mpxtractor: A flexible R package to process data from different plate reader machines

Student: Martin Banchero

Supervisor: Douwe Molenaar, PhD

Introduction

High-throughput experiments

Broad popularity:

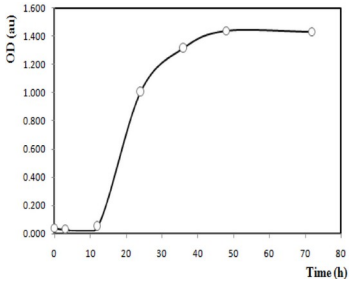
- Drug discovery
- Food research
- Biofuels

96 well microplate

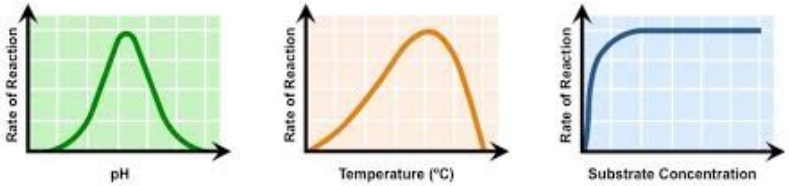
384 well microplate

dimensions of, 1536, 3456 and more

- Test hundreds of samples simultaneously.
- Allow to collect, analyze data about complex interactions within biological systems.



- i.e Growth rates



- i.e Enzyme rates

Introduction

Microplate reader machines in the lab

FluoStar Omega

- Fluorescence intensity,
- Time-resolved fluorescence
- Absorbance
- Luminescence.

Multiskan GO

- Spectral scanning
- Single point in time (endpoint)
- Over specified period of time (Kinetic)

SpectraMax

- Optical density (OD)
- endpoint
- kinetic
- spectral scan

Introduction

Microplate reader machines: Raw data

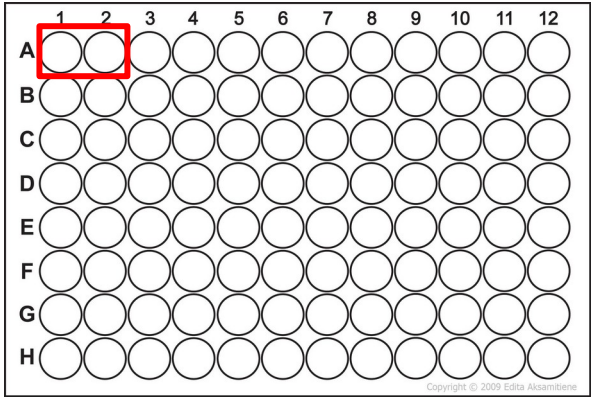
Output file FLUOStar Omega

- Measures at 6 time points

```
1  User: USER      Path: C:\Program Files (x86)\BMG\Omega\User\Data\
2  Test ID
   221
3  Test Name: Test_Fluorescence    Date: 29/08/2019       Time:
   15:04:05
4  ID1: Reading
   1
5  ID2:
   Fluorescence
6  Fluorescence
   (FI)
7
8
9  "Well
10 Row"    "Well
11 Col"    Content         "Raw Data (485, 520)
12 1 - 0 h  "      "Raw Data (485, 520)
13 2 - 0 h 2 min "         "Raw Data (485, 520)
14 3 - 0 h 7 min "         "Raw Data (485, 520)
15 4 - 0 h 12 min "        "Raw Data (485, 520)
16 5 - 0 h 17 min "        "Raw Data (485, 520)
17 6 - 0 h 22 min "        "Raw Data (485, 520)
18                         0       0.043333333     0.125555556     0.21    0.292222222     0.376666667
19 A       1       Sample X1       186705  186950  188978  189018  189620  191198
20 A       2       Sample X2       186784  186224  187730  187383  188480  188519
```

Fluorescence

Schematic representation 96 microplate



Introduction

Microplate reader machines: Raw data

Output file Multiskan GO: List format

	Plate	Well	Group	Type	Sample	Wavelength	Reading	Abs	Meas. Time [s]
2	Plate 1	C02	Assay	Calibrator	1 1/1	340	1	0.231	0.330
3	Plate 1	C02	Assay	Calibrator	1 1/1	340	2	0.233	30.320
4	Plate 1	C02	Assay	Calibrator	1 1/1	340	3	0.235	60.320
5	Plate 1	C02	Assay	Calibrator	1 1/1	340	4	0.237	90.320
6	Plate 1	D02	Assay	Calibrator	2 1/1	340	1	0.236	0.690
7	Plate 1	D02	Assay	Calibrator	2 1/1	340	2	0.239	30.690
8	Plate 1	D02	Assay	Calibrator	2 1/1	340	3	0.240	60.690
9	Plate 1	D02	Assay	Calibrator	2 1/1	340	4	0.242	90.690
10	Plate 1	E02	Assay	Calibrator	3 1/1	340	1	0.241	1.020
11	Plate 1	E02	Assay	Calibrator	3 1/1	340	2	0.244	31.020
12	Plate 1	E02	Assay	Calibrator	3 1/1	340	3	0.246	61.020
13	Plate 1	E02	Assay	Calibrator	3 1/1	340	4	0.250	91.020
14	Plate 1	F02	Assay	Calibrator	4 1/1	340	1	0.231	1.390
15	Plate 1	F02	Assay	Calibrator	4 1/1	340	2	0.235	31.380
16	Plate 1	F02	Assay	Calibrator	4 1/1	340	3	0.238	61.380
17	Plate 1	F02	Assay	Calibrator	4 1/1	340	4	0.242	91.380
18	Plate 1	C03	Assay	Control	5 1/1	340	1	0.240	0.350
19	Plate 1	C03	Assay	Control	5 1/1	340	2	0.246	30.340
20	Plate 1	C03	Assay	Control	5 1/1	340	3	0.251	60.340
21	Plate 1	C03	Assay	Control	5 1/1	340	4	0.257	90.340
22	Plate 1	D03	Assay	Control	6 1/1	340	1	0.240	0.680

- 4 readings every 30 seconds

Output file Multiskan GO: Table format

```
Results of Photometric1

        Plate: Plate 1 - Wavelength: 600

        Reading: 1
        0.091   0.081   0.080   0.080   0.082   0.081   0.083   0.081   0.083   0.081   0.082   0.084   0.082   0.082   0.081   0.082
  0.081 0.081   0.080   0.081   0.081   0.082   0.082   0.082   0.081
        0.082   0.086   0.087   0.085   0.085   0.083   0.083   0.083   0.084   0.084   0.082   0.086   0.082   0.084   0.084   0.085
  0.084 0.082   0.083   0.082   0.083   0.083   0.082   0.083
        0.081   0.085   0.087   0.085   0.085   0.083   0.083   0.083   0.083   0.083   0.082   0.086   0.084   0.082   0.083   0.084
  0.082 0.082   0.083   0.083   0.083   0.083   0.084   0.082
        0.082   0.086   0.085   0.085   0.082   0.082   0.083   0.083   0.083   0.083   0.082   0.085   0.082   0.082   0.083   0.082
  0.083 0.082   0.082   0.082   0.083   0.084   0.083   0.082
        0.083   0.086   0.085   0.084   0.083   0.084   0.083   0.084   0.084   0.083   0.082   0.086   0.083   0.083   0.083   0.083
  0.083 0.082   0.082   0.082   0.083   0.083   0.084   0.082
        0.083   0.085   0.084   0.085   0.084   0.084   0.083   0.083   0.083   0.084   0.083   0.085   0.083   0.082   0.083   0.083
  0.082 0.083   0.082   0.082   0.083   0.083   0.084
        0.084   0.086   0.086   0.085   0.085   0.085   0.084   0.085   0.085   0.084   0.083   0.086   0.087   0.083   0.083   0.084
  0.083 0.083   0.083   0.085   0.083   0.083   0.093   0.083
        0.084   0.085   0.084   0.085   0.085   0.084   0.083   0.085   0.084   0.084   0.083   0.085   0.084   0.083   0.083   0.082
  0.083 0.084   0.084   0.085   0.084   0.084   0.084   0.083
        0.084   0.087   0.085   0.087   0.086   0.086   0.085   0.085   0.086   0.086   0.086   0.087   0.086   0.083   0.084   0.085
  0.086 0.084   0.084   0.086   0.086   0.087   0.086   0.083
        0.083   0.086   0.086   0.086   0.087   0.085   0.085   0.085   0.084   0.084   0.086   0.086   0.083   0.085   0.083   0.084
  0.085 0.083   0.085   0.084   0.085   0.086   0.088   0.083
        0.084   0.086   0.085   0.085   0.087   0.084   0.086   0.085   0.085   0.086   0.086   0.085   0.087   0.086   0.084   0.085
  0.086 0.084   0.083   0.085   0.088   0.087   0.085   0.083
        0.083   0.084   0.087   0.086   0.087   0.086   0.083   0.086   0.086   0.086   0.086   0.086   0.086   0.085   0.085   0.084
  0.086 0.084   0.084   0.084   0.086   0.087   0.083   0.084
        0.082   0.086   0.086   0.087   0.086   0.085   0.086   0.086   0.087   0.087   0.086   0.085   0.086   0.083   0.083   0.086
  0.086 0.085   0.084   0.087   0.086   0.087   0.085   0.083
        0.082   0.085   0.086   0.087   0.087   0.086   0.083   0.086   0.084   0.085   0.085   0.085   0.084   0.083   0.083   0.083
  0.086 0.085   0.083   0.085   0.087   0.086   0.083   0.083
        0.082   0.084   0.084   0.085   0.085   0.085   0.083   0.084   0.083   0.083   0.085   0.087   0.084   0.082   0.084   0.083
  0.084 0.083   0.083   0.084   0.085   0.084   0.083   0.083
        0.079   0.080   0.079   0.081   0.081   0.081   0.079   0.080   0.080   0.080   0.081   0.081   0.081   0.081   0.081   0.080
  0.080 0.080   0.080   0.082   0.082   0.081   0.081   0.082
```

