

Description of affyILM package

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Contents

1	Introduction	1
2	Getting started	2
2.1	Preliminaries	2
2.2	First Steps	3
3	More Examples with options	5

1 Introduction

affyILM is a preprocessing tool which estimates gene expression levels for Affymetrix Gene Expression Chips. The computation is divided into two parts:

1. Background estimation for each feature based on input from physical chemistry (nearest neighbor model) as well as on the physical location of the features and their neighboring probes.
2. Computation of gene expression levels from background-subtracted data using the Langmuir model. In contrast to other measures, this method outputs the gene expression level as concentrations measured in pM (picoMolar).

A linear function with 50 parameters is used to describe the background intensity for each probe. The linear least square method is used to determine these 50 parameters. In order to obtain these parameters, *affyILM* uses a standard SVD (Singular Value Decomposition) algorithm based on Golub&Reinsch (see e.g. Golub and Reinsch (1970); Golub and Loan (1996)).

18 of the 50 parameters reflect the influence of the neighboring spots on the background intensity of a particular feature. The next 16 parameters incorporate the nearest-neighbor free energies (sequence dependence) and the last 16 parameters modify the strength of the sequence dependence based upon the position of a nucleotide along the

sequence. For a more detailed description on the theoretical background of *affyILM* we refer the interested reader to Kroll et al. (2008, 2009).

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*. In case more than one CEL-file is analyzed, a simple *in-between* array normalization is done such that the median values of intensities of the CEL-files are identical.

2 Getting started

2.1 Preliminaries

To build the package from source, you have to have the following components installed on your system:

- a C compiler
- GNU Scientific Library (GSL version 1.12)
- Basic Linear Algebra Subprograms (BLAS)

affyILM makes use of a few routines of the GNU Scientific Library (GSL), a software library which is freely distributed under the GNU General Public license and can be downloaded at <http://www.gnu.org/software/gsl/>.

GSL requires a BLAS (Basic Linear Algebra Subprograms) library for basic vector and matrix operations. We recommend the user to replace the default BLAS library (supplied with GSL) by ATLAS (Automatically Tuned Linear Algebra Software), an optimized BLAS version which is freely available under <http://math-atlas.sourceforge.net>. A list of optimized BLAS libraries for a variety of computer architectures can be found here: <http://www.netlib.org/blas/faq.html#5> For instance, Mac users may use the built-in vecLib framework, while users of Intel machines may use the Math Kernel Library (MKL). A C compiler is needed to build the package as the core of the *affyILM* function is coded in C.

For the package to be installed properly you might have to type the following command before installation:

```
export LD_LIBRARY_PATH='/path/to/GSL/:/path/to/BLAS/':$LD_LIBRARY_PATH
```

which will tell **R** where your GSL and BLAS libraries (see below for more details about BLAS libraries) are. Note that this might have already been configured on your system, so you might not have to do so.

In case you need to do it, you might consider copying and pasting the line in your `.bashrc` so that you do not have to do it every time.

Now you are ready to install the package:

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

The package will look for a BLAS library on your system, and by default it will choose `gslcblas`, which is not optimized for your system. To use an optimized BLAS library, you can use the `--with-blas` argument which will be passed to the `configure.ac` file. For example, on a Mac with `vecLib` pre-installed the package may be installed via:

```
R CMD INSTALL affyILM_x.y.z.tar.gz --configure-args="--with-blas='-framework
vecLib'"
```

On a 64-bit Intel machine which has MKL as the optimized BLAS library, the command may look like:

```
R CMD INSTALL affyILM_x.y.z.tar.gz --configure-args="--
-with-blas='-L/usr/local/mkl/lib/em64t/ -lmkl -lguide -lpthread'"
```

where `/usr/local/mkl/lib/em64t/` is the path to MKL.

If you prefer to install a prebuilt binary, you need GSL for successful installation.

