

Description of affyILM package

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1 Introduction

affyILM is a preprocessing tool which estimates gene expression levels for Affymetrix Gene Expression Chips. *affyILM* computes gene expression levels using the Langmuir model. In contrast to other measures, this method outputs the gene expression level as concentrations measured in pM (picoMolar).

affyILM allows the user to simultaneously read-in several CEL-files; it does *not* require raw data (CEL-files) to be specifically formatted like e.g. as *AffyBatch*.

2 Getting started

2.1 Preliminaries

To install the package:

```
R CMD INSTALL affyILM_x.y.z.tar.gz
```

affyILM imports several functions from other packages. Make sure to have the following installed:

affxparser, *affy Biobase* and *germa*. Chip-specific probe packages which are not yet installed on your system will be automatically downloaded from the bioconductor webpage if needed.

2.2 First Steps

For demonstration purposes we use a test CEL-file supplied by *AffymetrixDataTestFiles*.

```
> require(AffymetrixDataTestFiles)
```

Load the library

```
> library(affyILM)
```

and locate the test CEL-file

```
> path <- system.file("rawData", "FusionSDK_HG-Focus", "HG-Focus", "2.Calvin",  
+ package="AffymetrixDataTestFiles")  
> file1 <- file.path(path, "HG-Focus-1-121502.CEL")
```

Calculation of the hybridization free energies for each probe, and estimation of concentrations using the Langmuir isotherm:

```
> result <- ilm(file1);
```

Chip dimension 448 x 448

```
[1] "Checking to see if your internet connection works..."
```

Probepackage hgfocusprobe loaded

Reading intensities...[1] ...done

Now let's have a look at the output printed on the screen:

- Chip dimension
- probe package downloaded if missing

Take a look at the experimental PM's

```
> getIntens(result, "AFFX-r2-Ec-bioD-5_at")
```

	HG-Focus-1-121502.CEL	HG-Focus-1-121502.CEL
ps.AFFX-r2-Ec-bioD-5_at.id	644.5	0
ps.AFFX-r2-Ec-bioD-5_at.id	694.3	0
ps.AFFX-r2-Ec-bioD-5_at.id	602.5	0
ps.AFFX-r2-Ec-bioD-5_at.id	1384.0	0
ps.AFFX-r2-Ec-bioD-5_at.id	1329.8	0
ps.AFFX-r2-Ec-bioD-5_at.id	1410.8	0
ps.AFFX-r2-Ec-bioD-5_at.id	1404.0	0
ps.AFFX-r2-Ec-bioD-5_at.id	3265.8	0
ps.AFFX-r2-Ec-bioD-5_at.id	2616.3	0
ps.AFFX-r2-Ec-bioD-5_at.id	1045.5	0
ps.AFFX-r2-Ec-bioD-5_at.id	628.3	0

Plot the result:

```
> plotIntens(result, "AFFX-r2-Ec-bioD-5_at", "HG-Focus-1-121502.CEL")
```

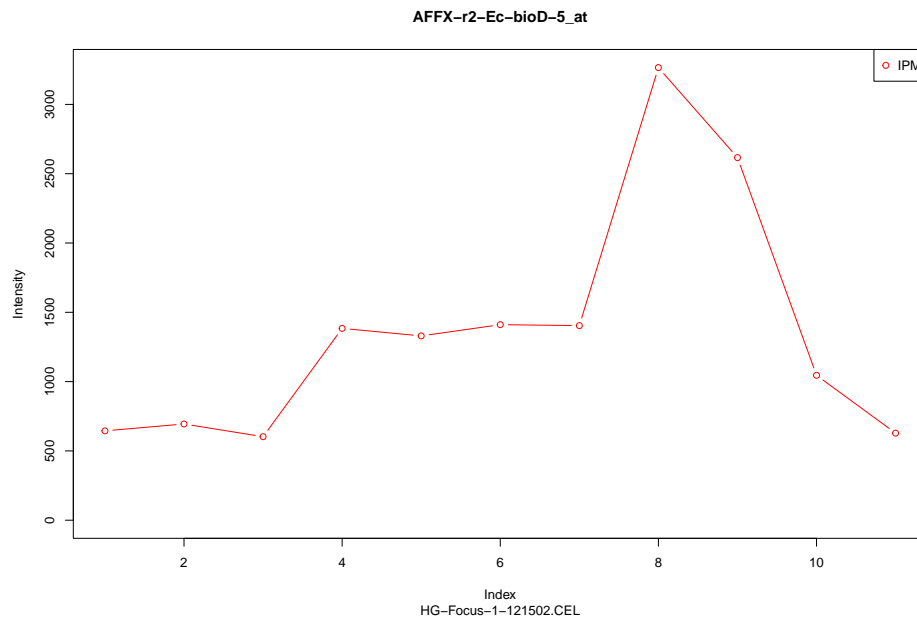


Figure 1: Probes intensities

3 More Examples with options

Analyze two or more CEL-files

```
> file2 <- file.path(path, "HG-Focus-2-121502.CEL")
> result2files <- ilm(c(file1, file2), satLim=12000)
```

```
Chip dimension 448 x 448
Probepackage hgfocusprobe loaded
Reading intensities...[1] ...done
```

where the saturation limit of the Langmuir isotherm is increased to 12000 (default: 10000)

