curves with the methods intended for PM data is straightforward, but some adaptations are necessary due to the deviations between the distinct kinds of data. For instance, the expected maximum for PM data can seldom be used for delimiting the y axes, and the data are not measured in OmniLog® units, and a negative control might not be present:

The result is shown in Figure 1. The TECAN data (which contain a negative control) can be visualised in the same way as the potato data, yielding Figure 2. Note the rm.num argument, which causes the removal of the numbering from the end of the full well names (which is not needed any more after applying the split function as described in Section 3.3):

[Lea, please enter an appropriate y-axis description for the E. coli plot.]

```
R> xy_plot(tecan, theor.max = FALSE, include = "Strain",
    main = list(in.parens = FALSE), ylab = "Value", rm.num = TRUE)
```

# 5. Estimating parameters from growth curves

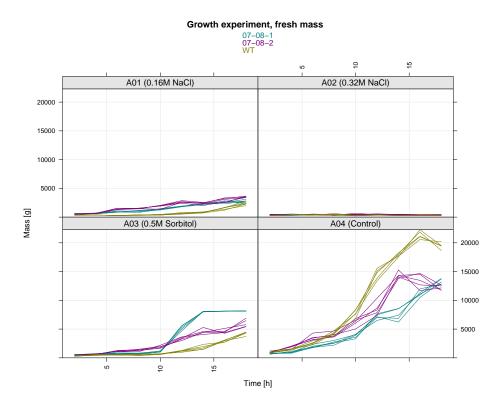


Figure 1: Potato cell line growth measurements, recorded as fresh mass, visualised using the xy\_plot method. See the main tutorial for details on this kind of plotting. The plot indicates that the wild type grows better than the genetically modified cell lines under non-stress (control) conditions. It also indicates that the stresses impair growth but that the genetically modified cells grow better then the wild type under moderate stress conditions.

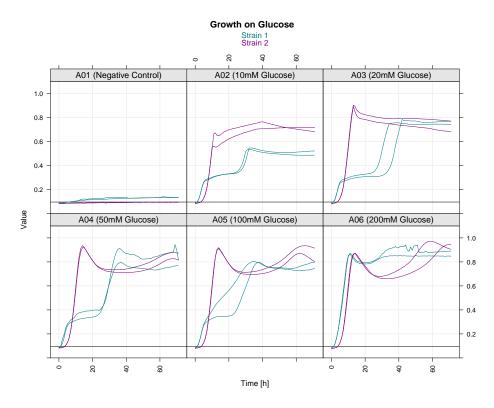


Figure 2: Growth of two *E. coli* strains on Glucose, visualised using the xy\_plot method. See the main tutorial for details on this kind of plotting. The plot indicates that one of the strains outgrows the other unless high concentrations of Glucose are applied.

TODO: Lea, please make recommendations regarding the spline method to be used with these data. For issues not specific to growth data refer to the main tutorial.

## 6. Statistical analysis of growth curves

TODO: Lea, please conduct selected analyses that make sense for these data. For instance, the interpretations given in the figure captions should be assessed using <code>opm\_mcp</code>. For issues not specific to growth data refer to the main tutorial.

## 7. Acknowledgements

We are grateful to Victoria Michael (Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ)) for providing growth curves measured with a TECAN instrument.

#### References

Vaas LAI, Marheine M, Sikorski J, Göker M, Schumacher HM (2013). "Impacts of pr-10a Overexpression at the Molecular and the Phenotypic Level." *International Journal of Molecular Sciences*, 14, 15141–15166. doi:10.3390/ijms140715141. URL http://www.mdpi.com/1422-0067/14/7/15141.

#### **Affiliation:**

Markus Göker Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures Braunschweig

Telephone: +49/531-2616-272 Fax: +49/531-2616-237

E-mail: markus.goeker@dsmz.de

URL: www.dsmz.de