GS01 0163
Analysis of Microarray Data

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Lecture 9: Exploring BioConductor

- How do we load CEL files into an AffyBatch? how can we merge batches? how can we partition batches?
- How do we check that it worked?
- How do we supply the associated phenoData?
- Given an AffyBatch, how do we look at it? boxplot, hist, ma-plots, ratio plots, PLM
- Given an AffyBatch, how do we fit it? expresso, justRMA
- Given an eset, what can we say about its contents?
- How can we get the probe level values for a probeset?

- How can we figure out what probeset corresponds to a given gene?

- How can we get the probe sequences for a probeset?

Loading CEL files into an AffyBatch

One theme for today is TMTOWTDI. We're going to try to extend this from Perl over to BioConductor, and to the analysis of Affy data.

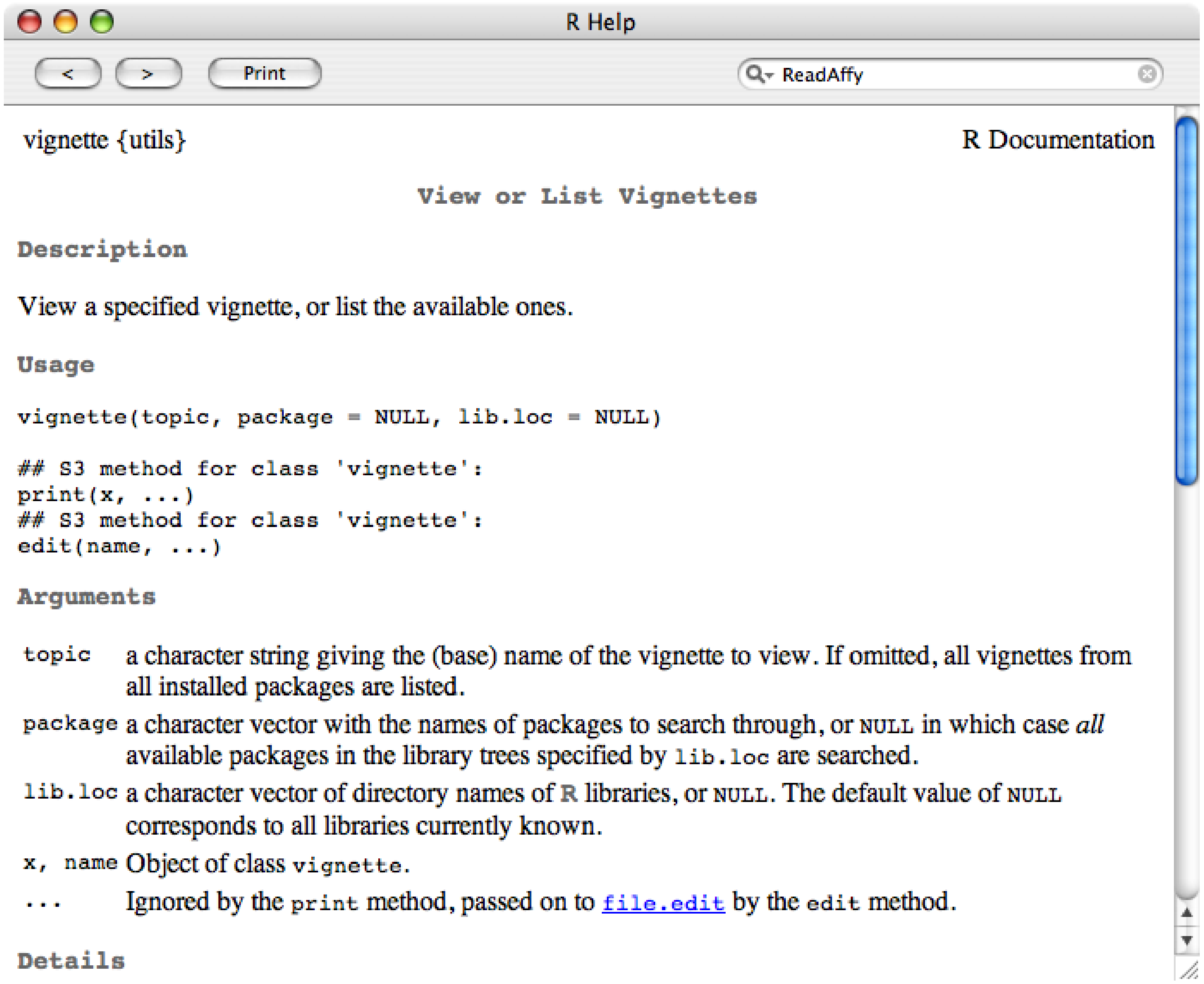
To begin with, let's say that we've got a set of CEL files. How do we pull them in?

There are several options. How do I survey them?

```
> library("affy");
> vignette("affy");
```

Don't panic, it's not really 271 pages...

Reading the Fine Manual: Vignettes



vignette {utils} R Documentation

View or List Vignettes

Description

View a specified vignette, or list the available ones.

Usage

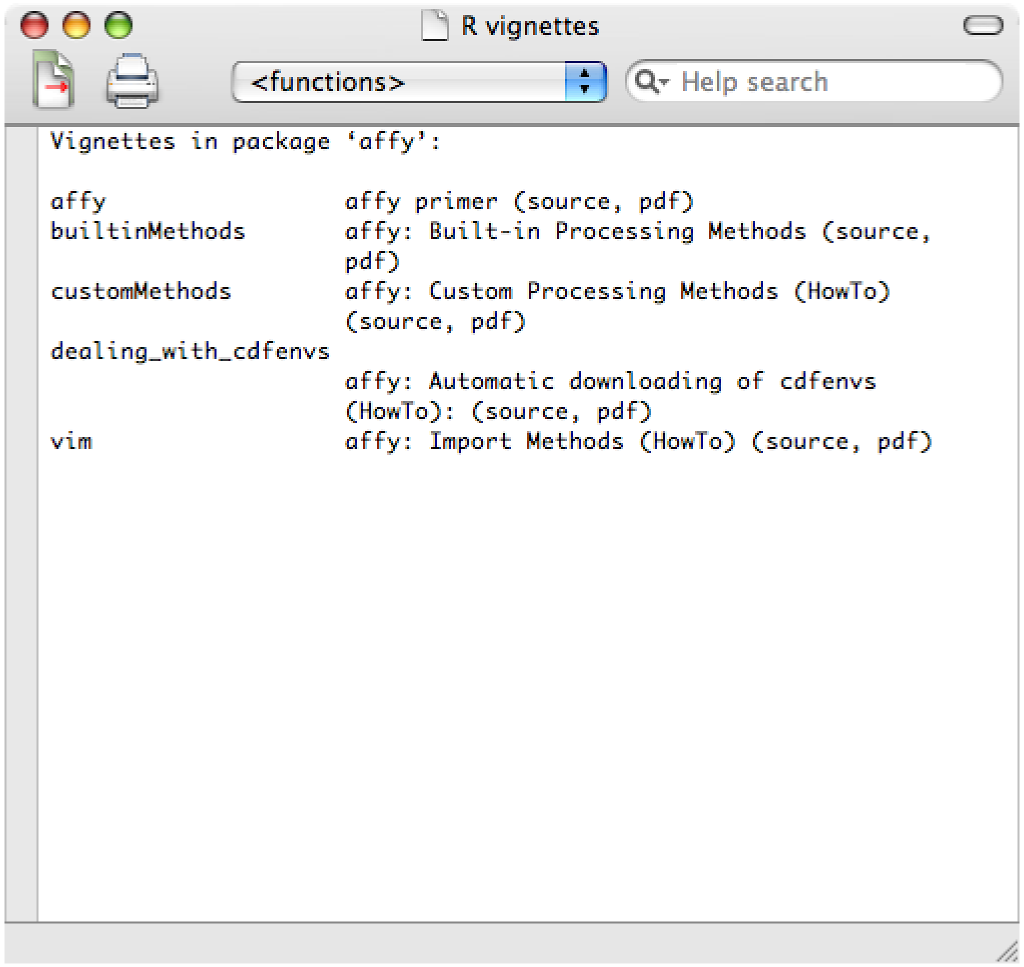
```
vignette(topic, package = NULL, lib.loc = NULL)

## S3 method for class 'vignette':
print(x, ...)
## S3 method for class 'vignette':
edit(name, ...)
```

