Dose-response Explorer: Exploring different models and how outliers affect model fitting

Kevin R Coombes and Pan Tong

October 1, 2014

Contents

List of Figures

List of Tables

1 Introduction

There is a great variety of models developed for dose-response data, many of which have been implemented in the *drc* and *DoseFinding* packages. *drexplorer* combines both packages to aid the user to visually examine and compare how existing models perform on the data. We also incorporate model selection. Another important feature for *drexplorer* is to allow the user to identify outlier measurements and visually examine how these outliers affect the fitted model.

In addition to fit dose-response models, *drexplorer* also implements methods previously published to assess drug-drug interaction. Graphical User Interfaces (GUIs) have been designed to allow users without advanced programming skills to perform dose-response analysis.

The main entry for *drexplorer* is the drFit() function and computeIC() function. drFit() fits a model. Outlier detection is also embedded into drFit(). Once a model is fitted, computeIC() computes IC values at specified percentiles.

2 Outlier identification

This package implements the method by Newman, D. The test statistic q=w/s where w is the range of the data and s is sample standard deviation estimated from controls. The null distribution for q has been derived and 1% and 5% quantiles have been given in the paper.

We implement this procedure. In particular, NewmanTest() returns a logic vector specifying whether the observations are outliers. Usually, drug-response data contains multiple doses. Therefore, we write a wrapper drOutlier() that compute the result for all doses each dose at a time.

We use the `ryegrass` data from *drc* package for illustration purpose.

First, we load the *drexplorer* package and attach the *ryegrass* data.

```
library(drexplorer)
data(ryegrass)
```

At dose=3.75 and significance level 0.05, we find there is one outlier identified:

```
dose <- ryegrass[, 2]
response <- ryegrass[, 1]
## potential outlier at dose 3.75
NewmanTest(ref = response[dose == 0], obs = response[dose ==
    3.75], alpha = 0.05)

## [1] FALSE FALSE  TRUE
```

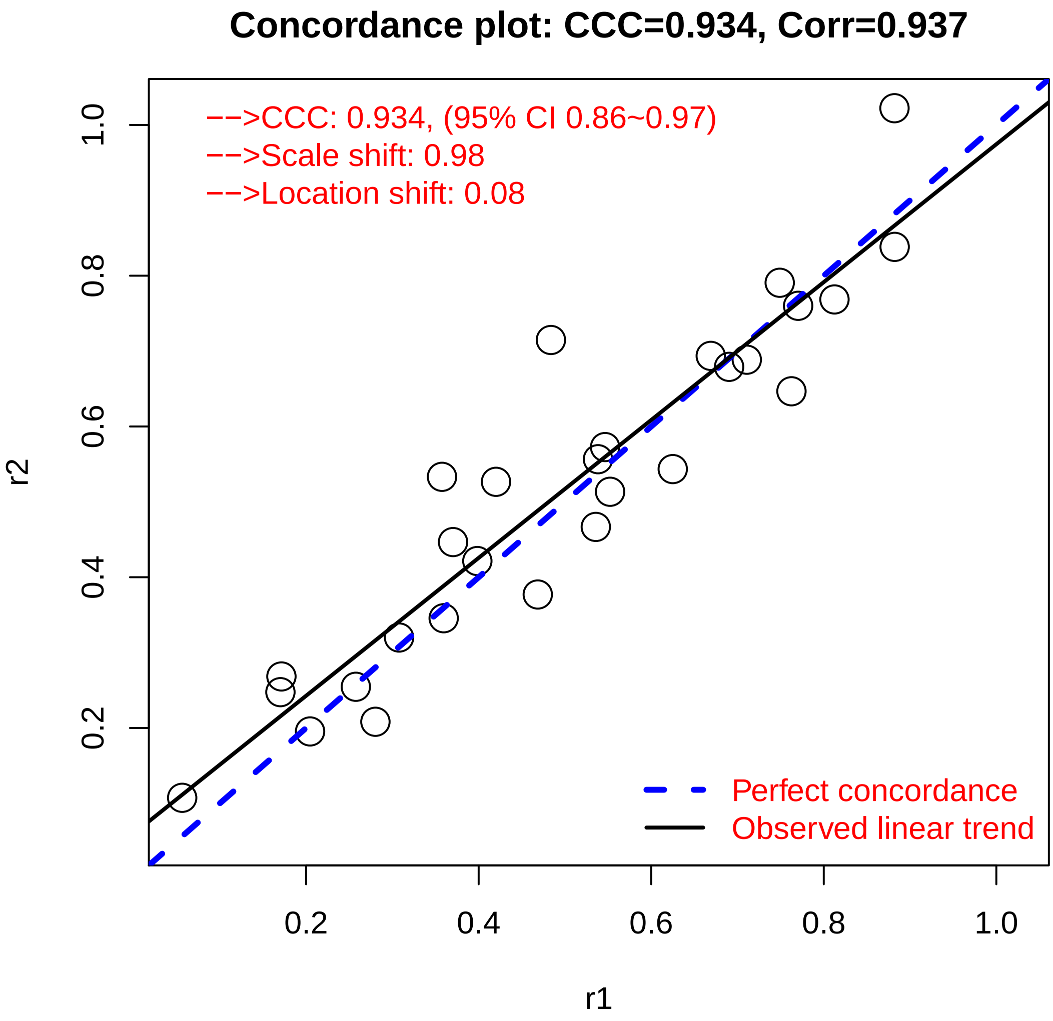
We also examine all dose levels and find no further outliers:

```
drOutlier(drMat = ryegrass[, c(2, 1)], alpha = 0.05)

##  [1] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
## [13] FALSE FALSE  TRUE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
```