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

affxparser, *affy* and *gcrma*. 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

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

Calculation of the background intensity as well as of the concentrations:

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

Chip dimension 448 x 448

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

```
package 'hgfocusprobe' successfully unpacked and MD5 sums checked
```

```

The downloaded packages are in
      E:\biocbld\bbs-2.8-bioc\tmpdir\RtmpORXTFk\downloaded_packages
Probepackage hgfocusprobe loaded
Reading intensities...done
72482 features used for parameter estimation
Residual value is 0.057654
Background intensities calculated
Concentrations calculated
Done
-----

```

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

- Chip dimension
- probe package downloaded if missing
- the number of features used for background estimation depends on the threshold intensity according to which features to be used for the calculation are selected. The default value is 350.
- Residual value of the linear least square problem

Take a look at the calculated background intensities as well as the experimental PM's

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

Probeset	Intensities	
	IPM-HG-Focus-1-121502.CEL	IOPM-HG-Focus-1-121502.CEL
AFFX-r2-Ec-bioD-5_at	1384.0	162.38
AFFX-r2-Ec-bioD-5_at	1410.8	136.65
AFFX-r2-Ec-bioD-5_at	2616.3	127.28
AFFX-r2-Ec-bioD-5_at	1404.0	96.57
AFFX-r2-Ec-bioD-5_at	602.5	213.75
AFFX-r2-Ec-bioD-5_at	694.3	190.60
AFFX-r2-Ec-bioD-5_at	1329.8	96.07
AFFX-r2-Ec-bioD-5_at	644.5	120.89
AFFX-r2-Ec-bioD-5_at	628.3	114.54
AFFX-r2-Ec-bioD-5_at	3265.8	172.70
AFFX-r2-Ec-bioD-5_at	1045.5	137.40

Note: The order in which the probes are displayed do not correspond to the original order!

Plot the result:

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

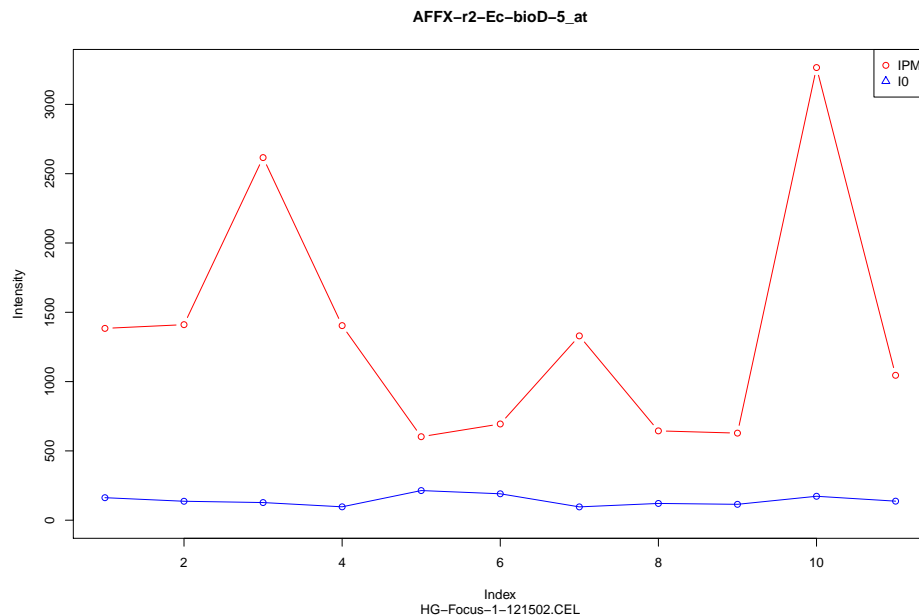


Figure 1: Comparison between PM intensity and the background values

3 More Examples with options

Analyze two or more CEL-files

```
> file2 <- system.file("rawData", "FusionSDK_HG-Focus", "HG-Focus",
+   "2.Calvin", "HG-Focus-2-121502.CEL", package = "AffymetrixDataTestFiles")
> result2files <- ilm(c(file1, file2), 400, 12000)
```

```
Chip dimension 448 x 448
Probepackage hgfocusprobe loaded
Reading intensities...done
File analyzed: HG-Focus-1-121502.CEL
75612 features used for parameter estimation
Residual value is 0.062521
Background intensities calculated
Concentrations calculated
Done
```

```
-----
File analyzed: HG-Focus-2-121502.CEL
76424 features used for parameter estimation
Residual value is 0.063496
Background intensities calculated
```

Concentrations calculated

Done

where only probes with an intensity up to 400 are used for background calculation and the saturation limit of the Langmuir isotherm is increased to 12000 (default: 10000).