- No time present in table format

```
Results of Photometric1

        Plate: Plate 1 - Wavelength: 600

        Reading: 1
        0.088   0.090   0.090   0.088   0.090   0.091   0.090   0.090   0.090   0.089
  0.091 0.093   0.090   0.090   0.089   0.089   0.089   0.088   0.088   0.089   0.089
  0.088 0.089   0.089
```

Introduction

Microplate reader machines: Raw data

Output file SpectraMax(.txt)

Time(hh:mm:ss)	Temperature(°C)	A1	A2	A3	A4	A5	A6	A7	A8
A17	A18	A19	A20	A21	A22	A23	A24	B1	B2	B3	B4
B13	B14	B15	B16	B17	B18	B19	B20	B21	B22	B23	B24
C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20
D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
E21	E22	E23	E24	F1	F2	F3	F4	F5	F6	F7	F8
F17	F18	F19	F20	F21	F22	F23	F24	G1	G2	G3	G4
G13	G14	G15	G16	G17	G18	G19	G20	G21	G22	G23	G24
H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20
I5	I6	I7	I8	I9	I10	I11	I12	I13	I14	I15	I16
J1	J2	J3	J4	J5	J6	J7	J8	J9	J10	J11	J12
J21	J22	J23	J24	K1	K2	K3	K4	K5	K6	K7	K8
K17	K18	K19	K20	K21	K22	K23	K24	L1	L2	L3	L4
L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24
M9	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20
N5	N6	N7	N8	N9	N10	N11	N12	N13	N14	N15	N16
O1	O2	O3	O4	O5	O6	O7	O8	O9	O10	O11	O12
O21	O22	O23	O24	P1	P2	P3	P4	P5	P6	P7	P8
P17	P18	P19	P20	P21	P22	P23	P24				
0:00	30,00	0,0821	0,1387	0,1438	0,1558	0,1416	0,1425	0,15	0,144	0,1404	0,1396
0,1526	0,1343	0,1551	0,1399	0,1458	0,0833	0,1552	0,2537	0,2535	0,2781	0,2708	0,2755
0,2769	0,2466	0,2459	0,2505	0,2529	0,2623	0,2626	0,2708	0,2559	0,134	0,1536	0,2529
0,3049	0,2984	0,2645	0,2621	0,26	0,2732	0,2662	0,2632	0,2606	0,2302	0,2265	0,2956
0,2775	0,2796	0,2774	0,2822	0,2748	0,2748	0,2737	0,2878	0,2792	0,2786	0,273	0,282
0,1655	0,17	0,1709	0,1728	0,1718	0,1803	0,1702	0,171	0,1694	0,1779	0,1707	0,1744
0,1715	0,1757	0,1418	0,2444	0,2708	0,2746	0,2765	0,2851	0,2832	0,2881	0,2845	0,284
0,2801	0,2912	0,294	0,296	0,2817	0,1929	0,1425	0,2675	0,2616	0,2755	0,281	0,2843

- Different researchers using different formats, .txt, .csv, xlsx

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI
1	Time(hh:mm:ss)	Temperature(°C)	A1	A2	A3	A4	A5	A6	A7	A8	A9				B1	B2	B3	B4	B5	B6	B7	B8	B9			B12	C1	C2	C3	C4	C5	C6	C7	C8	C9
2	0:00	37							0.3911	0.3963	0.3995										0.7936	0.6018	0.8342										1.2167	0.8148	1.2405
3	0:05	37							0.3902	0.3969	0.4003										0.7962	0.6028	0.836										1.2196	0.8231	1.2427
4	0:10	37							0.3908	0.3969	0.4011										0.7971	0.6031	0.8368										1.221	0.8243	1.2438
5	0:15	37							0.3907	0.3969	0.4013										0.7979	0.6034	0.8374										1.2218	0.8259	1.2442
6	0:20	37							0.3909	0.3969	0.4007										0.7985	0.6039	0.8377										1.2234	0.8284	1.2459
7	0:25	37							0.3909	0.3973	0.4004										0.7993	0.6042	0.8381										1.2241	0.8289	1.2464
8	0:30	37							0.391	0.3974	0.4008										0.8	0.6046	0.8388										1.2252	0.8299	1.247
9	0:35	37							0.3911	0.3975	0.4008										0.8004	0.6048	0.84										1.2255	0.8307	1.2471

Introduction

Experimental design: Layout files

Layout files contain:

- A description of the location and content of each well.
- Indicating if the well is control or a sample
- Control variables, like concentration or pH.
- Information in layout files not present in raw output files from plate readers.

	A	B	C	D	E	F	G	H	I	J	K
37	strain	1	2	3	4	5	6	7	8	9	10
38	A	water	water	water	water	water	water	water	water	water	water
39	B	water	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363
40	C	water	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363
41	D	water	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363
42	E	water	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363
43	F	water	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363
44	G	water	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363
45	H	water	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363
46	I	water	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363
47	J	water	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363
48	K	water	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363
49	L	water	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363
50	M	water	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363	MG1363
51	N	water	NCDO712	NCDO712	NCDO712	NCDO712	NCDO712	NCDO712	NCDO712	NCDO712	NCDO712
52	O	water	water	water	water	water	water	water	water	water	water
53	P	water	water	water	water	water	water	water	water	water	water
54											
55	concentration	1	2	3	4	5	6	7	8	9	10
56	A	100	20	4	0.8	0.16	0.032	0.0681	0.00128	0.000256	5.12E-05
57	B	100	20	4	0.8	0.16	0.032	0.0681	0.00128	0.000256	5.12E-05
58	C	100	20	4	0.8	0.16	0.032	0.0681	0.00128	0.000256	5.12E-05
59	D	100	20	4	0.8	0.16	0.032	0.0681	0.00128	0.000256	5.12E-05
60	E	100	20	4	0.8	0.16	0.032	0.0681	0.00128	0.000256	5.12E-05
61	F	100	20	4	0.8	0.16	0.032	0.0681	0.00128	0.000256	5.12E-05
62	G	100	20	4	0.8	0.16	0.032	0.0681	0.00128	0.000256	5.12E-05
63	H	100	20	4	0.8	0.16	0.032	0.0681	0.00128	0.000256	5.12E-05
64	I	100	20	4	0.8	0.16	0.032	0.0681	0.00128	0.000256	5.12E-05
65	J	100	20	4	0.8	0.16	0.032	0.0681	0.00128	0.000256	5.12E-05
66	K	100	20	4	0.8	0.16	0.032	0.0681	0.00128	0.000256	5.12E-05
67	L	100	20	4	0.8	0.16	0.032	0.0681	0.00128	0.000256	5.12E-05
68	M	100	20	4	0.8	0.16	0.032	0.0681	0.00128	0.000256	5.12E-05
69	N	100	20	4	0.8	0.16	0.032	0.0681	0.00128	0.000256	5.12E-05
70	O	100	20	4	0.8	0.16	0.032	0.0681	0.00128	0.000256	5.12E-05
71	P	100	20	4	0.8	0.16	0.032	0.0681	0.00128	0.000256	5.12E-05
72	I	100	20	4	0.8	0.16	0.032	0.0681	0.00128	0.000256	5.12E-05
73											
74	basic	1	2	3	4	5	6	7	8	9	10
75	A	control	control	control	control	control	control	control	control	control	control
76	B	control	sample	sample	sample	sample	sample	sample	sample	sample	sample
77	C	control	sample	sample	sample	sample	sample	sample	sample	sample	sample
78	D	control	sample	sample	sample	sample	sample	sample	sample	sample	sample
79	E	control	sample	sample	sample	sample	sample	sample	sample	sample	sample
80	F	control	sample	sample	sample	sample	sample	sample	sample	sample	sample

Introduction

Recap:

- **Different possible output files formats.**
  - .txt, csv, xlsx depends on the user
  - Different data representation, i.e multiskan GO list or table format depending on the manufacturer.
- **Information in layout files not included in raw data files.**

Introduction

Previous packages to process microplate raw data

- **cellHTS2(2006)**
  - Framework for RNAi screening
  - Raw files has to be formatted before analysis
  - *Ref: M. Boutros, L. P. Brás, and W. Huber, "Analysis of cell-based RNAi screens," Genome Biol.*

- **Douwe first package(2010)**
  - Parser only for SpectraMax, also need for update

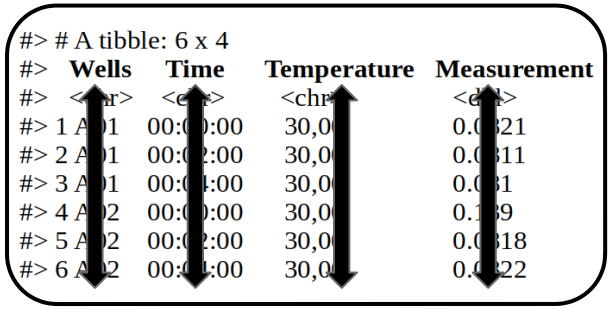
- **Plater package(2016)**
  - Designed to read plate-shaped file format of experimental design into tidy data frame.
  - Also, allows the conversion of a tidy data frame into plate-shaped data.
  - *Ref: S. M. Hughes, "plater: Read, Tidy, and Display Data from Microtiter Plates."*