3 Assessing Reproducibility

Sometimes replicated viability assays are performed. In such case, it is useful to examine if the experiments are reproducible. A good metric is the Concordance Correlation Coefficient (CCC) that captures both the location shift as well as scale shift between the replicates. The plotCCC function can be used to compute CCC and visualize the replicated data.

```
set.seed(100)
r1 <- runif(28)
r2 <- r1 + rnorm(28, 0, 0.1)
ccc <- plotCCC(r1, r2)
```



```
ccc

##     ccc s_shift l_shift  ccc_lo  ccc_hi      Cb    corr  mean_x  mean_y    sd_x
## 0.93365 0.97649 0.07578 0.86264 0.96857 0.99686 0.93660 0.50119 0.51820 0.23138
##    sd_y
## 0.22594
```

Here we simulate two response vectors and calculate CCC. The computed CCC value is 0.934, location shift is 0.076, scale shift is 0.976, Pearson correlation is 0.937.

4 Fit dose-response models

Below we show how to fit a dose-response model. The fitDRC() function is a wrapper to the *drc* and *DoseFinding* packages. Therefore, all models implemented by either package can be fitted. A model is specified by a modelName and package name to be passed to this function.

Outliers can be identified and removed from model fitting by specifying the parameter alpha (either at significance level of 0.01 or 0.05). To disable outlier identification, set alpha=1.

To remove controls (responses at dose=0) during model fitting, we can set fitCtr=FALSE.

Note that the responses are scaled by mean response at dose=0 before model fitting.

Below we fit a sigmaEmax model. We set alpha=1 to disable outlier removal and fitCtr=FALSE to exclude controls.

```
fit_sigEmax_alpha1 <- drFit(drMat = ryegrass[, c(2,
    1)], modelName = "sigEmax", alpha = 1, fitCtr = FALSE)

## Message:  Need bounds in "bnds" for nonlinear models, using default
bounds from "defBnds".
```

The result is slightly different when outliers passing significance level of 0.05 is removed.

```
fit_sigEmax_alpha_o5 <- drFit(drMat = ryegrass[, c(2,
    1)], modelName = "sigEmax", alpha = 0.05, fitCtr = FALSE)

## Message:  Need bounds in "bnds" for nonlinear models, using default
bounds from "defBnds".

fit_sigEmax_alpha1@fit

## Dose Response Model
##
## Model: sigEmax
## Fit-type: normal
##
## Coefficients dose-response model
##      e0    eMax    ed50       h
##  1.0290 -0.9703  2.9920  2.8090
##
## Degrees of freedom: 14
## Residual standard error: 0.073
```

```
fit_sigEmax_alpha_o5@fit
```

```
## Dose Response Model
##
## Model: sigEmax
## Fit-type: normal
##
## Coefficients dose-response model
##     e0   eMax   ed50      h
##  1.017 -0.945  2.789  3.384
##
## Degrees of freedom: 13
## Residual standard error: 0.05673
```

5 Predict response

One a model is fitted, it can be used to make predictions.