Get PM intensities and corresponding background values:

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

Probeset	IPM-HG-Focus-1-121502.CEL	IOPM-HG-Focus-1-121502.CEL
AFFX-r2-Ec-bioD-5_at	1384.0	172.92
AFFX-r2-Ec-bioD-5_at	1410.8	144.02
AFFX-r2-Ec-bioD-5_at	2616.3	133.21
AFFX-r2-Ec-bioD-5_at	1404.0	100.46
AFFX-r2-Ec-bioD-5_at	602.5	233.47
AFFX-r2-Ec-bioD-5_at	694.3	204.48
AFFX-r2-Ec-bioD-5_at	1329.8	99.43
AFFX-r2-Ec-bioD-5_at	644.5	124.50
AFFX-r2-Ec-bioD-5_at	628.3	120.37
AFFX-r2-Ec-bioD-5_at	3265.8	184.76
AFFX-r2-Ec-bioD-5_at	1045.5	145.41

Probeset	IPM-HG-Focus-2-121502.CEL	IOPM-HG-Focus-2-121502.CEL
AFFX-r2-Ec-bioD-5_at	1404.39	169.55
AFFX-r2-Ec-bioD-5_at	1351.20	137.58
AFFX-r2-Ec-bioD-5_at	2738.08	134.22
AFFX-r2-Ec-bioD-5_at	1510.84	97.64
AFFX-r2-Ec-bioD-5_at	564.96	234.11
AFFX-r2-Ec-bioD-5_at	665.41	203.08
AFFX-r2-Ec-bioD-5_at	1244.10	95.44
AFFX-r2-Ec-bioD-5_at	569.07	123.70
AFFX-r2-Ec-bioD-5_at	613.21	118.51
AFFX-r2-Ec-bioD-5_at	3275.92	183.89
AFFX-r2-Ec-bioD-5_at	905.84	140.50

- 1st column: Probeset name
- Following columns (read pairwise,i.e. 2 columns per CEL-file): Measured PM intensities IPM and background estimates IOPM

The function of the background intensity per probe is characterized by its 50 optimized parameters:

```
> getParams(result2files)
```

	HG-Focus-1-121502.CEL	HG-Focus-2-121502.CEL
[1,]	1.0302856768	0.541052675
[2,]	0.0611188541	0.059458658
[3,]	0.0556656444	0.058960849
[4,]	-0.0146940677	-0.013512307
[5,]	-0.0140267281	-0.014351281
[6,]	0.2372035264	0.229545898
[7,]	0.0071061005	0.008841897
[8,]	0.0053782089	0.007204471
[9,]	0.0217924529	0.025816610
[10,]	0.1971714464	-0.295573078
[11,]	0.0812876929	0.079056320
[12,]	0.0721215982	0.069481893
[13,]	-0.0207139593	-0.016798366
[14,]	-0.0102032255	-0.007726154
[15,]	0.3944131736	0.388778215
[16,]	0.0099561961	0.011616956
[17,]	0.0096446474	0.010878876
[18,]	0.0256766106	0.030683355
[19,]	0.2331673766	0.312768882
[20,]	0.1527224605	0.240539062
[21,]	-0.2214175584	-0.107401023
[22,]	-0.1261764284	-0.023923073
[23,]	-0.0767014024	-0.032216816
[24,]	0.1038319120	0.174265887
[25,]	-0.3390762483	-0.253065883
[26,]	-0.2312138499	-0.154092231
[27,]	0.2809728475	0.297399105
[28,]	0.3149427653	0.349188162
[29,]	-0.0422039762	0.012249172
[30,]	0.0031445805	0.045198388
[31,]	0.3047878227	0.342881456
[32,]	0.3456039967	0.401658597
[33,]	-0.0513767293	0.023576924
[34,]	-0.0011116773	0.060619855
[35,]	-0.0048070487	-0.015077861
[36,]	-0.0006150782	-0.011701380
[37,]	0.0245474479	0.011264808
[38,]	0.0160387487	0.003866209
[39,]	0.0166789290	0.008777454
[40,]	0.0035895030	-0.006551098