Arguments

`topic`	a character string giving the (base) name of the vignette to view. If omitted, all vignettes from all installed packages are listed.
`package`	a character vector with the names of packages to search through, or `NULL` in which case *all* available packages in the library trees specified by `lib.loc` are searched.
`lib.loc`	a character vector of directory names of R libraries, or `NULL`. The default value of `NULL` corresponds to all libraries currently known.
`x, name`	Object of class `vignette`.
`...`	Ignored by the `print` method, passed on to `file.edit` by the `edit` method.

Details

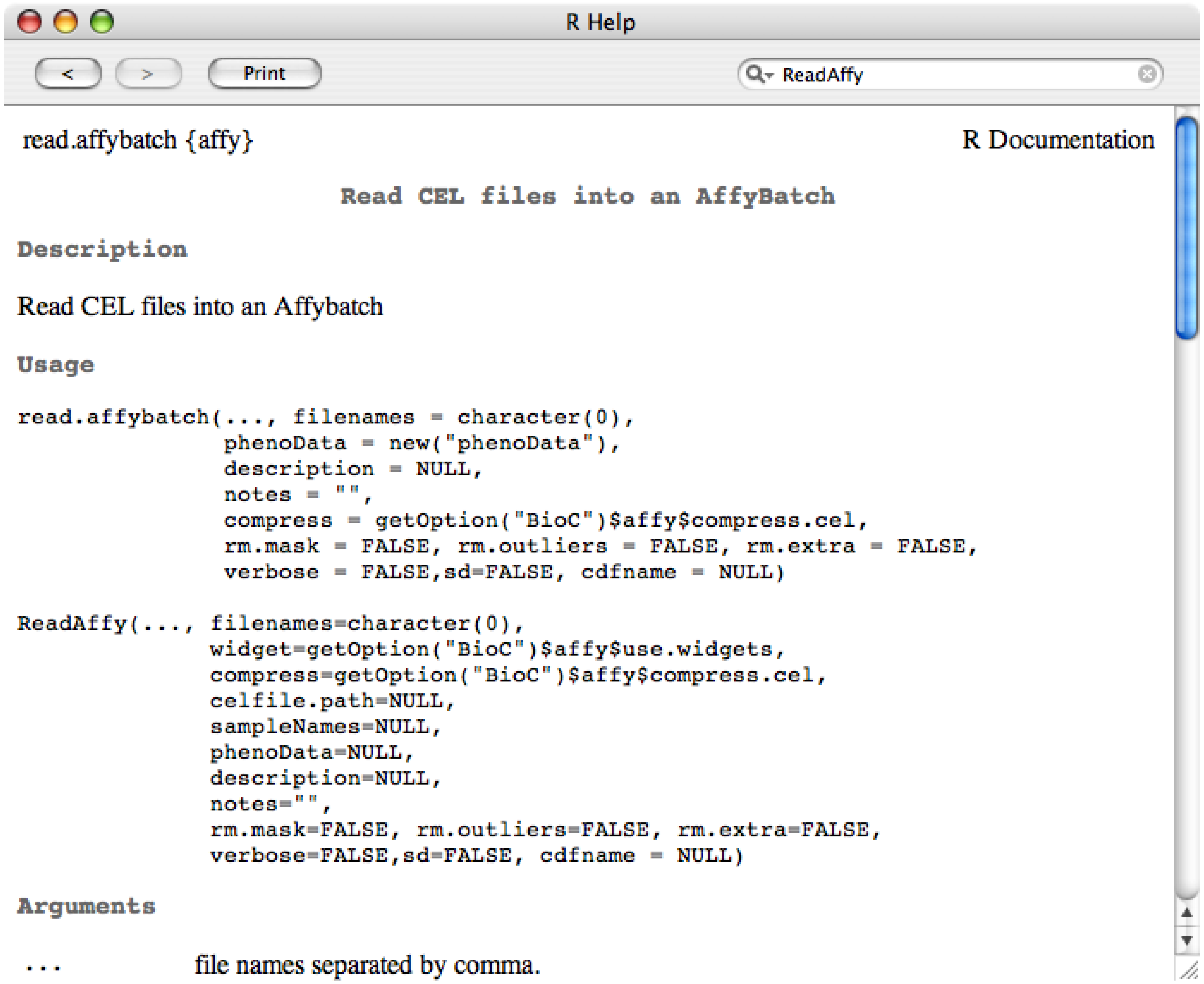
Listing Vignettes



```
Vignettes in package 'affy':

affy                    affy primer (source, pdf)
builtinMethods          affy: Built-in Processing Methods (source,
                        pdf)
customMethods           affy: Custom Processing Methods (HowTo)
                        (source, pdf)
dealing_with_cdfenvs
                        affy: Automatic downloading of cdfenvs
                        (HowTo): (source, pdf)
vim                     affy: Import Methods (HowTo) (source, pdf)
```

```
> vignette(package = "affy");
```