Introduction

Tidy data

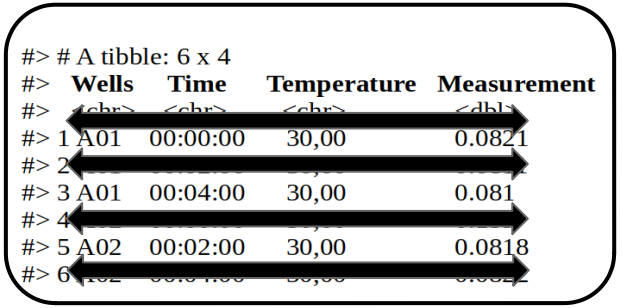
- Tidy data is a standard way to organize data
- Well suited for vectorised programming languages like R
- Easy to plug into tidyverse collection of R packages for data analysis

Variables



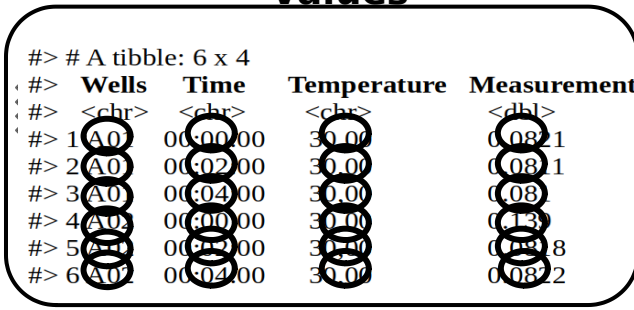
- Each variable represented as column

Observations



- Each observation represented by a row

Values



- Each value belong to one cell

H. Wickham, "Tidy data," J. Stat. Softw., vol. 59, no. 10, pp. 1–23, Sep. 2014.

Introduction

Project goal

The main goal of this research project is to develop an R package to:

- Reduce the time of data pre-processing in high-throughput experiments and improve standardization.
  - Process different output files from micro-plates readers. Store this information as tidy data.
  - Process layout files. Store this into tidy data
  - Combine output files with layout files into one tidy tibble object.

Introduction

mpxtractor

- **The main function of mpxtractor is to provide a simple method that can process raw data from microplate readers:**
  - SpectraMax
  - FluorStar
  - MultiscanGO
- This package generates tidy tibble objects, this data can be easily combined with layout files.
- Growth rates can be calculated and plot over a microplate-shape format.

Package available here:
https://github.com/MartinBanchero/mpxtractor

open source license
GPL-3

Data

SpectraMax
2 files:
Time: 26 hs and 16 hs, interval 2 minutes.Temperature: 30°C

Multiskan GO:
2 files:
1000 readings
Time interval 2 minutes.
Demo files for list format.

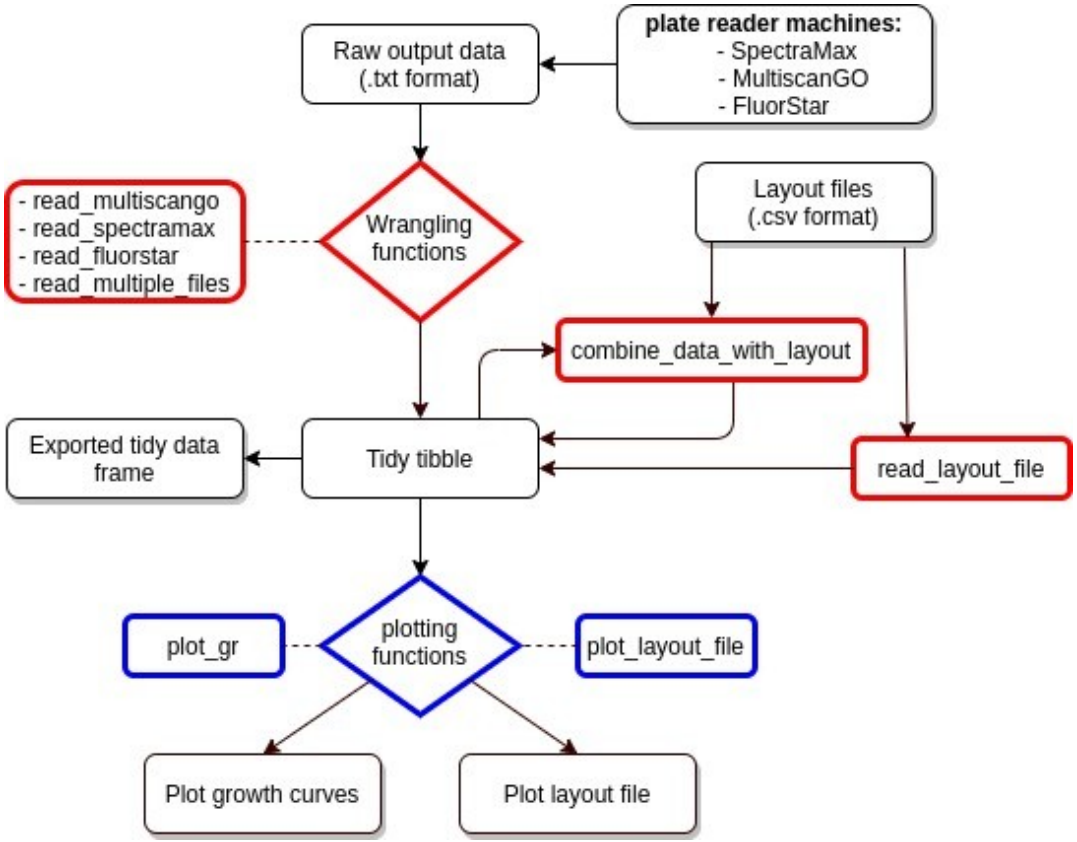
FluorStar:
2 files:
Time 20 hs 30 minutes
Time interval 2 minutes

Sieze Douwenga, PhD student at Systems Biology lab-VU

Methods

Mpxtractor: flowchart

- Wrangling functions
- Plotting functions



Methods

Wrangling functions: SpectraMax files

- **Caption raw file SpectraMax**

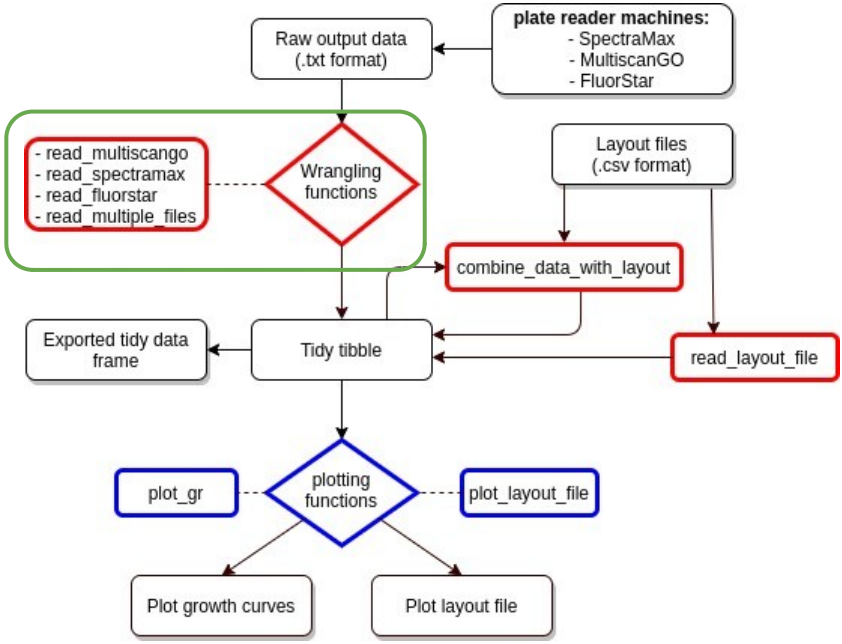
Time(hh:mm:ss)	Temperature(°C)	A1	A2	A3	A4	A5	A6	A7	A8
A17	A18	A19	A20	A21	A22	A23	A24	B1	B2	B3	B4
B13	B14	B15	B16	B17	B18	B19	B20	B21	B22	B23	B24
C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20
D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
E21	E22	E23	E24	F1	F2	F3	F4	F5	F6	F7	F8
F17	F18	F19	F20	F21	F22	F23	F24	G1	G2	G3	G4
G13	G14	G15	G16	G17	G18	G19	G20	G21	G22	G23	G24
H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20
I5	I6	I7	I8	I9	I10	I11	I12	I13	I14	I15	I16
J1	J2	J3	J4	J5	J6	J7	J8	J9	J10	J11	J12
J21	J22	J23	J24	K1	K2	K3	K4	K5	K6	K7	K8
K17	K18	K19	K20	K21	K22	K23	K24	L1	L2	L3	L4
L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24
M9	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20
N5	N6	N7	N8	N9	N10	N11	N12	N13	N14	N15	N16
O1	O2	O3	O4	O5	O6	O7	O8	O9	O10	O11	O12
O21	O22	O23	O24	P1	P2	P3	P4	P5	P6	P7	P8
P17	P18	P19	P20	P21	P22	P23	P24				
0:00	30,00	0,0821	0,1387	0,1438	0,1558	0,1416	0,1425	0,15	0,144	0,1404	0,1396
0,1526	0,1343	0,1551	0,1399	0,1458	0,0833	0,1552	0,2537	0,2535	0,2781	0,2708	0,2755
0,2769	0,2466	0,2459	0,2505	0,2529	0,2623	0,2626	0,2708	0,2559	0,134	0,1536	0,2529
0,3049	0,2984	0,2645	0,2621	0,26	0,2732	0,2662	0,2632	0,2606	0,2302	0,2265	0,2956
0,2775	0,2796	0,2774	0,2822	0,2748	0,2748	0,2737	0,2878	0,2792	0,2786	0,273	0,282
0,1655	0,17	0,1709	0,1728	0,1718	0,1803	0,1702	0,171	0,1694	0,1779	0,1707	0,1744
0,1715	0,1757	0,1418	0,2444	0,2708	0,2746	0,2765	0,2851	0,2832	0,2881	0,2845	0,284
0,2801	0,2912	0,294	0,296	0,2817	0,1929	0,1425	0,2675	0,2616	0,2755	0,281	0,2843