[41,]	0.0348306540	0.023749048
[42,]	0.0241440887	0.013852678
[43,]	-0.0117930683	-0.017866206
[44,]	-0.0143402270	-0.021632141
[45,]	0.0120752954	0.003427732
[46,]	0.0056486030	-0.002038731
[47,]	-0.0097682383	-0.017395112
[48,]	-0.0131986028	-0.022041660
[49,]	0.0151881593	0.004857502
[50,]	0.0079928288	-0.001574064

To obtain the concentrations (or expression levels) per probeset, use

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

genes	HG-Focus-1-121502.CEL	HG-Focus-2-121502.CEL
AFFX-r2-Ec-bioD-5_at	21.12639	20.49479

Use [to subset the results on one or more probesets

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

Probeset	Intensities	
	IPM-HG-Focus-1-121502.CEL	IOPM-HG-Focus-1-121502.CEL
AFFX-r2-Ec-bioD-5_at	1384.0	162.38
AFFX-r2-Ec-bioD-5_at	1410.8	136.65
AFFX-r2-Ec-bioD-5_at	2616.3	127.28
AFFX-r2-Ec-bioD-5_at	1404.0	96.57
AFFX-r2-Ec-bioD-5_at	602.5	213.75
AFFX-r2-Ec-bioD-5_at	694.3	190.60
AFFX-r2-Ec-bioD-5_at	1329.8	96.07
AFFX-r2-Ec-bioD-5_at	644.5	120.89
AFFX-r2-Ec-bioD-5_at	628.3	114.54
AFFX-r2-Ec-bioD-5_at	3265.8	172.70
AFFX-r2-Ec-bioD-5_at	1045.5	137.40

```
[1] 26.19
```

	HG-Focus-1-121502.CEL
[1,]	1.096441
[2,]	0.058993
[3,]	0.053599
[4,]	-0.014431
[5,]	-0.014164
[6,]	0.232392

[7,]	0.006346
[8,]	0.005312
[9,]	0.022097
[10,]	0.271669
[11,]	0.077861
[12,]	0.067479
[13,]	-0.020032
[14,]	-0.009434
[15,]	0.392230
[16,]	0.008602
[17,]	0.009852
[18,]	0.024806
[19,]	0.225314
[20,]	0.159353
[21,]	-0.200488
[22,]	-0.114104
[23,]	-0.081428
[24,]	0.105911
[25,]	-0.324216
[26,]	-0.225752
[27,]	0.263247
[28,]	0.305862
[29,]	-0.039616
[30,]	0.000477
[31,]	0.290430
[32,]	0.339369
[33,]	-0.043217
[34,]	0.001141
[35,]	-0.004699
[36,]	-0.001445
[37,]	0.022734
[38,]	0.014921
[39,]	0.016642
[40,]	0.002982
[41,]	0.033382
[42,]	0.023536
[43,]	-0.010844
[44,]	-0.014001
[45,]	0.011557
[46,]	0.005621
[47,]	-0.009108
[48,]	-0.013074

```
[49,]          0.014150
[50,]          0.007584
[1] 10000
```

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

Probeset	Intensities	
	IPM-HG-Focus-1-121502.CEL	IOPM-HG-Focus-1-121502.CEL
AFFX-r2-Ec-bioD-5_at	1384.0	162.38
AFFX-r2-Ec-bioD-5_at	1410.8	136.65
AFFX-r2-Ec-bioD-5_at	2616.3	127.28
AFFX-r2-Ec-bioD-5_at	1404.0	96.57
AFFX-r2-Ec-bioD-5_at	602.5	213.75
AFFX-r2-Ec-bioD-5_at	694.3	190.60
AFFX-r2-Ec-bioD-5_at	1329.8	96.07
AFFX-r2-Ec-bioD-5_at	644.5	120.89
AFFX-r2-Ec-bioD-5_at	628.3	114.54
AFFX-r2-Ec-bioD-5_at	3265.8	172.70
AFFX-r2-Ec-bioD-5_at	1045.5	137.40
207218_at	73.5	92.20
207218_at	133.0	97.29
207218_at	60.8	57.94
207218_at	191.5	154.48
207218_at	62.5	56.48
207218_at	82.5	85.11
207218_at	63.8	66.62
207218_at	58.8	72.79
207218_at	97.0	70.24
207218_at	83.5	84.93
207218_at	83.8	57.54