ReadAffy: Help from Top



read.affybatch {affy} R Documentation

Read CEL files into an AffyBatch

Description

Read CEL files into an Affybatch

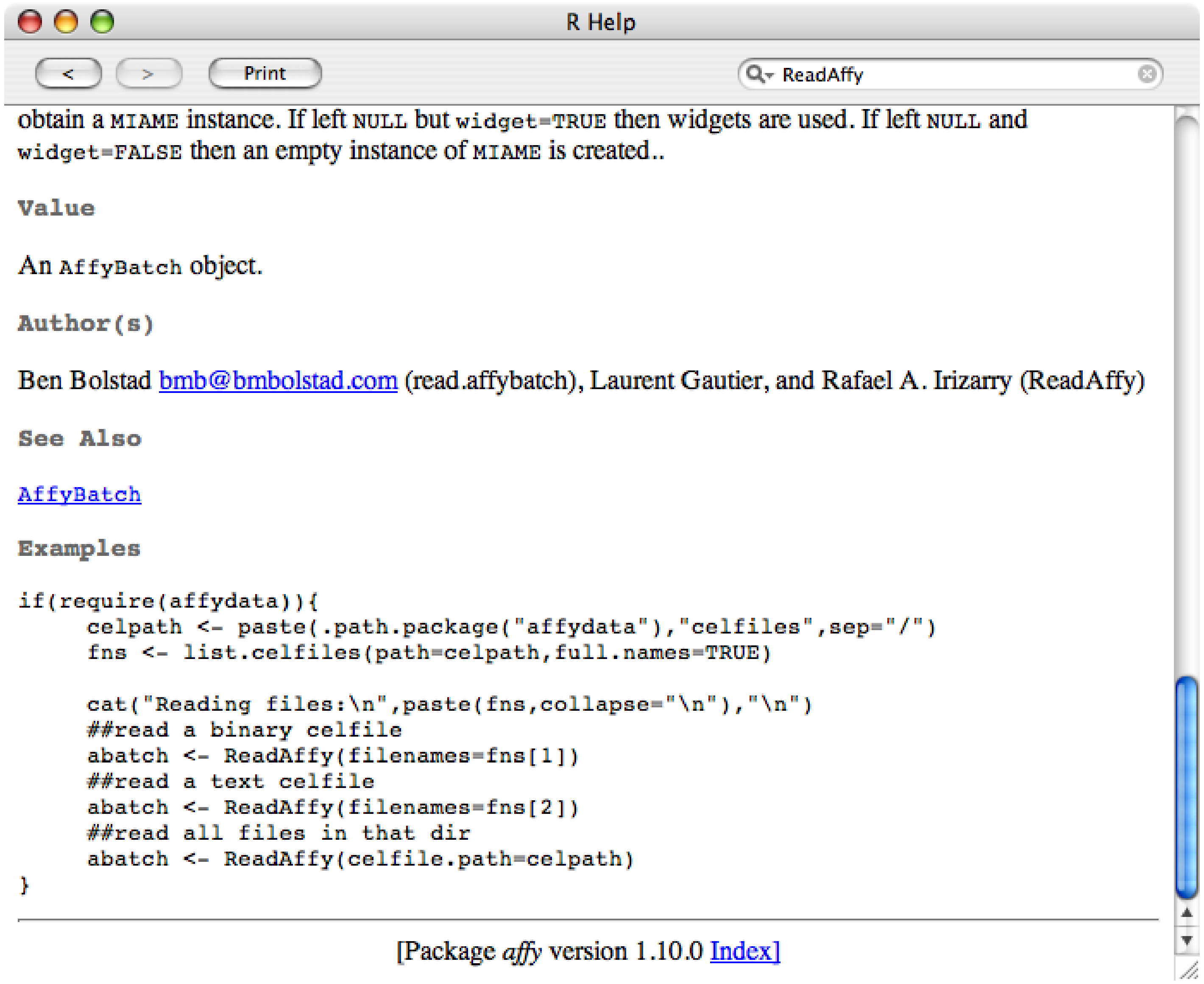
Usage

```
read.affybatch(..., filenames = character(0),
               phenoData = new("phenoData"),
               description = NULL,
               notes = "",
               compress = getOption("BioC")$affy$compress.cel,
               rm.mask = FALSE, rm.outliers = FALSE, rm.extra = FALSE,
               verbose = FALSE,sd=FALSE, cdfname = NULL)

ReadAffy(..., filenames=character(0),
              widget=getOption("BioC")$affy$use.widgets,
              compress=getOption("BioC")$affy$compress.cel,
              celfile.path=NULL,
              sampleNames=NULL,
              phenoData=NULL,
              description=NULL,
              notes="",
              rm.mask=FALSE, rm.outliers=FALSE, rm.extra=FALSE,
              verbose=FALSE,sd=FALSE, cdfname = NULL)
```

Arguments

`...`	file names separated by comma.

ReadAffy: ... to Bottom



obtain a `MIAME` instance. If left `NULL` but `widget=TRUE` then widgets are used. If left `NULL` and `widget=FALSE` then an empty instance of `MIAME` is created..

Value

An `AffyBatch` object.

Author(s)

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See Also

`AffyBatch`

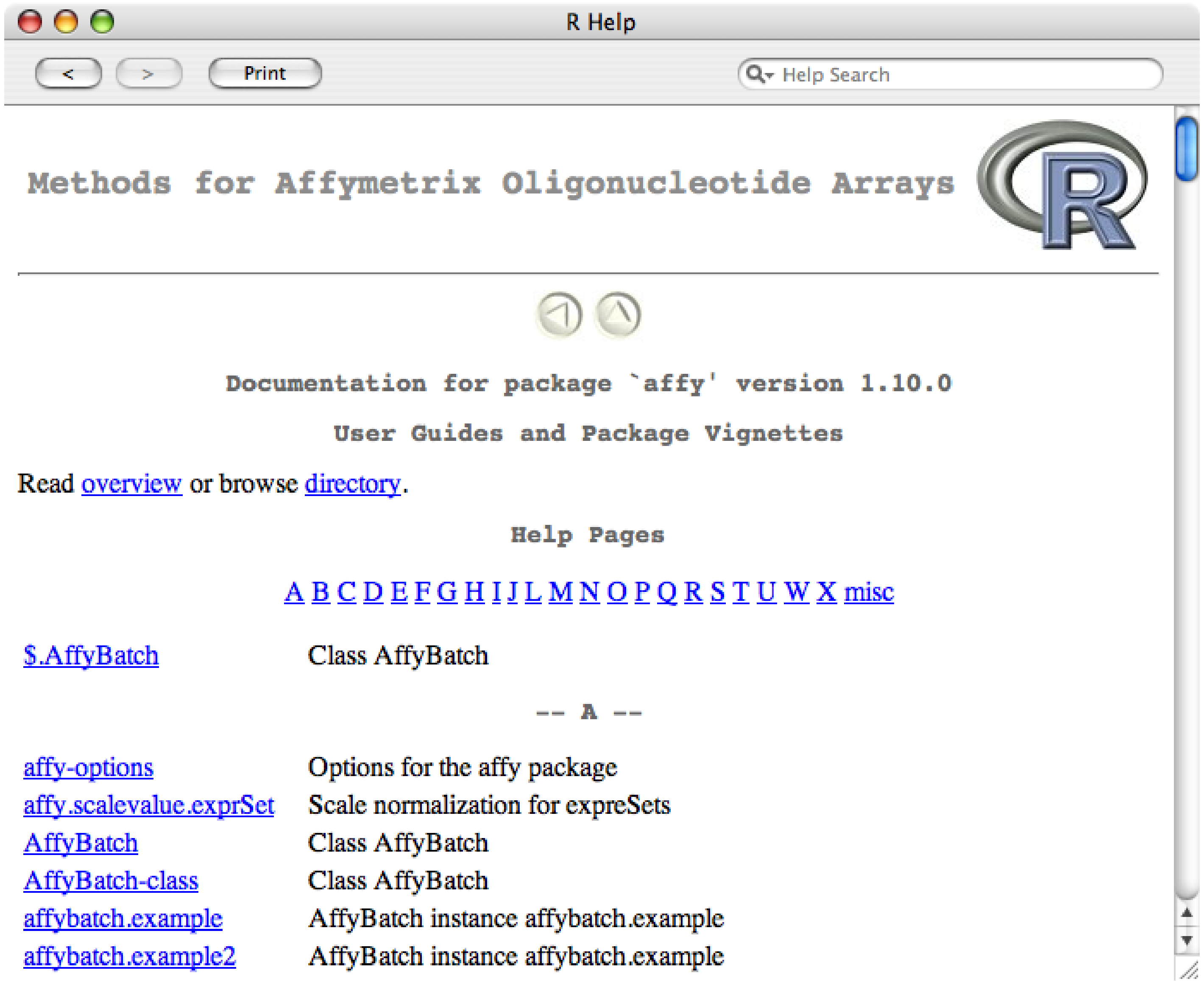
Examples

```
if(require(affydata)){
     celpath <- paste(.path.package("affydata"),"celfiles",sep="/")
     fns <- list.celfiles(path=celpath,full.names=TRUE)

     cat("Reading files:\n",paste(fns,collapse="\n"),"\n")
     ##read a binary celfile
     abatch <- ReadAffy(filenames=fns[1])
     ##read a text celfile
     abatch <- ReadAffy(filenames=fns[2])
     ##read all files in that dir
     abatch <- ReadAffy(celfile.path=celpath)
}
```