Below we make predictions at the observed dose levels with a previously fitted model. Since the responses are scaled by mean response at dose=0 in model fitting, the predicted responses are also scaled by the mean response from controls. By default, the predict function makes predictions at observed doses.

```
y <- predict(fit_sigEmax_alpha_o5)
y
```

```
##       1       2       3       4       5       6       7       8       9      10
## 1.01741 1.01741 1.01741 1.01741 1.01741 1.01741 0.99418 0.99418 0.99418 0.82053
##      11      12      13      14      15      16      17      18      19      20
## 0.82053 0.82053 0.32623 0.32623 0.32623 0.10454 0.10454 0.10454 0.07561 0.07561
##      21      22      23      24
## 0.07561 0.07274 0.07274 0.07274
```

6 Obtain IC values

We implement two approaches for IC value computation. One is to interpolate the observed dosages and try to use the dose that has the predicted response closest to the specified percentile of IC value. The second approach is to use root finding by setting the fitted model to equal to the specified percentile. In most cases, the result are similar. However, the latter approach may give IC50 values beyond observed dosages and sometimes not robust. The computeIC() function implements both approaches. By setting interpolation=TRUE (the default value) in the computeIC() function, the interpolation approach will be selected.

Computing IC values at different quantiles is also easy. Similar to the fitDRC() function, different models as well as other options (alpha and fitCtr) can be specified in estimating IC value.

Below we estimate IC50 at different percentiles with the sigmoid Emax model with outlier removal (alpha=0.05) fitted previously. We see that estimates from interpolation and prediction by the model are quite similar.

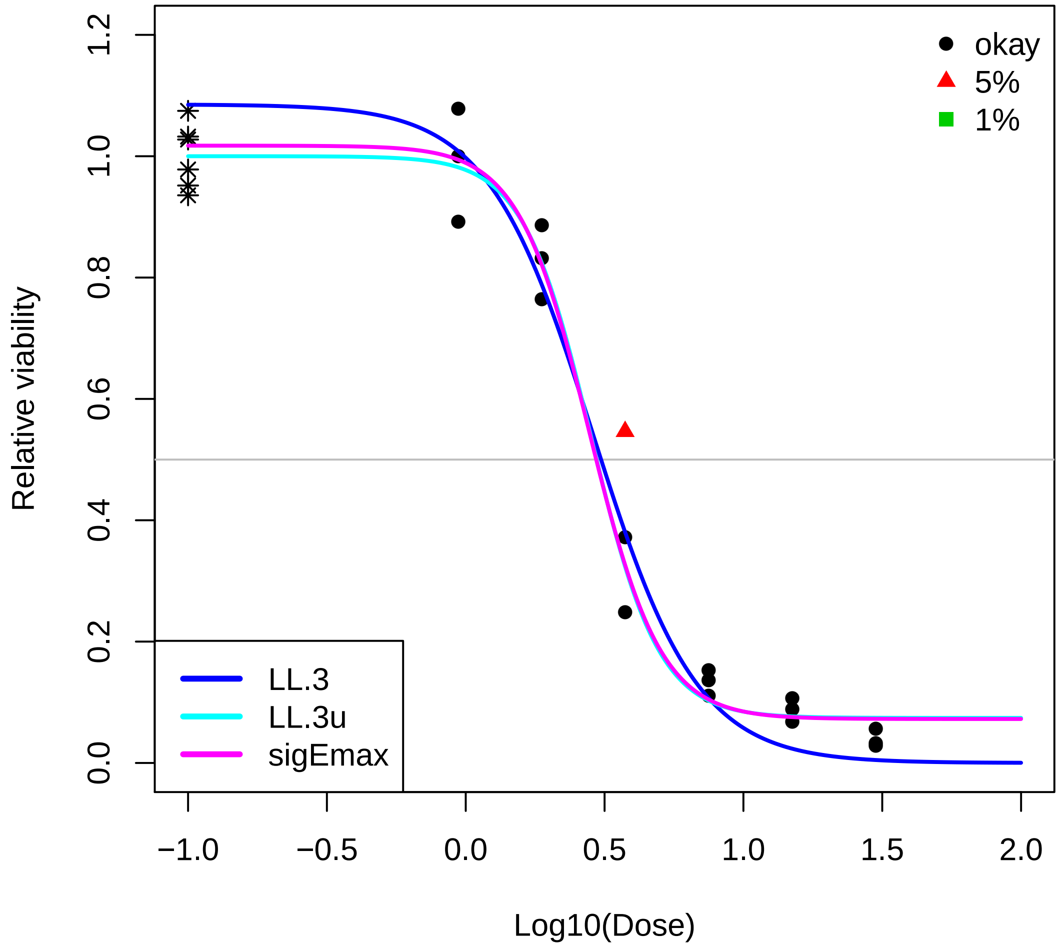
```
computeIC(fit_sigEmax_alpha_o5, percent = seq(0, 1,
    by = 0.1), log.d = FALSE, interpolation = TRUE)

##     IC0   IC10   IC20   IC30   IC40   IC50   IC60   IC70   IC80   IC90  IC100
##   0.940  1.566  1.952  2.281  2.600  2.948  3.362  3.916  4.828  7.855 30.000

computeIC(fit_sigEmax_alpha_o5, percent = seq(0, 1,
    by = 0.1), log.d = FALSE, interpolation = FALSE)

##       IC0      IC10      IC20      IC30      IC40      IC50      IC60      IC70
## 8.616e-01 1.566e+00 1.952e+00 2.280e+00 2.603e+00 2.951e+00 3.364e+00 3.916e+00
##      IC80      IC90     IC100
## 4.829e+00 7.857e+00 1.000e+30
```