- **Example of usage and output of read_spectramax_data()**

```
R> data_spectramax <- mpxtractor::read_spectramax_data(file = file_path)
R> head(data_spectramax)
```

```
#> # A tibble: 6 x 4
```

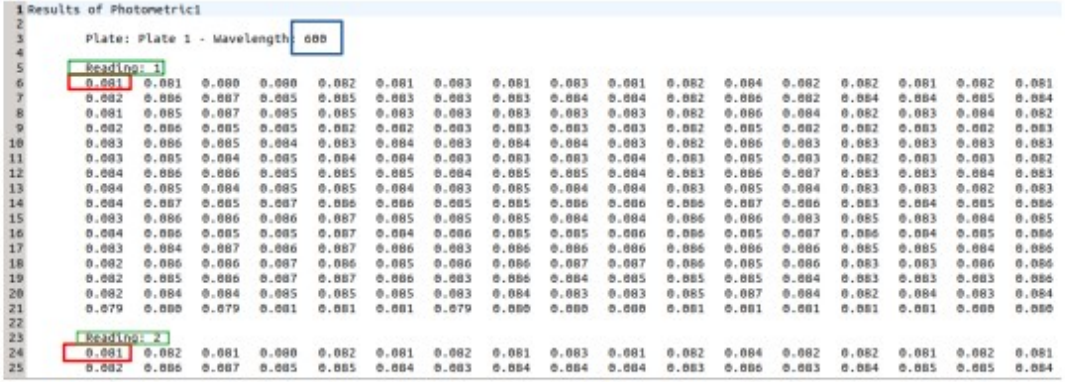
	Wells	Time	Temperature	Measurement
#>	<chr>	<chr>	<chr>	<dbl>
#> 1	A01	00:00:00	30,00	0.0821
#> 2	A01	00:02:00	30,00	0.0811
#> 3	A01	00:04:00	30,00	0.081
#> 4	A02	00:00:00	30,00	0.139
#> 5	A02	00:02:00	30,00	0.0818
#> 6	A02	00:04:00	30,00	0.0822



Methods

Wrangling functions: Multiskan GO files

- **Caption raw file: table format**



Results of Photometric1

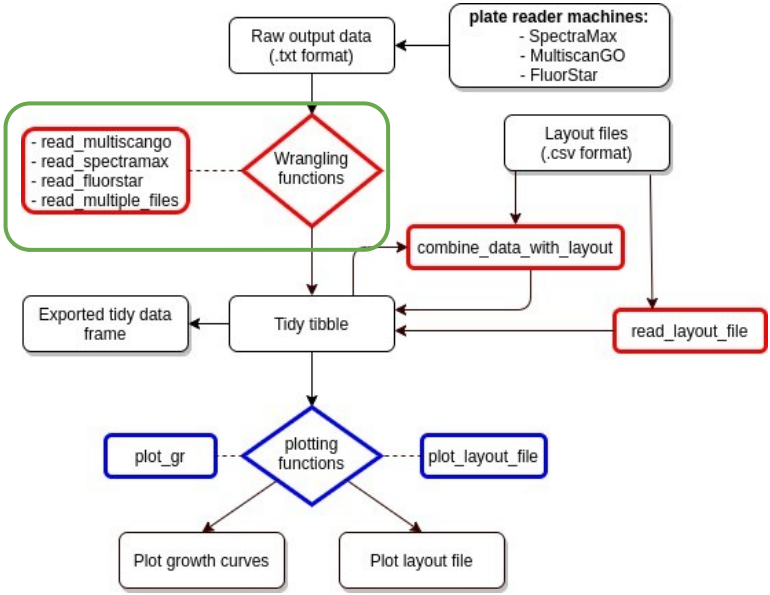
Plate: Plate 1 - Wavelength: 600

Reading: 1

0.081	0.081	0.080	0.080	0.082	0.081	0.083	0.081	0.083	0.081	0.082	0.084	0.082	0.082	0.081	0.082	0.081
0.082	0.086	0.087	0.085	0.085	0.083	0.083	0.083	0.084	0.084	0.082	0.086	0.082	0.084	0.084	0.085	0.084
0.081	0.085	0.087	0.085	0.085	0.083	0.083	0.083	0.083	0.083	0.082	0.086	0.084	0.082	0.083	0.084	0.082
0.082	0.086	0.085	0.085	0.082	0.082	0.083	0.083	0.083	0.083	0.082	0.085	0.082	0.082	0.083	0.082	0.083
0.083	0.086	0.085	0.084	0.083	0.084	0.083	0.084	0.084	0.083	0.082	0.086	0.083	0.083	0.083	0.083	0.083
0.083	0.085	0.084	0.085	0.084	0.084	0.083	0.083	0.083	0.084	0.083	0.085	0.083	0.082	0.083	0.083	0.082
0.084	0.086	0.086	0.085	0.085	0.085	0.084	0.085	0.085	0.084	0.083	0.086	0.087	0.083	0.083	0.084	0.083
0.084	0.085	0.084	0.085	0.085	0.084	0.083	0.085	0.084	0.084	0.083	0.085	0.084	0.083	0.083	0.082	0.083
0.084	0.087	0.085	0.087	0.086	0.086	0.085	0.085	0.086	0.086	0.086	0.087	0.086	0.083	0.084	0.085	0.086
0.083	0.086	0.086	0.086	0.087	0.085	0.085	0.085	0.084	0.084	0.086	0.086	0.083	0.085	0.083	0.084	0.085
0.084	0.086	0.085	0.085	0.087	0.084	0.086	0.085	0.085	0.086	0.086	0.085	0.087	0.086	0.084	0.085	0.086
0.083	0.084	0.087	0.086	0.087	0.086	0.083	0.086	0.086	0.086	0.086	0.086	0.086	0.085	0.085	0.084	0.086
0.082	0.086	0.086	0.087	0.086	0.085	0.086	0.086	0.087	0.087	0.086	0.085	0.086	0.083	0.083	0.086	0.086
0.082	0.085	0.086	0.087	0.087	0.086	0.083	0.086	0.084	0.085	0.085	0.085	0.084	0.083	0.083	0.083	0.086
0.082	0.084	0.084	0.085	0.085	0.085	0.083	0.084	0.083	0.083	0.085	0.087	0.084	0.082	0.084	0.083	0.084
0.079	0.080	0.079	0.081	0.081	0.081	0.079	0.080	0.080	0.080	0.081	0.081	0.081	0.081	0.081	0.080	0.080

Reading: 2

0.081	0.082	0.081	0.080	0.082	0.081	0.082	0.081	0.083	0.081	0.082	0.084	0.082	0.082	0.081	0.082	0.081
0.082	0.086	0.087	0.085	0.085	0.084	0.083	0.084	0.084	0.084	0.083	0.086	0.083	0.084	0.085	0.085	0.084




```
R> data_multiscan <- mpxtractor::read_multiscango_data(file = file_path,
    time_interval = "2.5 min" )
R> head(data_multiscan)
```

```
#> # A tibble: 6 x 5
#>   Wells Time     Reading wavelength Measurement
#>   <chr> <chr>      <dbl>      <dbl>       <dbl>
#> 1 A01   00:00:00       1        600       0.081
#> 2 A01   00:02:30       2        600       0.081
#> 3 A01   00:05:00       3        600       0.081
#> 4 A01   00:07:30       4        600       0.081
#> 5 A01   00:10:00       5        600       0.081
#> 6 A01   00:12:30       6        600       0.081
```