AFFX-r2-Ec-bioD-5_at	207218_at
26.1909	0.5886

HG-Focus-1-121502.CEL

```
[1,] 1.096441
[2,] 0.058993
[3,] 0.053599
[4,] -0.014431
[5,] -0.014164
[6,] 0.232392
[7,] 0.006346
[8,] 0.005312
[9,] 0.022097
```

[10,]	0.271669
[11,]	0.077861
[12,]	0.067479
[13,]	-0.020032
[14,]	-0.009434
[15,]	0.392230
[16,]	0.008602
[17,]	0.009852
[18,]	0.024806
[19,]	0.225314
[20,]	0.159353
[21,]	-0.200488
[22,]	-0.114104
[23,]	-0.081428
[24,]	0.105911
[25,]	-0.324216
[26,]	-0.225752
[27,]	0.263247
[28,]	0.305862
[29,]	-0.039616
[30,]	0.000477
[31,]	0.290430
[32,]	0.339369
[33,]	-0.043217
[34,]	0.001141
[35,]	-0.004699
[36,]	-0.001445
[37,]	0.022734
[38,]	0.014921
[39,]	0.016642
[40,]	0.002982
[41,]	0.033382
[42,]	0.023536
[43,]	-0.010844
[44,]	-0.014001
[45,]	0.011557
[46,]	0.005621
[47,]	-0.009108
[48,]	-0.013074
[49,]	0.014150
[50,]	0.007584
[1] 10000	

and/or on one or more files:

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

Probeset	IPM-HG-Focus-1-121502.CEL	IOPM-HG-Focus-1-121502.CEL
AFFX-r2-Ec-bioD-5_at	1384.0	172.92
AFFX-r2-Ec-bioD-5_at	1410.8	144.02
AFFX-r2-Ec-bioD-5_at	2616.3	133.21
AFFX-r2-Ec-bioD-5_at	1404.0	100.46
AFFX-r2-Ec-bioD-5_at	602.5	233.47
AFFX-r2-Ec-bioD-5_at	694.3	204.48
AFFX-r2-Ec-bioD-5_at	1329.8	99.43
AFFX-r2-Ec-bioD-5_at	644.5	124.50
AFFX-r2-Ec-bioD-5_at	628.3	120.37
AFFX-r2-Ec-bioD-5_at	3265.8	184.76
AFFX-r2-Ec-bioD-5_at	1045.5	145.41

Probeset	IPM-HG-Focus-2-121502.CEL	IOPM-HG-Focus-2-121502.CEL
AFFX-r2-Ec-bioD-5_at	1404.4	169.55
AFFX-r2-Ec-bioD-5_at	1351.2	137.58
AFFX-r2-Ec-bioD-5_at	2738.1	134.22
AFFX-r2-Ec-bioD-5_at	1510.8	97.64
AFFX-r2-Ec-bioD-5_at	565.0	234.11
AFFX-r2-Ec-bioD-5_at	665.4	203.08
AFFX-r2-Ec-bioD-5_at	1244.1	95.44
AFFX-r2-Ec-bioD-5_at	569.1	123.70
AFFX-r2-Ec-bioD-5_at	613.2	118.51
AFFX-r2-Ec-bioD-5_at	3275.9	183.89
AFFX-r2-Ec-bioD-5_at	905.8	140.50

	HG-Focus-1-121502.CEL	HG-Focus-2-121502.CEL
	21.13	20.49
	HG-Focus-1-121502.CEL	HG-Focus-2-121502.CEL
[1,]	1.0302857	0.541053
[2,]	0.0611189	0.059459
[3,]	0.0556656	0.058961
[4,]	-0.0146941	-0.013512
[5,]	-0.0140267	-0.014351
[6,]	0.2372035	0.229546
[7,]	0.0071061	0.008842
[8,]	0.0053782	0.007204
[9,]	0.0217925	0.025817
[10,]	0.1971714	-0.295573