7 Comparing multiple dose-response curves

We provide S4 generic functions (plot and lines) for fitted model. As a result, it is easy to compare different models and graphically examine outliers through multiple dose-response curves.

Outliers at significance levels 0.01 and 0.05 are indicated by different colors and symbols. Below we show the LL.3, LL.3u and sigEmax curves in this example corresponding to the three-parameter log-logistic model with lower limit 0, three-parameter log-logistic with upper limit 1 and the sigmoid Emax model.

```
fit.LL.3 <- drFit(drMat = ryegrass[, c(2, 1)], modelName = "LL.3",
    alpha = 0.05, fitCtr = FALSE)
fit.LL.3u <- drFit(drMat = ryegrass[, c(2, 1)], modelName = "LL.3u",
    alpha = 0.05, fitCtr = FALSE)
fit.sigEmax <- drFit(drMat = ryegrass[, c(2, 1)], modelName = "sigEmax",
    alpha = 0.05, fitCtr = FALSE)

## Message:  Need bounds in "bnds" for nonlinear models, using default
bounds from "defBnds".

###
plot(fit.LL.3, main = "", col = 4, lwd = 2)
lines(fit.LL.3u, col = 5, lwd = 2)
lines(fit.sigEmax, col = 6, lwd = 2)
legend("bottomleft", c("LL.3", "LL.3u", "sigEmax"),
    col = 4:6, lwd = 3)
```