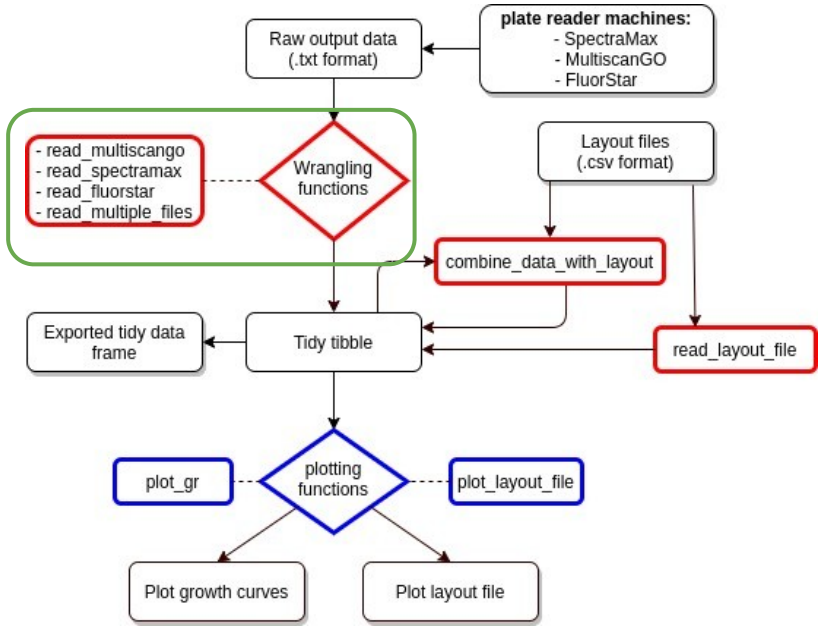
- **Example of usage and output of read_multiscan_data()**

Methods

Wrangling functions: Multiskan GO files

- **Caption raw file: List format**

	Plate	Well	Group	Type	Sample	Wavelength	Reading	Abs	Meas. Time [s]
1	Plate 1	C02	Assay	Calibrator	1 1/1	340	1	0.231	0.330
2	Plate 1	C02	Assay	Calibrator	1 1/1	340	2	0.233	30.320
3	Plate 1	C02	Assay	Calibrator	1 1/1	340	3	0.235	60.320
4	Plate 1	C02	Assay	Calibrator	1 1/1	340	4	0.237	90.320
5	Plate 1	D02	Assay	Calibrator	2 1/1	340	1	0.236	0.690
6	Plate 1	D02	Assay	Calibrator	2 1/1	340	2	0.239	30.690
7	Plate 1	D02	Assay	Calibrator	2 1/1	340	3	0.240	60.690
8	Plate 1	D02	Assay	Calibrator	2 1/1	340	4	0.242	90.690
9	Plate 1	E02	Assay	Calibrator	3 1/1	340	1	0.241	1.020
10	Plate 1	E02	Assay	Calibrator	3 1/1	340	2	0.244	31.020
11	Plate 1	E02	Assay	Calibrator	3 1/1	340	3	0.246	61.020
12	Plate 1	E02	Assay	Calibrator	3 1/1	340	4	0.250	91.020
13	Plate 1	F02	Assay	Calibrator	4 1/1	340	1	0.231	1.390
14	Plate 1	F02	Assay	Calibrator	4 1/1	340	2	0.235	31.380
15	Plate 1	F02	Assay	Calibrator	4 1/1	340	3	0.238	61.380
16	Plate 1	F02	Assay	Calibrator	4 1/1	340	4	0.242	91.380
17	Plate 1	C03	Assay	Control	5 1/1	340	1	0.240	0.350
18	Plate 1	C03	Assay	Control	5 1/1	340	2	0.246	30.340
19	Plate 1	C03	Assay	Control	5 1/1	340	3	0.251	60.340
20	Plate 1	C03	Assay	Control	5 1/1	340	4	0.257	90.340
21	Plate 1	D03	Assay	Control	6 1/1	340	1	0.240	0.680
22	Plate 1	D03	Assay	Control	6 1/1	340	2	0.246	30.670
23	Plate 1	D03	Assay	Control	6 1/1	340	3	0.252	60.670
24	Plate 1	D03	Assay	Control	6 1/1	340	4	0.259	90.670
25	Plate 1	E03	Assay	Control	7 1/1	340	1	0.274	1.040
26	Plate 1	E03	Assay	Control	7 1/1	340	2	0.281	31.030
27	Plate 1	E03	Assay	Control	7 1/1	340	3	0.289	61.030
28	Plate 1	E03	Assay	Control	7 1/1	340	4	0.296	91.040
29	Plate 1	B02	Assay	Blank	Blank_Assay 1/1	340	1	0.231	0.000
30	Plate 1	B02	Assay	Blank	Blank_Assay 1/1	340	2	0.230	30.000
31	Plate 1	B02	Assay	Blank	Blank_Assay 1/1	340	3	0.231	60.000
32	Plate 1	B02	Assay	Blank	Blank_Assay 1/1	340	4	0.231	90.000
33	Plate 1	B02	Assay	Blank	Blank_Assay 1/1	340	4	0.231	90.000




```
R> data_multiscan <- mpxtractor::read_multiscango_data(file_path, input_type = "list")
R> head(data_multiscan)
```

Wells	Time	Measurement	Plate	Group	Type	Sample	Wavelength	Reading	Meas.time.sec
<chr>	<chr>	<dbl>	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>	<dbl>
C02	00:00:00	0.231	Plate 1	Assay	Calibrator 1	1/1	340	1	0.33
C02	00:00:30	0.233	Plate 1	Assay	Calibrator 1	1/1	340	2	30.32
C02	00:01:00	0.235	Plate 1	Assay	Calibrator 1	1/1	340	3	60.32
C02	00:01:30	0.237	Plate 1	Assay	Calibrator 1	1/1	340	4	90.32
D02	00:00:00	0.236	Plate 1	Assay	Calibrator 2	1/1	340	1	0.69
D02	00:00:30	0.239	Plate 1	Assay	Calibrator 2	1/1	340	2	30.69

- **Example of usage and output of read_multiscan_data()**

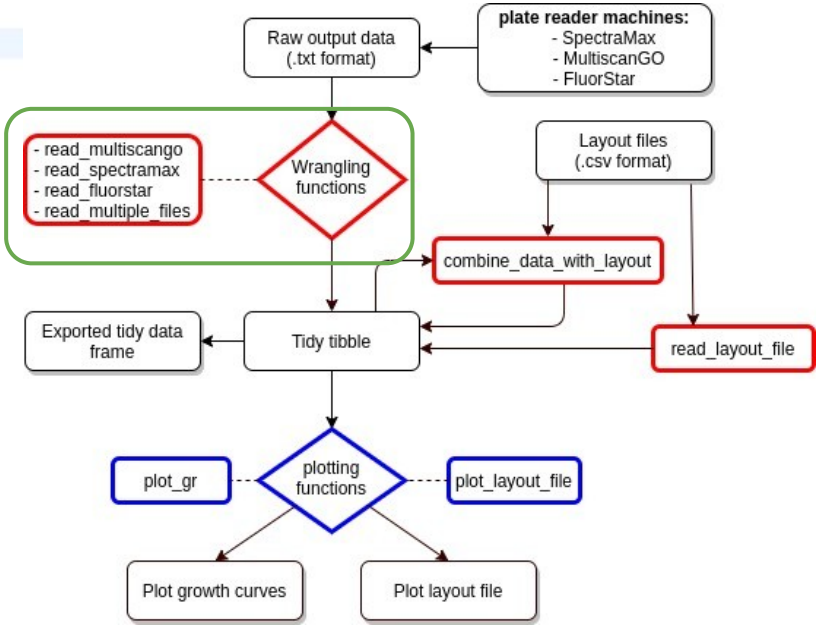
Methods

Wrangling functions: FluorStar files

- **Caption raw file**

```
User: USER                    Path: C:\Program Files (x86)\BMG\Omega\User\Data\
221
Test Name: Test_Fluorescence                                  Date: 29/08/2019        Time:
15:04:05
ID1: Reading
1
ID2:
Fluorescence
Fluorescence
(FI)


"Well
Row"    "Well
Col"    Content         "Raw Data (485, 520)
1 - 0 h "       "Raw Data (485, 520)
2 - 0 h 2 min "         "Raw Data (485, 520)
3 - 0 h 7 min "         "Raw Data (485, 520)
4 - 0 h 12 min "        "Raw Data (485, 520)
5 - 0 h 17 min "        "Raw Data (485, 520)
6 - 0 h 22 min "        "Raw Data (485, 520)
                0       0.041333333     0.125555556     0.21    0.292222222     0.376666667
A       1       Sample X1       186705  186950  188978  189018  189620  191198
```



- **Example of usage and output of read_fluorstar_data()**

```
R> data_fluorstar <- mpxtractor::read_fluorstar_data(file = file_path)
R> head(data_fluorstar)
```

```
#> # A tibble: 6 x 5
#>   Wells Sample Time     Measurement wavelength
#>   <chr> <chr>  <chr>          <int> <chr>
#> 1 A01   " X1"  00:00:00      186705 485, 520
#> 2 A01   " X1"  00:02:00      186950 485, 520
#> 3 A01   " X1"  00:07:00      188978 485, 520
#> 4 A01   " X1"  00:12:00      189018 485, 520
#> 5 A01   " X1"  00:17:00      189620 485, 520
#> 6 A01   " X1"  00:22:00      191198 485, 520
```