Get intensity values:

```
> getIntens(result2files, "AFFX-r2-Ec-bioD-5_at")
```

	HG-Focus-1-121502.CEL	HG-Focus-2-121502.CEL
ps.AFFX-r2-Ec-bioD-5_at.id	644.5	692.3
ps.AFFX-r2-Ec-bioD-5_at.id	694.3	809.5
ps.AFFX-r2-Ec-bioD-5_at.id	602.5	687.3

ps.AFFX-r2-Ec-bioD-5_at.id	1384.0	1708.5
ps.AFFX-r2-Ec-bioD-5_at.id	1329.8	1513.5
ps.AFFX-r2-Ec-bioD-5_at.id	1410.8	1643.8
ps.AFFX-r2-Ec-bioD-5_at.id	1404.0	1838.0
ps.AFFX-r2-Ec-bioD-5_at.id	3265.8	3985.3
ps.AFFX-r2-Ec-bioD-5_at.id	2616.3	3331.0
ps.AFFX-r2-Ec-bioD-5_at.id	1045.5	1102.0
ps.AFFX-r2-Ec-bioD-5_at.id	628.3	746.0
	HG-Focus-1-121502.CEL	HG-Focus-2-121502.CEL
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0

- 1st column: Probeset name
- 2nd and 3rd column: Measured PM intensities IPM of each file.
- 4th and 5th column: IO intensities of each file (default value is 0 in current release, no background estimation).

To obtain the probe concentrations (or expression levels), use

```
> getProbeConcs(result2files, "AFFX-r2-Ec-bioD-5_at")
```

	HG-Focus-1-121502.CEL	HG-Focus-2-121502.CEL
ps.AFFX-r2-Ec-bioD-5_at.id	265.98771	286.9227
ps.AFFX-r2-Ec-bioD-5_at.id	225.46838	265.5849
ps.AFFX-r2-Ec-bioD-5_at.id	587.82734	675.5887
ps.AFFX-r2-Ec-bioD-5_at.id	511.07926	650.8028
ps.AFFX-r2-Ec-bioD-5_at.id	255.39839	295.7714
ps.AFFX-r2-Ec-bioD-5_at.id	149.64574	178.2833
ps.AFFX-r2-Ec-bioD-5_at.id	132.74302	181.1978
ps.AFFX-r2-Ec-bioD-5_at.id	447.36533	594.9351
ps.AFFX-r2-Ec-bioD-5_at.id	131.99693	181.9098
ps.AFFX-r2-Ec-bioD-5_at.id	352.33599	373.3020
ps.AFFX-r2-Ec-bioD-5_at.id	94.30074	113.1372

Use | to subset the results on one or more probesets

```
> res_1 <- result["AFFX-r2-Ec-bioD-5_at"]
> res_1
```

	HG-Focus-1-121502.CEL
ps.AFFX-r2-Ec-bioD-5_at.id	644.5
ps.AFFX-r2-Ec-bioD-5_at.id	694.3
ps.AFFX-r2-Ec-bioD-5_at.id	602.5
ps.AFFX-r2-Ec-bioD-5_at.id	1384.0
ps.AFFX-r2-Ec-bioD-5_at.id	1329.8
ps.AFFX-r2-Ec-bioD-5_at.id	1410.8
ps.AFFX-r2-Ec-bioD-5_at.id	1404.0
ps.AFFX-r2-Ec-bioD-5_at.id	3265.8
ps.AFFX-r2-Ec-bioD-5_at.id	2616.3
ps.AFFX-r2-Ec-bioD-5_at.id	1045.5
ps.AFFX-r2-Ec-bioD-5_at.id	628.3

	HG-Focus-1-121502.CEL
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0

[1] 10000

```
> res_2 <- result[c("AFFX-r2-Ec-bioD-5_at", "207218_at")]
> res_2
```