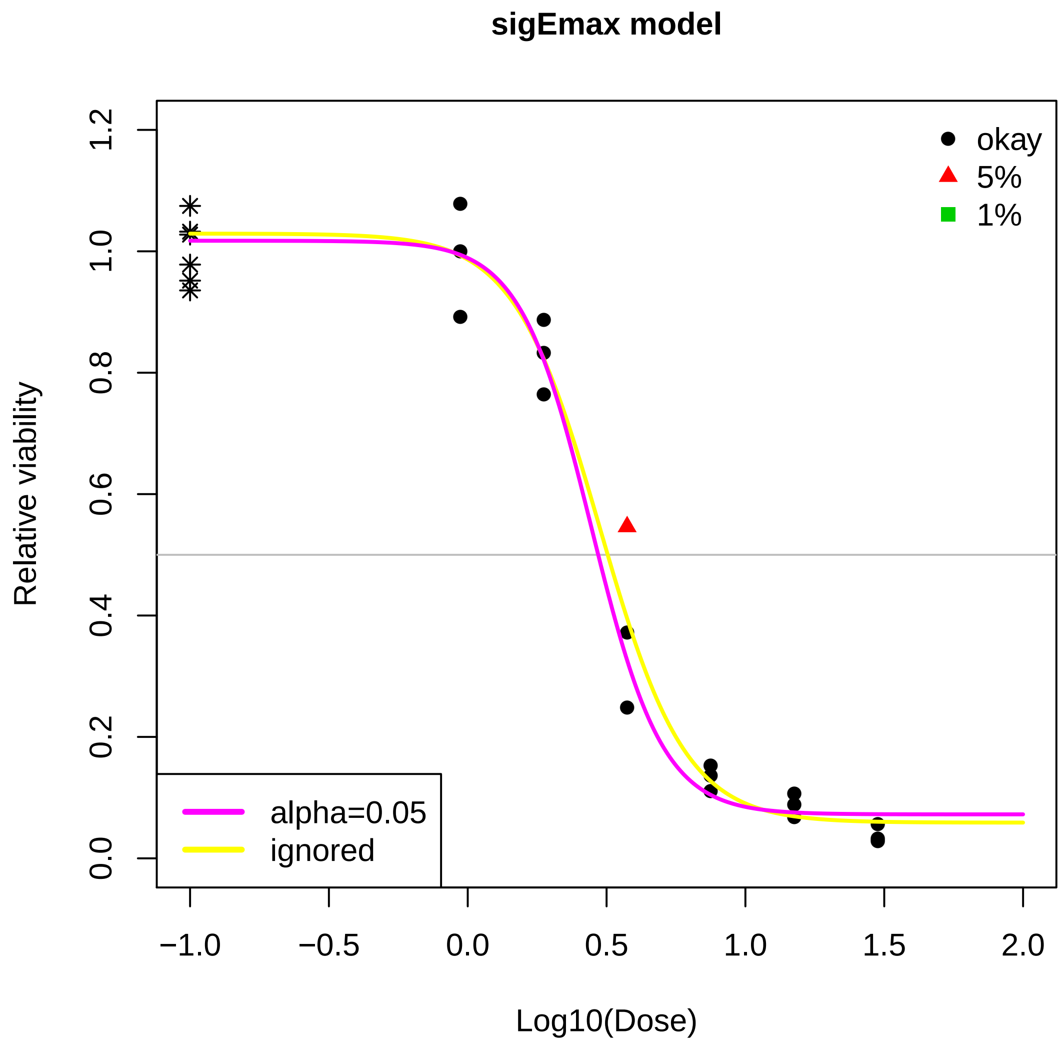


With these many models fitted, which one should be preferred? One way is to look at the Residual Standard Error (RSE) as below. We see that the LL.3u model is best by the RSE criteria.

```
sapply(list(fit.LL.3, fit.LL.3u, fit.sigEmax), function(x) x@info$RSE)

## [1] 0.06987 0.05501 0.05673
```

We also compare the curve using sigEmax model with and without outlier identification.

```
# no outlier excluded
fit.sigEmax0 <- drFit(drMat = ryegrass[, c(2, 1)],
    modelName = "sigEmax", alpha = 1, fitCtr = FALSE)

## Message:  Need bounds in "bnds" for nonlinear models, using default
bounds from "defBnds".

###
plot(fit.sigEmax0, main = "sigEmax model", col = 7,
    lwd = 2)
lines(fit.sigEmax, col = 6, lwd = 2)
legend("bottomleft", c("alpha=0.05", "ignored"), col = c(6,
    7), lwd = 3)
```



8 Drug Interaction Index

Administering two drugs simultaneously might induce stronger effect than if administered separately. This is called synergism. Experiments to detect synergism (or antagonism which is the opposite) are usually in two forms. One is the fixed ratio design (ray design) where the ratio of doses between two drugs is a constant. Another one is grid design which means all-possible combinations of drug doses are available.

Two papers have been published regarding to drug interaction index (IAI) by Lee et al, one in 2007 (Lee2007) [1] and on in 2009 (Lee2009) [2]. The Lee2007 paper described five methods to assess interaction: (1) Lowewe additivity model using interaction index (IAI) (2) Model of Greco et al 1990. This approach uses α as the metric and it can be related to IAI (3) Model of Machado and Robinson which uses a metric denoted as η (4) Model of Plummer and Short which can also be linked to IAI through the parameter β_4 (5) Model of Carter et al that can be linked to IAI through the parameter β_{12}. For more details of these models, please refer to Lee2007 [1].