[Package *affy* version 1.10.0 Index]

The Affy Index



Methods for Affymetrix Oligonucleotide Arrays

Documentation for package `affy' version 1.10.0

User Guides and Package Vignettes

Read overview or browse directory.

Help Pages

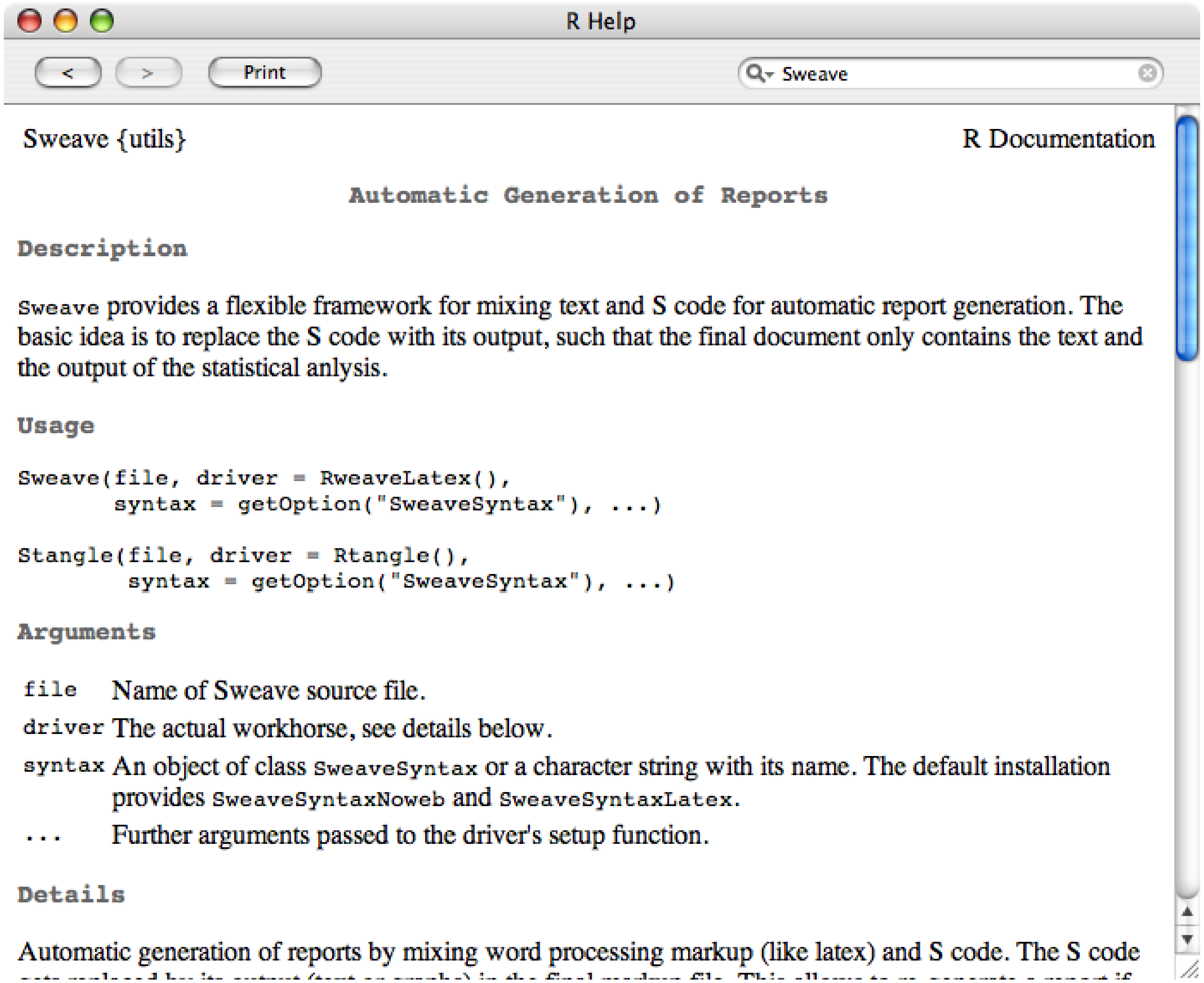
A B C D E F G H I J L M N O P Q R S T U W X misc

$.AffyBatch	Class AffyBatch

-- A --

affy-options	Options for the affy package
affy.scalevalue.exprSet	Scale normalization for expreSets
AffyBatch	Class AffyBatch
AffyBatch-class	Class AffyBatch
affybatch.example	AffyBatch instance affybatch.example
affybatch.example2	AffyBatch instance affybatch.example

R documentation: Sweave



Sweave {utils} R Documentation

Automatic Generation of Reports

Description

`Sweave` provides a flexible framework for mixing text and S code for automatic report generation. The basic idea is to replace the S code with its output, such that the final document only contains the text and the output of the statistical anlysis.

Usage

```
Sweave(file, driver = RweaveLatex(),
       syntax = getOption("SweaveSyntax"), ...)

Stangle(file, driver = Rtangle(),
        syntax = getOption("SweaveSyntax"), ...)
```

Arguments

`file`	Name of Sweave source file.
`driver`	The actual workhorse, see details below.
`syntax`	An object of class `SweaveSyntax` or a character string with its name. The default installation provides `SweaveSyntaxNoweb` and `SweaveSyntaxLatex`.
`...`	Further arguments passed to the driver's setup function.

Details

Automatic generation of reports by mixing word processing markup (like latex) and S code. The S code

Reading a list of files

some_cels.txt:

```
../../DataSets/SinghProstate/N01__normal.CEL
../../DataSets/SinghProstate/N05__normal.CEL
../../DataSets/SinghProstate/N11__normal.CEL
../../DataSets/SinghProstate/N15__normal.CEL
../../DataSets/SinghProstate/N21__normal.CEL
../../DataSets/SinghProstate/N25__normal.CEL
```

```
> celList <- readTable("some_cels.txt");
> ABatch  <- ReadAffy(celList);
```

The Evolution...

▮

oops...

```
> celList <- readTable("some_cels.txt");
> celList <- as.character(celList$V1);
> ABatch  <- ReadAffy(celList);
```

▮

oops...

```
> celList <- readTable("some_cels.txt");
> celList <- as.character(celList$V1);
> ABatch  <- ReadAffy(filenames = celList);
```

Ta Da!