	HG-Focus-1-121502.CEL
ps.AFFX-r2-Ec-bioD-5_at.id	644.5
ps.AFFX-r2-Ec-bioD-5_at.id	694.3
ps.AFFX-r2-Ec-bioD-5_at.id	602.5
ps.AFFX-r2-Ec-bioD-5_at.id	1384.0
ps.AFFX-r2-Ec-bioD-5_at.id	1329.8
ps.AFFX-r2-Ec-bioD-5_at.id	1410.8
ps.AFFX-r2-Ec-bioD-5_at.id	1404.0
ps.AFFX-r2-Ec-bioD-5_at.id	3265.8

ps.AFFX-r2-Ec-bioD-5_at.id	2616.3
ps.AFFX-r2-Ec-bioD-5_at.id	1045.5
ps.AFFX-r2-Ec-bioD-5_at.id	628.3
ps.207218_at.id	63.8
ps.207218_at.id	62.5
ps.207218_at.id	83.5
ps.207218_at.id	97.0
ps.207218_at.id	83.8
ps.207218_at.id	191.5
ps.207218_at.id	60.8
ps.207218_at.id	73.5
ps.207218_at.id	133.0
ps.207218_at.id	58.8
ps.207218_at.id	82.5

HG-Focus-1-121502.CEL

ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.AFFX-r2-Ec-bioD-5_at.id	0
ps.207218_at.id	0
ps.207218_at.id	0
ps.207218_at.id	0
ps.207218_at.id	0
ps.207218_at.id	0
ps.207218_at.id	0
ps.207218_at.id	0
ps.207218_at.id	0
ps.207218_at.id	0
ps.207218_at.id	0

[1] 10000

and/or on one or more files:

```
> res2_1 <- result2files["AFFX-r2-Ec-bioD-5_at"]
> res2_1
```

	HG-Focus-1-121502.CEL	HG-Focus-2-121502.CEL
ps.AFFX-r2-Ec-bioD-5_at.id	644.5	692.3
ps.AFFX-r2-Ec-bioD-5_at.id	694.3	809.5
ps.AFFX-r2-Ec-bioD-5_at.id	602.5	687.3
ps.AFFX-r2-Ec-bioD-5_at.id	1384.0	1708.5
ps.AFFX-r2-Ec-bioD-5_at.id	1329.8	1513.5
ps.AFFX-r2-Ec-bioD-5_at.id	1410.8	1643.8
ps.AFFX-r2-Ec-bioD-5_at.id	1404.0	1838.0
ps.AFFX-r2-Ec-bioD-5_at.id	3265.8	3985.3
ps.AFFX-r2-Ec-bioD-5_at.id	2616.3	3331.0
ps.AFFX-r2-Ec-bioD-5_at.id	1045.5	1102.0
ps.AFFX-r2-Ec-bioD-5_at.id	628.3	746.0

	HG-Focus-1-121502.CEL	HG-Focus-2-121502.CEL
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0

[1] 12000

```
> res2_2 <- result2files[c("AFFX-r2-Ec-bioD-5_at", "207218_at")]
> res2_2
```

	HG-Focus-1-121502.CEL	HG-Focus-2-121502.CEL
ps.AFFX-r2-Ec-bioD-5_at.id	644.5	692.3
ps.AFFX-r2-Ec-bioD-5_at.id	694.3	809.5
ps.AFFX-r2-Ec-bioD-5_at.id	602.5	687.3
ps.AFFX-r2-Ec-bioD-5_at.id	1384.0	1708.5
ps.AFFX-r2-Ec-bioD-5_at.id	1329.8	1513.5
ps.AFFX-r2-Ec-bioD-5_at.id	1410.8	1643.8
ps.AFFX-r2-Ec-bioD-5_at.id	1404.0	1838.0
ps.AFFX-r2-Ec-bioD-5_at.id	3265.8	3985.3
ps.AFFX-r2-Ec-bioD-5_at.id	2616.3	3331.0
ps.AFFX-r2-Ec-bioD-5_at.id	1045.5	1102.0
ps.AFFX-r2-Ec-bioD-5_at.id	628.3	746.0
ps.207218_at.id	63.8	66.3
ps.207218_at.id	62.5	66.0

ps.207218_at.id	83.5	105.3
ps.207218_at.id	97.0	109.5
ps.207218_at.id	83.8	98.5
ps.207218_at.id	191.5	240.3
ps.207218_at.id	60.8	77.5
ps.207218_at.id	73.5	102.5
ps.207218_at.id	133.0	147.8
ps.207218_at.id	58.8	63.0
ps.207218_at.id	82.5	85.3

	HG-Focus-1-121502.CEL	HG-Focus-2-121502.CEL
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.AFFX-r2-Ec-bioD-5_at.id	0	0
ps.207218_at.id	0	0
ps.207218_at.id	0	0
ps.207218_at.id	0	0
ps.207218_at.id	0	0
ps.207218_at.id	0	0
ps.207218_at.id	0	0
ps.207218_at.id	0	0
ps.207218_at.id	0	0
ps.207218_at.id	0	0
ps.207218_at.id	0	0
[1] 12000		

The output objects are of class `ILM`.