Methods

Read multiple data files

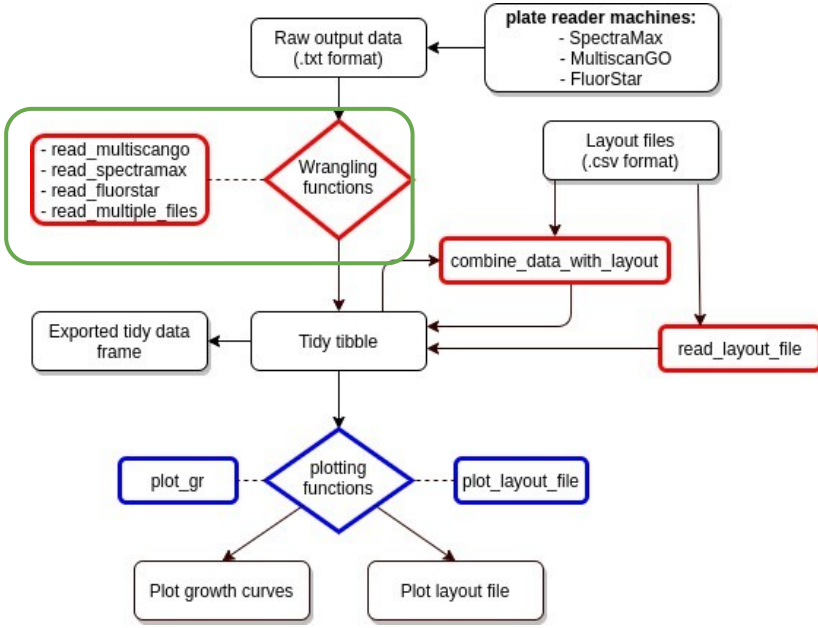
Usage and output of read_multiple_data_files

```
R> data_multiple_spectramax <- mpxtractor::read_multiple_data_files(
reader_type = "spectramax",
filesname = file_path,
plate_names = c("plate_1", "plate_2")
)
R> head(data_multiple_spectramax)

#> # A tibble: 6 x 5
#>   Wells  Time     Temperature Measurement plate_filename
#>   <chr>  <chr>    <chr>       <dbl>      <chr>
#> 1 A01    00:00:00 30,00       0.0821     plate_1
#> 2 A01    00:02:00 30,00       0.0811     plate_1
#> 3 A01    00:04:00 30,00       0.081      plate_1
#> 4 A02    00:00:00 30,00       0.139      plate_1
#> 5 A02    00:02:00 30,00       0.0818     plate_1
#> 6 A02    00:04:00 30,00       0.0822     plate_1

R> tail(data_multiple_spectramax)

#> # A tibble: 6 x 5
#>   Wells  Time     Temperature Measurement plate_filename
#>   <chr>  <chr>    <chr>       <dbl>      <chr>
#> 1 P23    00:00:00 30,00       0.0801     plate_2
#> 2 P23    00:02:00 30,00       0.0799     plate_2
#> 3 P23    00:04:00 30,00       0.08       plate_2
#> 4 P24    00:00:00 30,00       0.0812     plate_2
#> 5 P24    00:02:00 30,00       0.0811     plate_2
#> 6 P24    00:04:00 30,00       0.0815     plate_2
```



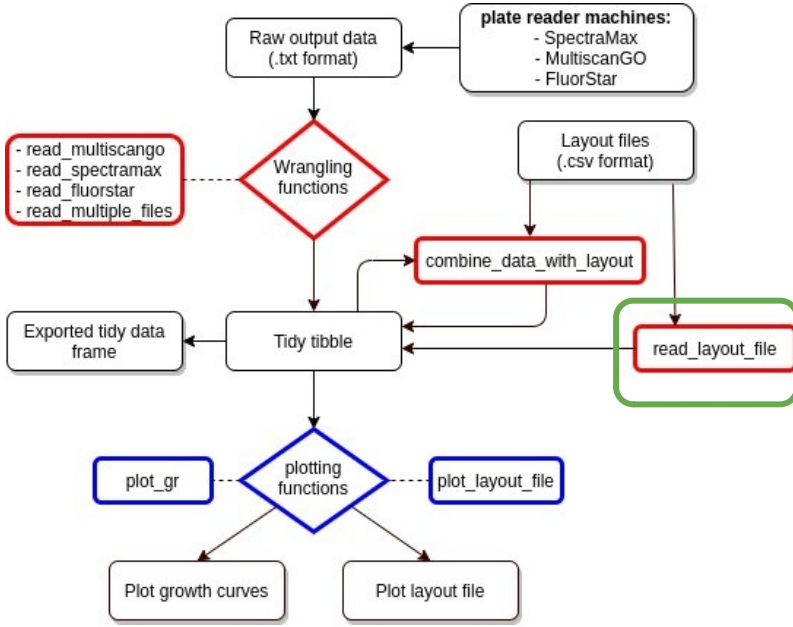
This function take as argument
the type of reader machine(blue)
and the name of the file(red)

Methods

Read layout files

- **Caption layout file**

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	basic	1	2	3	4	5	6	7	8	9	10	11	12
2	A	control	sample	sample	sample	sample	sample	sample	control	sample	sample	sample	control
3	B	control	sample	sample	sample	sample	sample	sample	control	sample	sample	sample	control
4	C	control	sample	sample	sample	sample	sample	sample	control	sample	sample	sample	control
5	D	control	sample	sample	control	sample	sample	sample	control	sample	sample	sample	control
6	E	control	sample	sample	sample	sample	sample	sample	control	sample	sample	sample	control
7	F	control	sample	sample	sample	sample	sample	sample	control	sample	sample	sample	control
8	G	control	sample	sample	sample	sample	sample	sample	control	sample	sample	sample	control
9	H	control	sample	sample	sample	sample	sample	sample	control	sample	sample	sample	control
10													
11	medium	1	2	3	4	5	6	7	8	9	10	11	12
12	A	milk	milk	milk	milk	milk_sup	milk_sup	milk_sup	milk_sup	filt_milk_sup	filt_milk_sup	filt_milk_sup	filt_milk_sup
13	B	milk	milk	milk	milk	milk_sup	milk_sup	milk_sup	milk_sup	filt_milk_sup	filt_milk_sup	filt_milk_sup	filt_milk_sup
14	C	milk	milk	milk	milk	milk_sup	milk_sup	milk_sup	milk_sup	filt_milk_sup	filt_milk_sup	filt_milk_sup	filt_milk_sup
15	D	milk	milk	milk	milk	milk_sup	milk_sup	milk_sup	milk_sup	filt_milk_sup	filt_milk_sup	filt_milk_sup	filt_milk_sup
16	E	milk	milk	milk	milk	milk_sup	milk_sup	milk_sup	milk_sup	filt_milk_sup	filt_milk_sup	filt_milk_sup	filt_milk_sup
17	F	milk	milk	milk	milk	milk_sup	milk_sup	milk_sup	milk_sup	filt_milk_sup	filt_milk_sup	filt_milk_sup	filt_milk_sup
18	G	milk	milk	milk	milk	milk_sup	milk_sup	milk_sup	milk_sup	filt_milk_sup	filt_milk_sup	filt_milk_sup	filt_milk_sup
19	H	milk	milk	milk	milk	milk_sup	milk_sup	milk_sup	milk_sup	filt_milk_sup	filt_milk_sup	filt_milk_sup	filt_milk_sup
20													
21	concentration	1	2	3	4	5	6	7	8	9	10	11	12
22	A	100	20	4	0.8	0.16	0.032	0.0064	0.00128	0.000256	5.12E-05	1.024E-05	0
23	B	100	20	4	0.8	0.16	0.032	0.0064	0.00128	0.000256	5.12E-05	1.024E-05	0
24	C	100	20	4	0.8	0.16	0.032	0.0064	0.00128	0.000256	5.12E-05	1.024E-05	0
25	D	100	20	4	0.8	0.16	0.032	0.0064	0.00128	0.000256	5.12E-05	1.024E-05	0
26	E	100	20	4	0.8	0.16	0.032	0.0064	0.00128	0.000256	5.12E-05	1.024E-05	0
27	F	100	20	4	0.8	0.16	0.032	0.0064	0.00128	0.000256	5.12E-05	1.024E-05	0
28	G	100	20	4	0.8	0.16	0.032	0.0064	0.00128	0.000256	5.12E-05	1.024E-05	0
29	H	100	20	4	0.8	0.16	0.032	0.0064	0.00128	0.000256	5.12E-05	1.024E-05	0




```
R> df_layout <- mpxtractor::read_layout_file(file)
R> head(df_layout)
```

Wells	basic	medium	concentration
<chr>	<chr>	<chr>	<dbl>
1 A01	control	milk	100
2 A02	sample	milk	20
3 A03	sample	milk	4
4 A04	sample	milk	0.8
5 A05	sample	milk_sup	0.16
6 A06	sample	milk_sup	0.032

- **Example of usage and output of read_layout_file()**

read_layout_file() similar to ***read_plate()*** from plater package

S. M. Hughes, "plater: Read, Tidy, and Display Datd from Microtiter Plates."

Methods

Combine data files with layout files

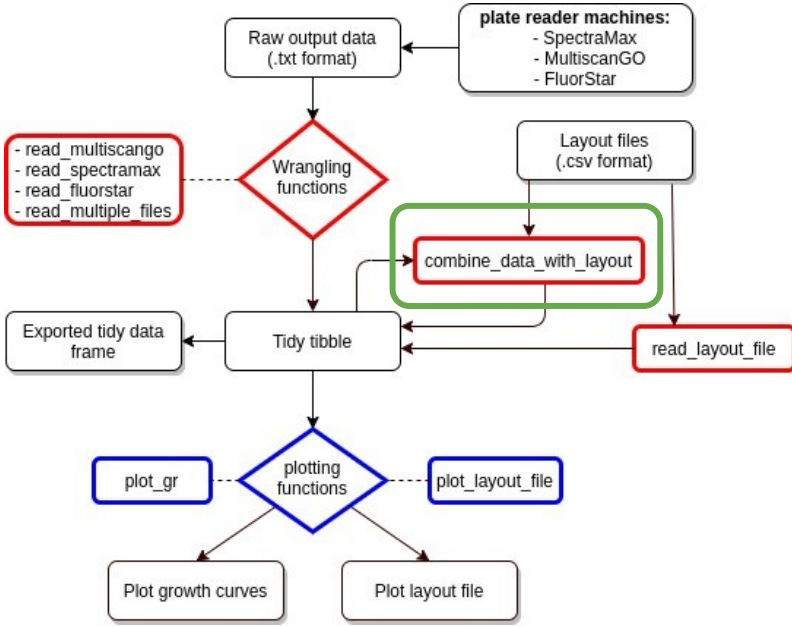
df_data: Raw data into tibble
Reader_type: Specified reader machine

```
R> df_data_fl <- mpxtractor::read_fluorstar_data(file_fl)
R> df_data_layout <- mpxtractor::combine_data_with_lay out(
  df_data = df_data_fl,
  reader_type = "fluorstar",
  layout_files = file
    )
R> head(df_data_layout)
```