Checking the Contents

```
> slotNames(ABatch)
 [1] "cdfName"      "nrow"           "ncol"
 [5] "se.exprs"     "description"    "annotation"
 [9] "reporterInfo" "phenoData"

> phenoData(ABatch)
 phenoData object with 1 variables and 6 cases
 varLabels
sample: arbitrary numbering
```

Looking at phenoData

```
> slotNames(phenoData(ABatch))
[1] "pData"        "varLabels"   "varMetadata"
> (phenoData(ABatch))@pData
                  sample
N01__normal.CEL        1
N05__normal.CEL        2
N11__normal.CEL        3
N15__normal.CEL        4
N21__normal.CEL        5
N25__normal.CEL        6
> (phenoData(ABatch))@varLabels
$sample
[1] "arbitrary numbering"
```

Assigning phenoData

some_pdata.txt:

```
Sample name      Concocted
N01_norm         A
N05_norm         A
N11_norm         A
N15_borm         B
N21_borm         B
N25_borm         B
```

```
> p1 <- read.phenoData("some_pdata.txt");# error
```

Assigning phenoData, pt 2

```
> p1 <- read.phenoData("some_pdata.txt",
                              sep="\t")
> p1
 phenoData object with 2 variables
         and 7 cases
 varLabels
V1: read from file
V2: read from file
# Not quite what we want.
```

Assigning phenoData, pt 3

```
> p1 <- read.phenoData("some_pdata.txt",
                     sep="\t", header=TRUE)
> p1
 phenoData object with 2 variables
         and 6 cases
 varLabels
Sample.name: read from file
Concocted: read from file
> phenoData(ABatch) <- p1
```

Other ways of Reading Data

Are they all in one directory?

What is the list of filenames?

read.affybatch vs ReadAffy

GUI?

Other ways of Reading Data 1

```
kabagg$ ls ../../DataSets/SinghSmall
N60__normal.CEL N61__normal.CEL N62__normal.CEL
```

```
> ABSmall <- ReadAffy(celfile.path=
       "../../DataSets/SinghSmall"); # works
```

Other ways of Reading Data 2

```
kabagg$ ls ../../DataSets/SinghSmall2
N60__normal.CEL.gz N61__normal.CEL.gz
N62__normal.CEL.gz
```

```
> ABSmall <- ReadAffy(celfile.path=
       "../../DataSets/SinghSmall2",
       compress=TRUE); # works
```

This takes only about 1/3 the space...

Other ways of Reading Data 3

```
kabagg$ ls ../../DataSets/SinghSmall3
N60.gz N61.gz N62.gz
```

```
> ABSmall <- ReadAffy(celfile.path=
       "../../DataSets/SinghSmall3",
       compress=TRUE); # fails
```

```
> ABSmall <- ReadAffy(filenames=
       "../../DataSets/SinghSmall3/N60.gz",
       compress=TRUE); # works
```

This still takes only about 1/3 the space...

Now let's Quantify

```
t0 <- date();
eset0 <- expresso(ABatch,
                  bgcorrect.method="rma",
                  normalize.method="quantiles",
                  pmcorrect.method="pmonly",
                  summary.method="medianpolish");
t1 <- date(); # 151s
eset1 <- justRMA(filenames = celList);
t2 <- date(); #  10s
```

The customized routines are better if they do what you want to do...

(also note that justRMA didn't build an AffyBatch.)

Just Because I'm Curious

```
> exprs(eset1)[1,]
N01__normal.CEL N05__normal.CEL N11__normal.CEL
       7.789481        7.314639        7.445363
N15__normal.CEL N21__normal.CEL N25__normal.CEL
       7.289881        7.503692        7.412608
```

Can we reconstruct this?

```
> ABatch.BG <- bg.correct.rma(ABatch)
> ABatch.BG.norm <-
    normalize.AffyBatch.quantiles(ABatch.BG)
```

These steps produce AffyBatch objects, with altered exprs.

What is the First Gene?

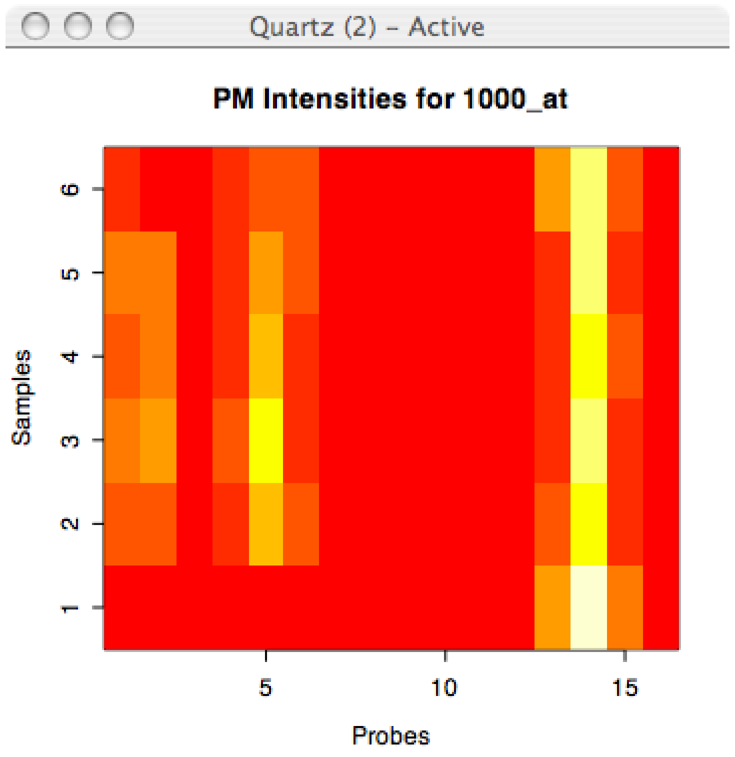
(well, ok, probeset)

```
> gn1 <- geneNames(ABatch.BG.norm)[1]
> gn1
[1] "1000_at"
```

Ok, now what are the values?

```
pr1 <- pm(ABatch.BG.norm, gn1);
```

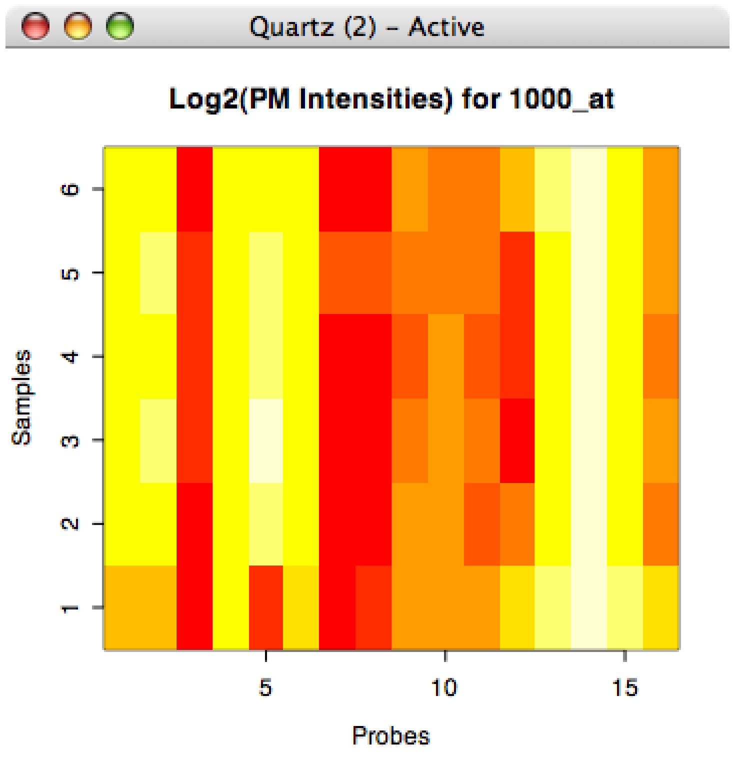
Looking at it, Take 1



```
image(1:nrow(pr1), 1:ncol(pr1), pr1,
      xlab="Probes", ylab="Samples",
      main="PM Intensities for 1000_at")
```