[11,]	0.0812877	0.079056
[12,]	0.0721216	0.069482
[13,]	-0.0207140	-0.016798
[14,]	-0.0102032	-0.007726
[15,]	0.3944132	0.388778
[16,]	0.0099562	0.011617
[17,]	0.0096446	0.010879
[18,]	0.0256766	0.030683
[19,]	0.2331674	0.312769
[20,]	0.1527225	0.240539
[21,]	-0.2214176	-0.107401
[22,]	-0.1261764	-0.023923
[23,]	-0.0767014	-0.032217
[24,]	0.1038319	0.174266
[25,]	-0.3390762	-0.253066
[26,]	-0.2312138	-0.154092
[27,]	0.2809728	0.297399
[28,]	0.3149428	0.349188
[29,]	-0.0422040	0.012249
[30,]	0.0031446	0.045198
[31,]	0.3047878	0.342881
[32,]	0.3456040	0.401659
[33,]	-0.0513767	0.023577
[34,]	-0.0011117	0.060620
[35,]	-0.0048070	-0.015078
[36,]	-0.0006151	-0.011701
[37,]	0.0245474	0.011265
[38,]	0.0160387	0.003866
[39,]	0.0166789	0.008777
[40,]	0.0035895	-0.006551
[41,]	0.0348307	0.023749
[42,]	0.0241441	0.013853
[43,]	-0.0117931	-0.017866
[44,]	-0.0143402	-0.021632
[45,]	0.0120753	0.003428
[46,]	0.0056486	-0.002039
[47,]	-0.0097682	-0.017395
[48,]	-0.0131986	-0.022042
[49,]	0.0151882	0.004858
[50,]	0.0079928	-0.001574
[1] 12000		

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

Probeset	IPM-HG-Focus-1-121502.CEL	IOPM-HG-Focus-1-121502.CEL
AFFX-r2-Ec-bioD-5_at	1384.0	172.92
AFFX-r2-Ec-bioD-5_at	1410.8	144.02
AFFX-r2-Ec-bioD-5_at	2616.3	133.21
AFFX-r2-Ec-bioD-5_at	1404.0	100.46
AFFX-r2-Ec-bioD-5_at	602.5	233.47
AFFX-r2-Ec-bioD-5_at	694.3	204.48
AFFX-r2-Ec-bioD-5_at	1329.8	99.43
AFFX-r2-Ec-bioD-5_at	644.5	124.50
AFFX-r2-Ec-bioD-5_at	628.3	120.37
AFFX-r2-Ec-bioD-5_at	3265.8	184.76
AFFX-r2-Ec-bioD-5_at	1045.5	145.41
207218_at	73.5	93.01
207218_at	133.0	98.20
207218_at	60.8	57.84
207218_at	191.5	155.70
207218_at	62.5	56.16
207218_at	82.5	86.50
207218_at	63.8	66.44
207218_at	58.8	73.30
207218_at	97.0	70.12
207218_at	83.5	84.97
207218_at	83.8	57.25

Probeset	IPM-HG-Focus-2-121502.CEL	IOPM-HG-Focus-2-121502.CEL
AFFX-r2-Ec-bioD-5_at	1404.39	169.55
AFFX-r2-Ec-bioD-5_at	1351.20	137.58
AFFX-r2-Ec-bioD-5_at	2738.08	134.22
AFFX-r2-Ec-bioD-5_at	1510.84	97.64
AFFX-r2-Ec-bioD-5_at	564.96	234.11
AFFX-r2-Ec-bioD-5_at	665.41	203.08
AFFX-r2-Ec-bioD-5_at	1244.10	95.44
AFFX-r2-Ec-bioD-5_at	569.07	123.70
AFFX-r2-Ec-bioD-5_at	613.21	118.51
AFFX-r2-Ec-bioD-5_at	3275.92	183.89
AFFX-r2-Ec-bioD-5_at	905.84	140.50
207218_at	84.26	100.53
207218_at	121.49	95.81
207218_at	63.71	56.90
207218_at	197.53	157.08

207218_at	54.25	51.43
207218_at	70.12	84.06
207218_at	54.50	63.19
207218_at	51.79	70.00
207218_at	90.01	66.75
207218_at	86.56	86.43
207218_at	80.97	55.29

genes	HG-Focus-1-121502.CEL	HG-Focus-2-121502.CEL
AFFX-r2-Ec-bioD-5_at	21.1264	20.4948
207218_at	0.4973	0.1623