A tibble: 6 x 9

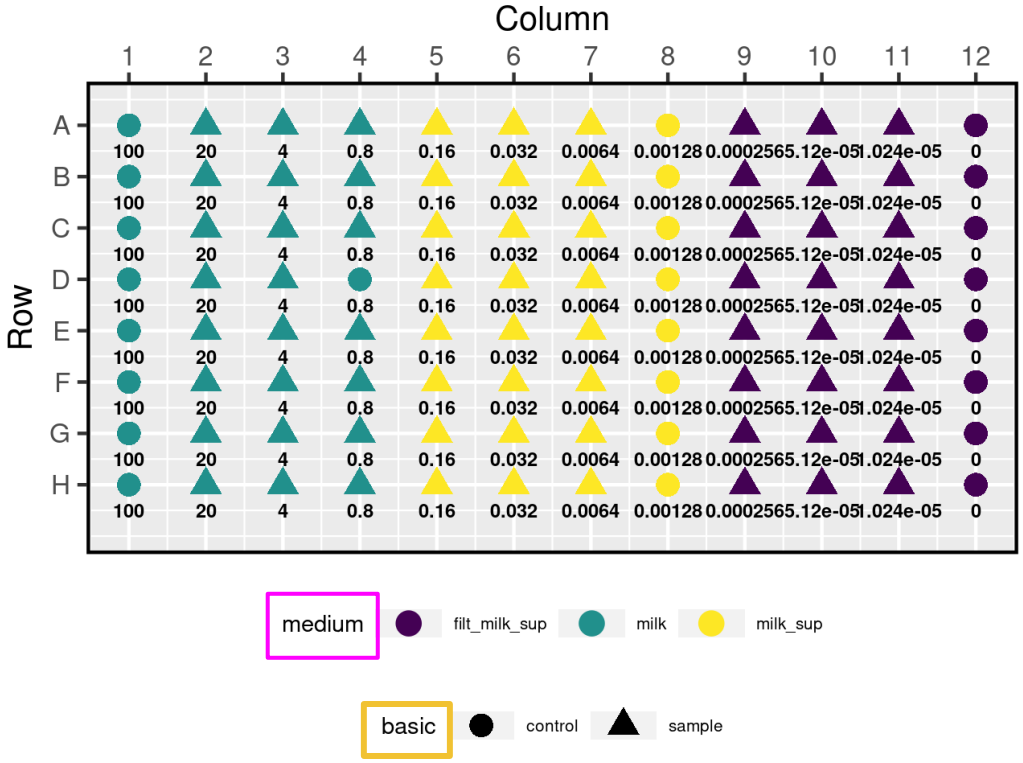
Wells	Sample	Time	Measurement	wavelength	basic	medium	concentration	layout_file
<chr>	<chr>	<chr>	<dbl>	<chr>	<chr>	<chr>	<dbl>	<chr>
1 A01	"X1"	00:00:00	3.5	600	control	milk	100	test_fluorstar_layout
2 A01	"X1"	00:02:00	3.44	600	control	milk	100	test_fluorstar_layout
3 A01	"X1"	00:07:00	3.5	600	control	milk	100	test_fluorstar_layout
4 A01	"X1"	00:12:00	3.5	600	control	milk	100	test_fluorstar_layout
5 A01	"X1"	00:17:00	3.21	600	control	milk	100	test_fluorstar_layout
6 A01	"X1"	00:22:00	3.35	600	control	milk	100	test_fluorstar_layout

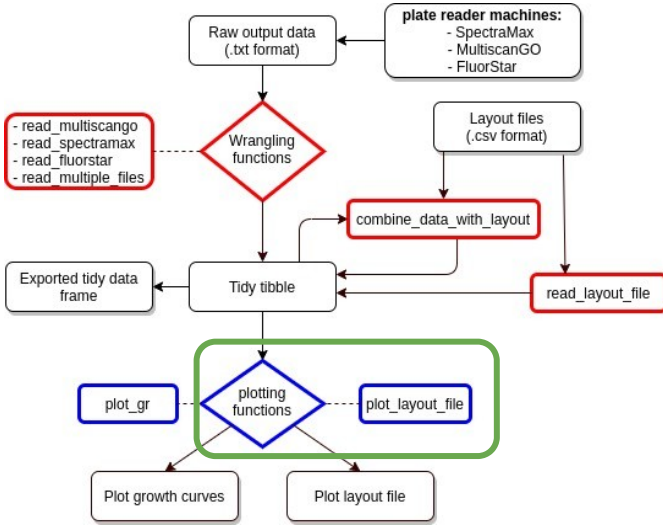
Raw data combined with layout file



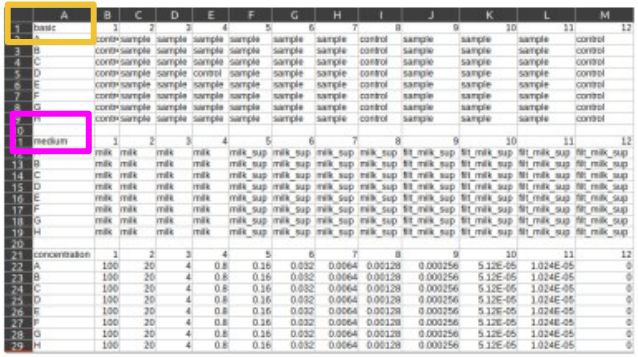
Methods

Plot layout file





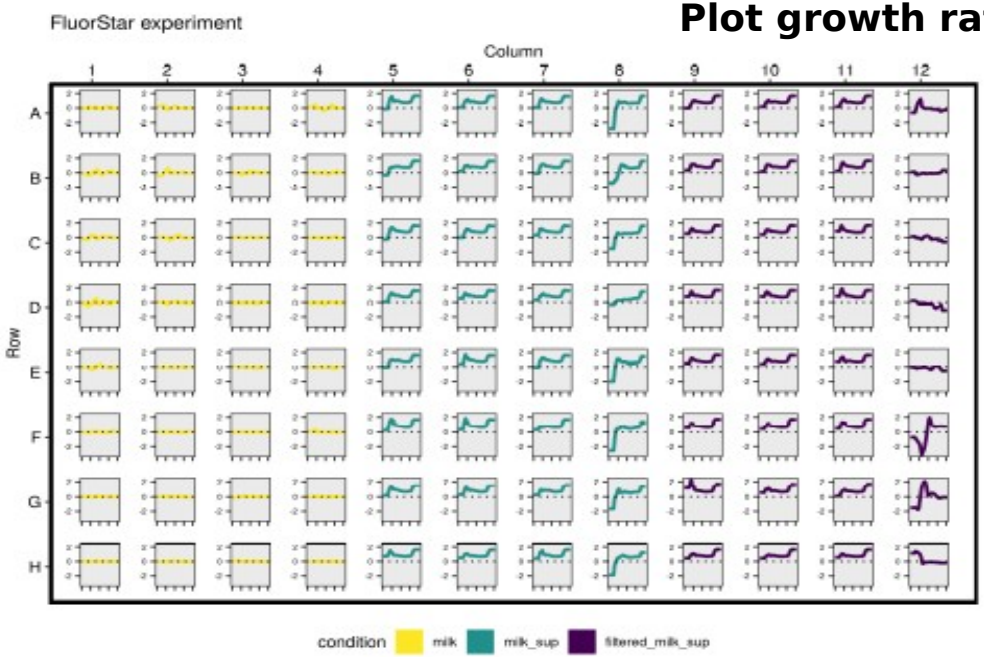
Layout file

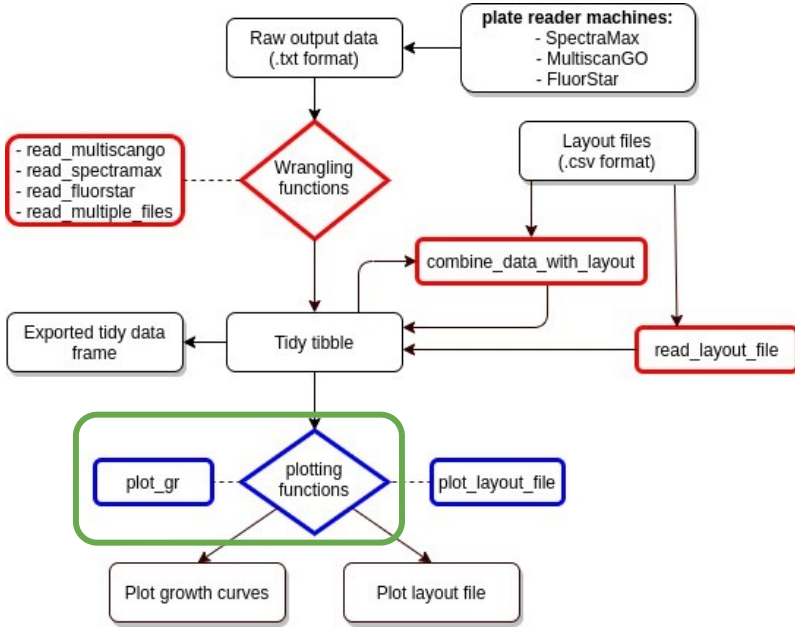


Representation up to 2 variables through shape, color and concentration(optional)