Plot the Langmuir Isotherm :

```
> pILM<-plotILM(result2files,"AFFX-r2-Ec-bioD-5_at","HG-Focus-1-121502.CEL")
```

Median = 255

M.a.d. = 181.85

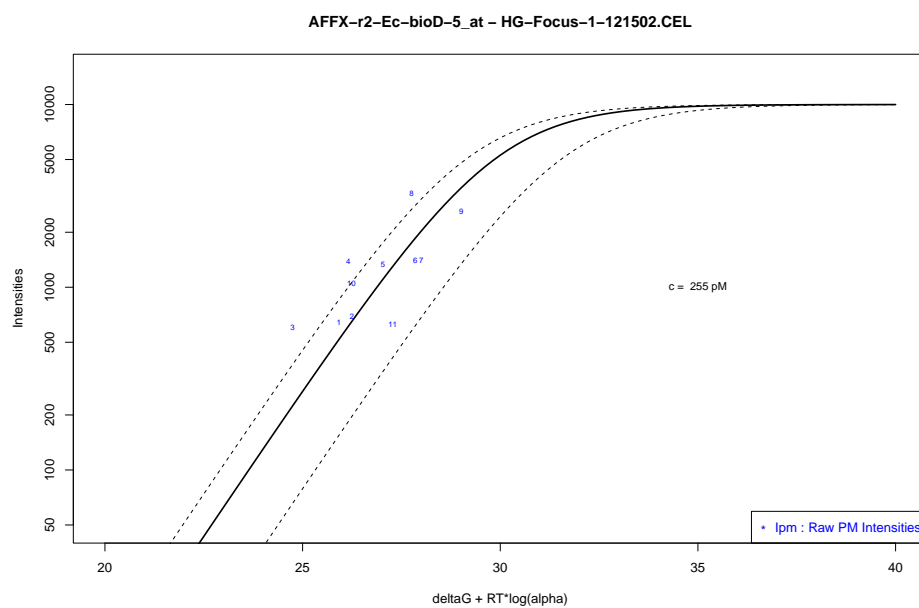


Figure 2: Illustration of the Langmuir Isotherm

This function also provides a list with computed values:

```
> print(str(pILM))
```

List of 8

```
$ Ipm          : Named num [1:11] 644 694 602 1384 1330 ...  
..- attr(*, "names")= chr [1:11] "ps.AFFX-r2-Ec-bioD-5_at.id" "ps.AFFX-r2-Ec-bioD-5_a  
$ I0pm         : Named num [1:11] 0 0 0 0 0 0 0 0 0 0 ...  
..- attr(*, "names")= chr [1:11] "ps.AFFX-r2-Ec-bioD-5_at.id" "ps.AFFX-r2-Ec-bioD-5_a  
$ ImI0         : Named num [1:11] 644 694 602 1384 1330 ...  
..- attr(*, "names")= chr [1:11] "ps.AFFX-r2-Ec-bioD-5_at.id" "ps.AFFX-r2-Ec-bioD-5_a  
$ deltaG       : Named num [1:11] 39.1 37 34.1 33.7 32.8 ...  
..- attr(*, "names")= chr [1:11] "ps.AFFX-r2-Ec-bioD-5_at.id" "ps.AFFX-r2-Ec-bioD-5_a  
$ deltaGp      : Named num [1:11] 60.7 58.1 56.6 54.6 52.7 ...  
..- attr(*, "names")= chr [1:11] "ps.AFFX-r2-Ec-bioD-5_at.id" "ps.AFFX-r2-Ec-bioD-5_a  
$ alpha        : Named num [1:11] 5.98e-05 3.60e-04 9.62e-04 3.80e-03 1.41e-02 ...  
..- attr(*, "names")= chr [1:11] "ps.AFFX-r2-Ec-bioD-5_at.id" "ps.AFFX-r2-Ec-bioD-5_a  
$ deltaGpRTlogA: Named num [1:11] 25.9 26.2 24.7 26.2 27 ...  
..- attr(*, "names")= chr [1:11] "ps.AFFX-r2-Ec-bioD-5_at.id" "ps.AFFX-r2-Ec-bioD-5_a  
$ Concs        : Named num [1:11] 266 225 588 511 255 ...  
..- attr(*, "names")= chr [1:11] "ps.AFFX-r2-Ec-bioD-5_at.id" "ps.AFFX-r2-Ec-bioD-5_a
```

NULL

References

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- N. Sugimoto, S. Nakano, M. Katoh, A. Matsumura, H. Nakamuta, T. Ohmichi, M. Yoneyama, and M. Sasaki. Thermodynamic parameters to predict stability of RNA/DNA hybrid duplexes. *Biochemistry*, 34:11211, 1995.