Some parallelism, but we may be missing something...

Looking at it, Take 2



```
image(1:nrow(pr1), 1:ncol(pr1), log2(pr1),
      xlab="Probes", ylab="Samples",
      main="Log2(PM Intensities) for 1000_at")
```

Logs!

Fitting the Probes

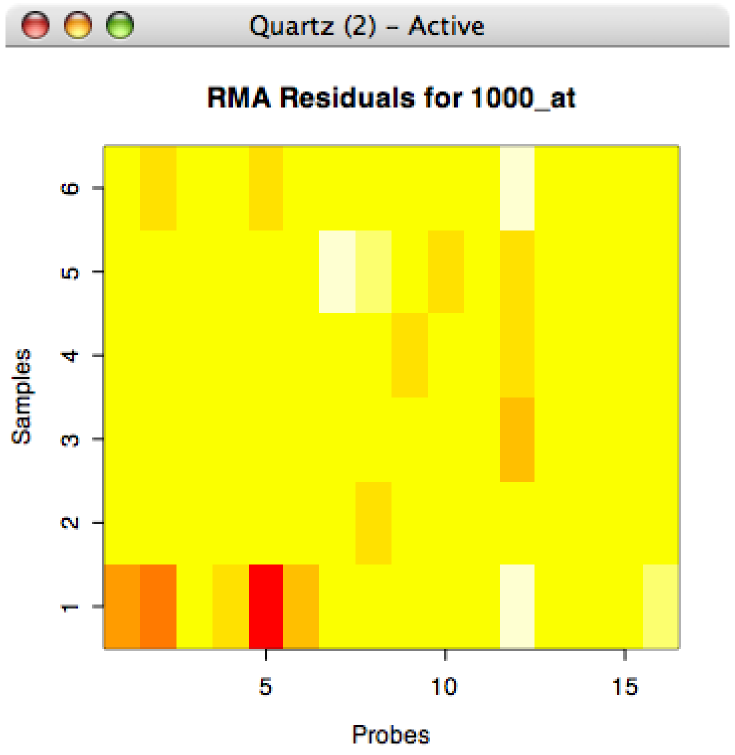
```
> pr1Fit <- medpolish(log2(pr1))
1 : 45.72612
2 : 45.08797
Final: 45.03615
> names(pr1Fit)
[1] "overall"  "row"       "col"       "residuals"
[5] "name"
> pr1Fit$overall + pr1Fit$col
N01__normal.CEL N05__normal.CEL N11__normal.CEL
       7.789481        7.314639        7.445363
N15__normal.CEL N21__normal.CEL N25__normal.CEL
       7.289881        7.503692        7.412608
```

This is what we found before!

We can Check the Code

```
> medpolish
function (x, eps = 0.01, maxiter = 10,
     trace.iter = TRUE, na.rm = FALSE)
{
    z <- as.matrix(x)
    nr <- nrow(z)
    nc <- ncol(z)
    t <- 0
    r <- numeric(nr)
    c <- numeric(nc)
    oldsum <- 0
    for (iter in 1:maxiter) {
        rdelta <- apply(z, 1, median, na.rm = na.
        z <- z - matrix(rdelta, nr = nr, nc = nc)
```

and Check the Residuals



```
image(1:nrow(pr1), 1:ncol(pr1), pr1Fit$residuals,
      xlab="Probes", ylab="Samples",
      main="RMA Residuals for 1000_at")
```

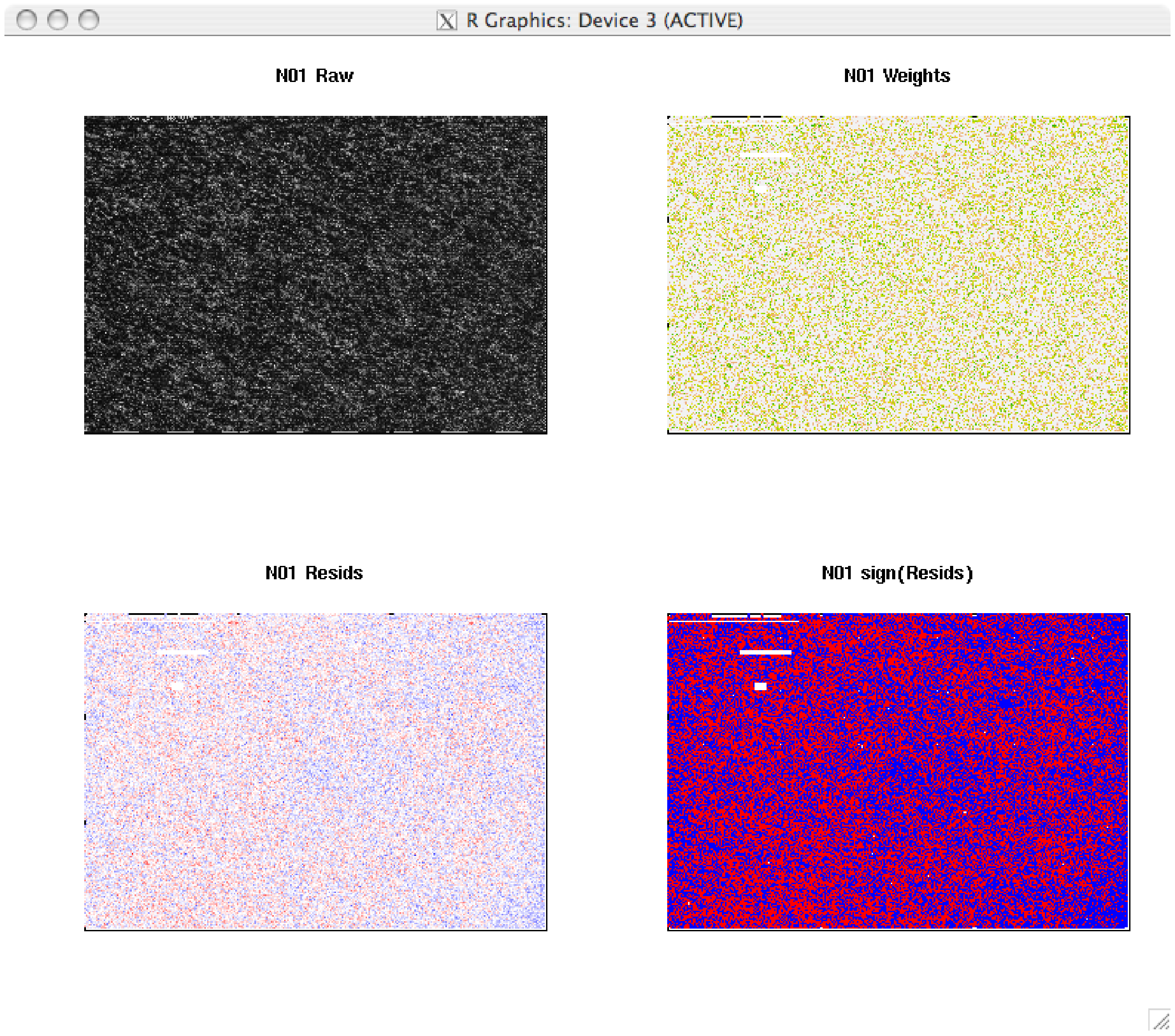
Trivia Q: Who introduced median polish?