Methods

Plot growth rates



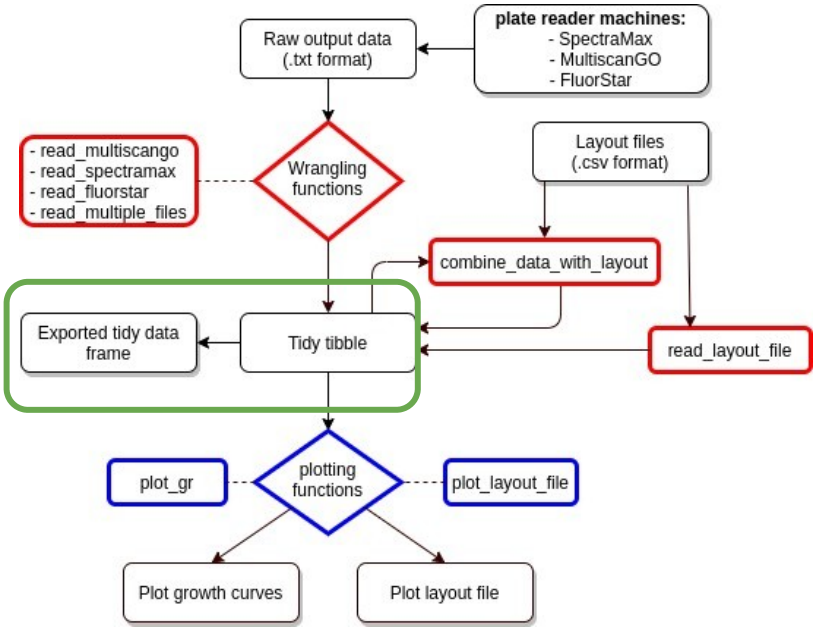


R> growth_rate_plot<- **mpxtractor::plot_gr_microplate**(
df_data = df_corrected,
var_gr = “Measurement”,
cond_to_col = ”condition”,
plate_file = NULL,
ws = “2hs”,
output_filename = NULL)

Methods

Plot growth rates

- **compute_growth_rates()**
  This function calculate growth rate using **Savitzky and Golay filter** from package Signal.



- **Sav-Gol filter :**
  - Type of low pass filter used to smooth the data, improve graphical output due to large number of data points and random noise.
  - It can be used to compute smoothed first order derivatives to obtain the growth rates.
  - Use a moving window average to fit a polynomial function through the data points

Methods

Time series

```
#> # A tibble: 6 x 9
#>   Wells Sample  Time  Measurement wavelength Basic condition concentration  layout_file
#>   <cr> <chr>  <chr> <dbl>       <chr>      <chr> <chr>       <dbl>         <chr>
#> 1 A01   " X1" 00:00:00  3.5          600       poor   milk          100    test_fluorstar_layout
#> 2 A01   " X1" 00:02:00  3.44         600       poor   milk          100    test_fluorstar_layout
#> 3 A01   " X1" 00:07:00  3.5          600       poor   milk          100    test_fluorstar_layout
#> 4 A01   " X1" 00:12:00  3.5          600       poor   milk          100    test_fluorstar_layout
#> 5 A01   " X1" 00:17:00  3.21         600       poor   milk          100    test_fluorstar_layout
#> 6 A01   " X1" 00:22:00  3.35         600       poor   milk          100    test_fluorstar_layout
```

Plot growth rates

Time series has to be fixed by user before calculate the growth rates, otherwise mpxtractor through an error.

Remark: missing values

- Missing values in column “Measurements” are imputed by taking the average between surrounding numbers.
- In this case mpxtractor shows a warning letting the user know about the imputation.

Results

Validation: Growth rates

Fluorstar data

	mpxtractor data			Validation data		
Wells	**Time(hs)**	**log(Abs)**	**Growth rate**	**Time(hs)**	**log(Abs)**	**Growth rate**
A01	0.03333	-0.84373	-0.07818094	0.00000	-0.843738	-0.078181
:	:	:	:	:	:	:
A01	1.28333	-1.175061	0.1089509	1.25000	-1.175061	0.108951
A01	1.36666	-0.717644	0.06203398	**1.3333**	-0.717645	**-inf**
:	:	:	:	:	:	:
A01	2.28333	-1.161871	-0.05384393	2.25000	-1.16187	**-inf**
A01	2.36666	**-1.010418**	0.057739	**2.3333**	**-inf**	**-inf**
A01	2.45000	-0.858965	0.00011882	2.41666	-0.858966	**inf**
:	:	:	:	:	:	:
A01	3.36666	-0.999400	0.06919943	**3.3333**	-0.999401	**inf**
A01	3.45000	-0.857078	0.08525832	3.41666	-0.857079	0.085258
:	:	:	:	:	:	:
A01	16.1166	-0.797175	-0.1556584	16.0833	-0.797175	0.073194
A01	--	--	--	20.4166	-0.920299	0.057739

Ws = 2hs

Value corresponding to background absorbance

Values imputed by taking the average of the surrounding values

Results

Validation: Growth rates

Multiskan Go data

		mpxtractor data		Validation data	
Wells	**Time(hs)**	**log(Abs)**	**Growth rate**	**log(Abs)**	**Growth rate**
A01	**0.000000**	**-6.907755**	0.6355529	**-Inf**	**NaN**
:	:	:	:	:	:
A01	**0.666667**	**-6.907755**	0.6355529	**-Inf**	**NaN**
A01	0.708333	-6.907755	0.6355529	-6.907755	**NaN**
:	:	:	:	:	:
A01	0.958333	-6.907755	0.6355529	-6.907755	**NaN**
A01	1.000000	-6.907755	0.6355529	-6.907755	**Inf**
:	:	:	:	:	:
A01	**1.666667**	-6.214608	0.8386713	-6.214608	**Inf**
A01	1.708333	-5.809143	0.8284531	-5.809143	0.828453
:	:	:	:	:	:
A01	41.625000	-4.710531	-0.0173069	-4.710531	-0.0173069

Value corresponding to background absorbance

Values imputed by taking the average of the surrounding values

Results

Validation: Growth rates

SpectraMax data

		mpxtractor data		Validation data	
Wells	Time(hs)	log(Abs)	Growth rate	log(Abs)	Growth rate
A01	0.0000	-7.264430	-0.238977	-7.264430	-0.238978
:	:	:	:	:	:
A01	1.1000	-6.907755	-0.384584	-6.907755	-0.384584
A01	1.1333	-6.907755	-0.412485	-6.907755	-Inf
:	:	:	:	:	:
A01	2.1000	-7.600902	0.033795	-7.600902	-Inf
A01	2.1333	-7.600902	0.031808	-Inf	-Inf
A01	2.1666	-7.600902	0.007807	-7.600902	Inf
:	:	:	:	:	:
A01	3.1333	-7.130899	0.357646	-7.130899	Inf
A01	3.1666	-7.600902	0.339011	-7.600902	0.339011
A01	26.1333	-6.645391	0.032598	-6.645391	0.032599

Value corresponding to background absorbance

Values imputed by taking the average of the surrounding values

Conclusion

- **Reduction** of the time to process raw files into tidy data.
- Improvement in **standardization**, only two types of files.
- plate readers, acceptable number of different plate machines rom three different
- taking the mean of the surrounding data points. ues in the data and imputes by
- Help to improve **reproducibility**

Future improvements

- Improve robustness of **mpxtractor** through users' reports of bugs in the package repository.
- More testing
- **Addition of features:**
  - To process data from other machines
  - Calculate enzyme rates
- Increase user friendliness using R-shiny

Acknowledgments

Systems Biology Lab-VU:

Supervisor: Douwe Molenaar PhD

Sieze Douwenga, PhD student

Vera Benavente, Lab technician

Dennis Botman, Phd student
Laura Guilherme Luzia, Phd student

Einde

Questions?

supplementary

Set of parameters

Type of argument	Value	R parameter	Python parameter
Window length = window size / diff time	wl= (2/ 0.0333) hs	n=60	window_length=60
Filter order	1	p=1	polyorder=1
Derivative order	1	m=1	derivative=1

supplementary

Input raw file for cellHTS with fluorescence values.

1	FT01-G01	A01	887763
2	FT01-G01	A02	958308
3	FT01-G01	A03	1012685
4	FT01-G01	A04	872603
5	FT01-G01	A05	1179875
6	FT01-G01	A06	1213279
7	FT01-G01	A07	1182103
8	FT01-G01	A08	1353041
9	FT01-G01	A09	1236055
10	FT01-G01	A10	1228895
11	FT01-G01	A11	1308768
12	FT01-G01	A12	1313472
13	FT01-G01	A13	1404374