	HG-Focus-1-121502.CEL	HG-Focus-2-121502.CEL
[1,]	1.0302857	0.541053
[2,]	0.0611189	0.059459
[3,]	0.0556656	0.058961
[4,]	-0.0146941	-0.013512
[5,]	-0.0140267	-0.014351
[6,]	0.2372035	0.229546
[7,]	0.0071061	0.008842
[8,]	0.0053782	0.007204
[9,]	0.0217925	0.025817
[10,]	0.1971714	-0.295573
[11,]	0.0812877	0.079056
[12,]	0.0721216	0.069482
[13,]	-0.0207140	-0.016798
[14,]	-0.0102032	-0.007726
[15,]	0.3944132	0.388778
[16,]	0.0099562	0.011617
[17,]	0.0096446	0.010879
[18,]	0.0256766	0.030683
[19,]	0.2331674	0.312769
[20,]	0.1527225	0.240539
[21,]	-0.2214176	-0.107401
[22,]	-0.1261764	-0.023923
[23,]	-0.0767014	-0.032217
[24,]	0.1038319	0.174266
[25,]	-0.3390762	-0.253066
[26,]	-0.2312138	-0.154092
[27,]	0.2809728	0.297399
[28,]	0.3149428	0.349188
[29,]	-0.0422040	0.012249
[30,]	0.0031446	0.045198

[31,]	0.3047878	0.342881
[32,]	0.3456040	0.401659
[33,]	-0.0513767	0.023577
[34,]	-0.0011117	0.060620
[35,]	-0.0048070	-0.015078
[36,]	-0.0006151	-0.011701
[37,]	0.0245474	0.011265
[38,]	0.0160387	0.003866
[39,]	0.0166789	0.008777
[40,]	0.0035895	-0.006551
[41,]	0.0348307	0.023749
[42,]	0.0241441	0.013853
[43,]	-0.0117931	-0.017866
[44,]	-0.0143402	-0.021632
[45,]	0.0120753	0.003428
[46,]	0.0056486	-0.002039
[47,]	-0.0097682	-0.017395
[48,]	-0.0131986	-0.022042
[49,]	0.0151882	0.004858
[50,]	0.0079928	-0.001574
[1]	12000	

The output objects are of class ILM.

Plot the intensities:

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

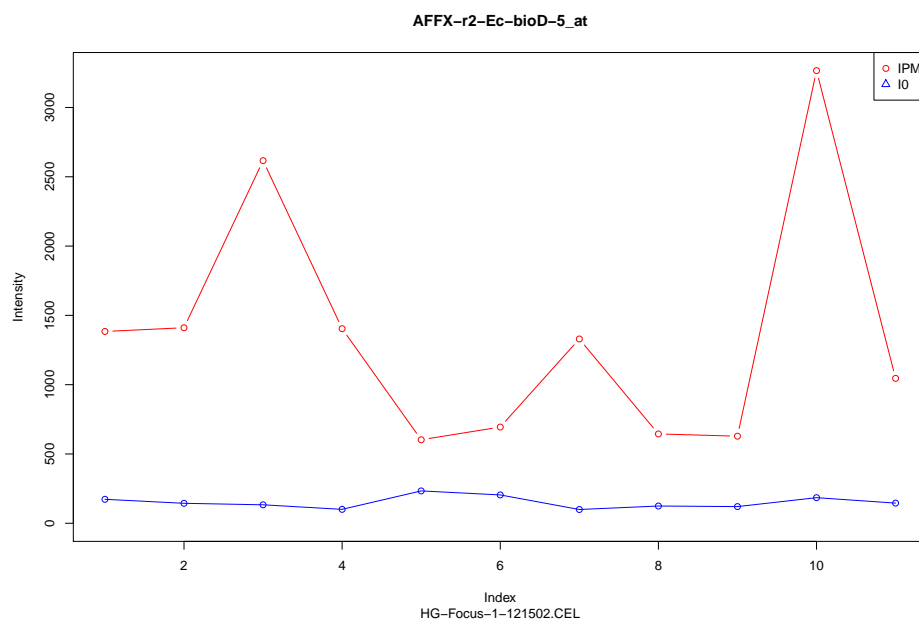


Figure 2: Comparison between PM intensity and the background values

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