One other Fitting Approach: PLM

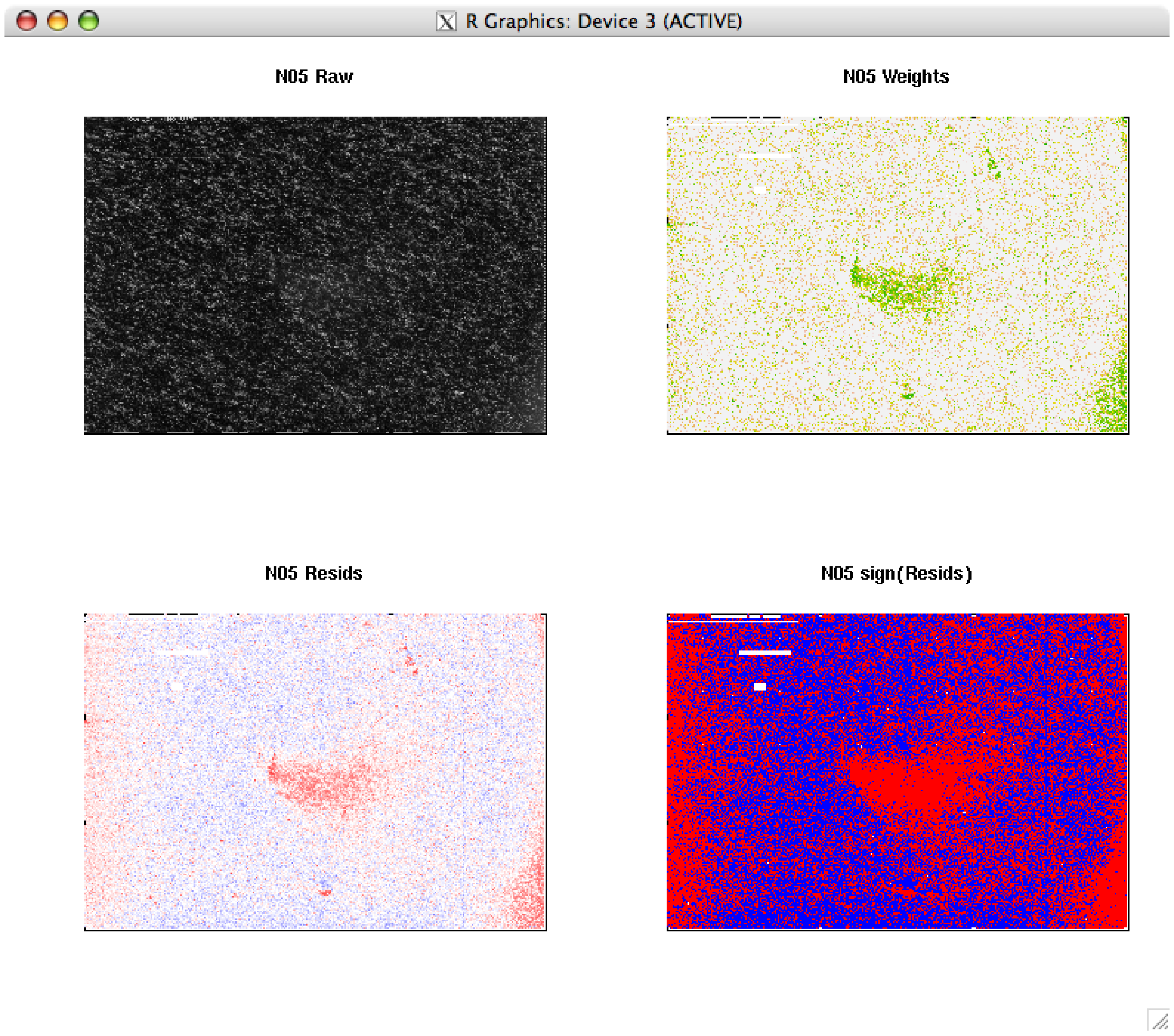
PLM = “Probe Level Model”

```
library("affyPLM");
plm1 <- fitPLM(ABatch); # takes a few minutes
par(mfrow=c(2,2));
image(ABatch[, 1],main="N01 Raw");
image(plm1, type="weights", which=1, main="N01 We
image(plm1, type="resids", which=1, main="N01 Res
image(plm1, type="sign.resids", which=1,
      main="N01 sign(Resids)");
```

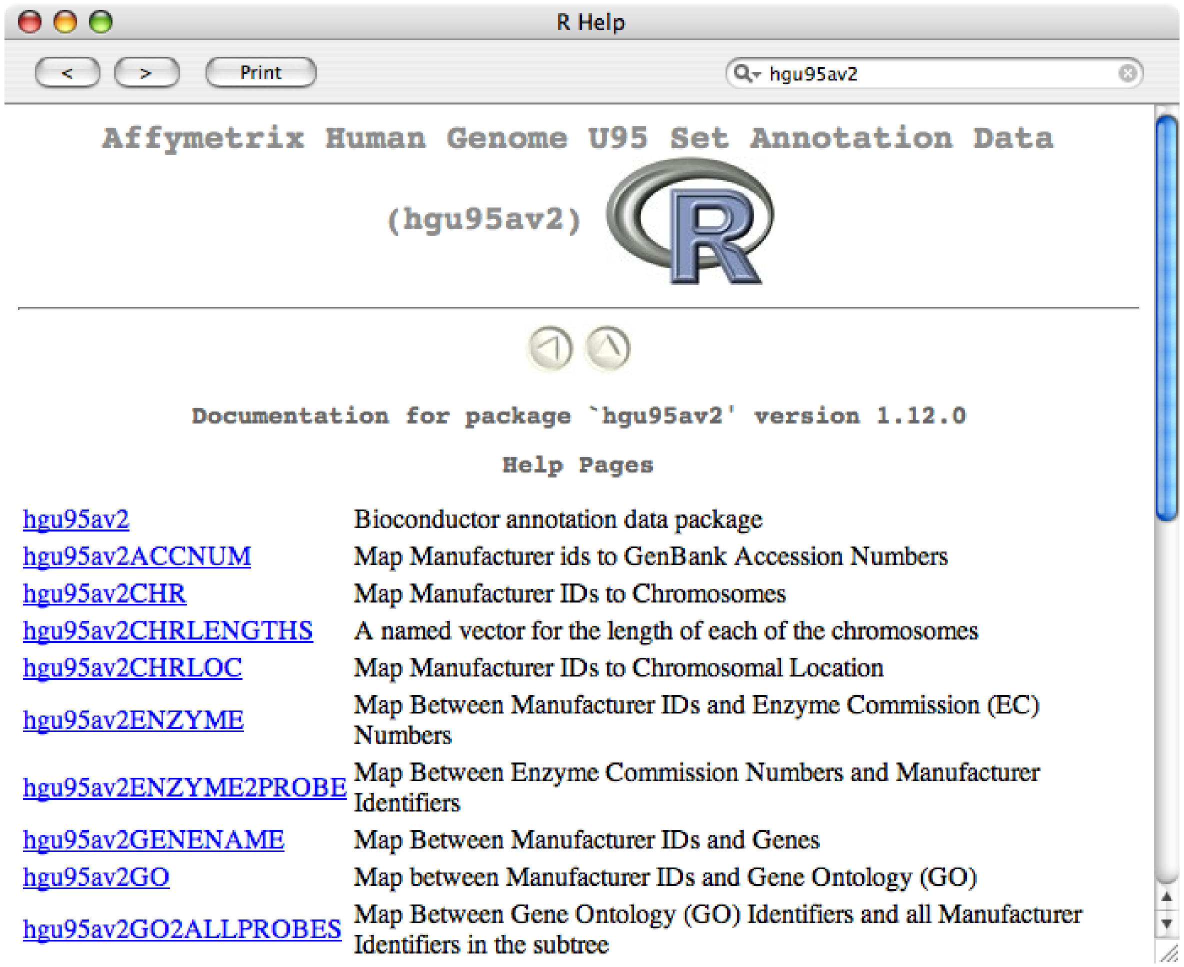
Looking at N01



Looking at N05



Whence the Gene Name Info?



Affymetrix Human Genome U95 Set Annotation Data

(hgu95av2)

Documentation for package `hgu95av2' version 1.12.0

Help Pages

hgu95av2	Bioconductor annotation data package
hgu95av2ACCNUM	Map Manufacturer ids to GenBank Accession Numbers
hgu95av2CHR	Map Manufacturer IDs to Chromosomes
hgu95av2CHRLENGTHS	A named vector for the length of each of the chromosomes
hgu95av2CHRLOC	Map Manufacturer IDs to Chromosomal Location
hgu95av2ENZYME	Map Between Manufacturer IDs and Enzyme Commission (EC) Numbers
hgu95av2ENZYME2PROBE	Map Between Enzyme Commission Numbers and Manufacturer Identifiers
hgu95av2GENENAME	Map Between Manufacturer IDs and Genes
hgu95av2GO	Map between Manufacturer IDs and Gene Ontology (GO)
hgu95av2GO2ALLPROBES	Map Between Gene Ontology (GO) Identifiers and all Manufacturer Identifiers in the subtree

```
> library("hgu95av2");
```

What Does This Package Contain?

```
> hgu95av2()

Quality control information for  hgu95av2
Date built: Created: Sun Mar 26 00:53:27 2006

Number of probes: 12625
Probe number missmatch: None
Probe missmatch: None
Mappings found for probe based rda files:
 hgu95av2ACCNUM found 12625 of 12625
 hgu95av2CHRLOC found 11716 of 12625
 hgu95av2CHR found 12171 of 12625
 hgu95av2ENZYME found 1922 of 12625
 hgu95av2GENENAME found 11660 of 12625
 hgu95av2GO found 11101 of 12625
 hgu95av2LOCUSID found 12238 of 12625
 hgu95av2MAP found 12140 of 12625
 hgu95av2OMIM found 9978 of 12625
 hgu95av2PATH found 4252 of 12625
 hgu95av2PMID found 12136 of 12625
 hgu95av2REFSEQ found 12039 of 12625
 hgu95av2SUMFUNC found 0 of 12625
 hgu95av2SYMBOL found 12184 of 12625
 hgu95av2UNIGENE found 12127 of 12625
Mappings found for non-probe based rda files:
   hgu95av2CHRLENGTHS found 25
 hgu95av2ENZYME2PROBE found 660
 hgu95av2GO2ALLPROBES found 6012
 hgu95av2GO2PROBE found 4274
```

```
hgu95av2ORGANISM found 1
hgu95av2PATH2PROBE found 173
hgu95av2PFAM found 10412
hgu95av2PMID2PROBE found 107253
hgu95av2PROSITE found 8193
```

(we can also see this using ls(”package:hgu95av2”).)

What Does This Package Contain?

```
> hgu95av2GENENAME
<environment: 0x26519bdc>
```

Almost everything in this package is an "environment", which is R's fancy name for a hash table. We can access things by name.

```
> hgu95av2GENENAME$"1000_at"
[1] "mitogen-activated protein kinase 3"
```

We can access a lot of annotation!

What was Needed for Quantification?

```
> library("hgu95av2cdf");
> hgu95av2cdf$"1000_at"
          pm     mm
 [1,] 358160 358800
 [2,] 118945 119585
 [3,] 323731 324371
 [4,] 223978 224618
...
[15,] 317054 317694
[16,] 404069 404709
```

These give the indices of the probes within the 409600-long vector of expression intensities.

What if We Want to Go in Reverse?

Given a probeset, I can find a gene name. What if I have a gene name, and I want something else?

Can we find “BAD”?

This is a gene symbol, so we probably want to work with the hgu95av2SYMBOL environment.

The key function for extracting items from an environment without the key is “contents”.

```
> tempSYM <- contents(hgu95av2SYMBOL);
> tempSYM[1]
$`986_at`
[1] "CYP19A1"
```

Finding BAD in the Contents

```
> tempSYM[tempSYM == "BAD"]
$`1861_at`
[1] "BAD"

> names(tempSYM[tempSYM == "BAD"])
[1] "1861_at"
```

This gives us the key!

Some of these queries are simplified if we invoke

```
> library("annotate"); # for example,
> getLL("1861_at", "hgu95av2");
[1] 572
```

One More Thing...

sequences?

```
> library("hgu95av2probe");
> data(hgu95av2probe); # a big file

> as.data.frame(hgu95av2probe[1,])
                   sequence   x   y
1 TCTCCTTTGCTGAGGCCTCCAGCTT 399 559

Probe.Set.Name   Probe.Interrogation.Position
1000_at                                 1367

Target.Strandedness
Antisense
```

So, What's BAD?

```
> as.data.frame(hgu95av2probe[hgu95av2probe$Probe
                          sequence   x   y Probe.Set
14006 CAACCTCTGGGCAGCACAGCGCTAT 403 485        18
14007 AACCTCTGGGCAGCACAGCGCTATG 402 485        18
14008 CCTCTGGGCAGCACAGCGCTATGGC 207 491        18
14009 TGGGCAGCACAGCGCTATGGCCGCG 436 421        18
14010 GCAGCACAGCGCTATGGCCGCGAGC 285 599        18
...
```

The midpoint of the first probe should be at position 384 within the cDNA sequence.