The two papers by Lee et al discussed the fixed ratio design and the source code for doing this is incorporated into *drexplorer*. To work on grid design, a fixed ratio from the data needs to be selected in order to apply their method. For example, the Lee2007 paper provided an example of grid design. A fixed ratio of 1 was specified in the paper. The specification of fixed ratio would affect the fitted median effect model (see definition in [1]) for the drug mixture as well as estimation of IAI. As a result, IAI has a ratio dependent interpretation.

Below we load the UMSCC22B data from [2]. This data has a fixed ratio design. The `fitIAI` function estimates IAI as well as its confidence interval after specifying dose1, dose2 and effect (between 0 and 1).

```
data(UMSCC22B)
fit_fixedRay <- fitIAI(d1 = UMSCC22B[, 1], d2 = UMSCC22B[,
    2], e = UMSCC22B[, 3], name1 = "SCH66336", name2 = "4HPR")

## Warning:  Fixed ratio design detected.  Fixed ratio is 1.000
```

The `plotIAI` function is then used to generate different plots including IAI versus response, IAI versus dose (predicted dose for the drug mixture, see equation (6) in [1]), median effect plot and dose response curves. We can also plot IAI versus response as well as IAI versus dose in one figure by specifying mode='both'.

The median effect equation [3] is as following:

$$E = \frac{(d/D_m)^m}{1 + (d/D_m)^m}$$

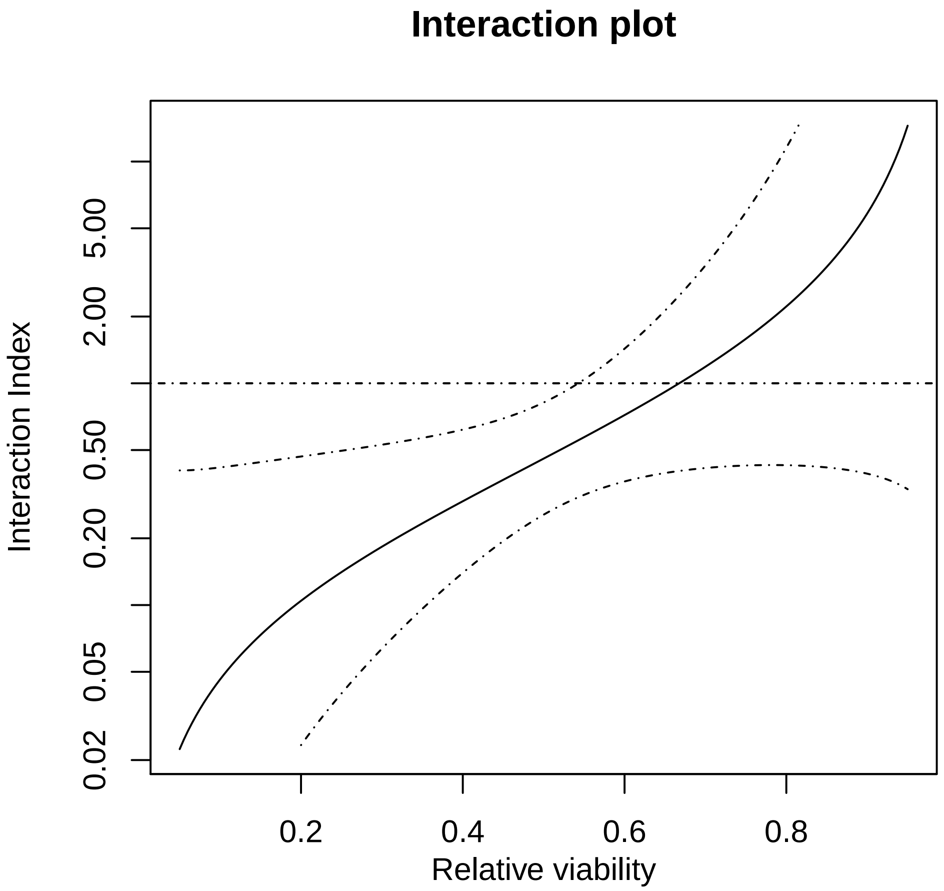
where E is the induced effect of a drug with dose d whose median effective dose is D_m and m is a slope parameter.

This equation can be arranged as:

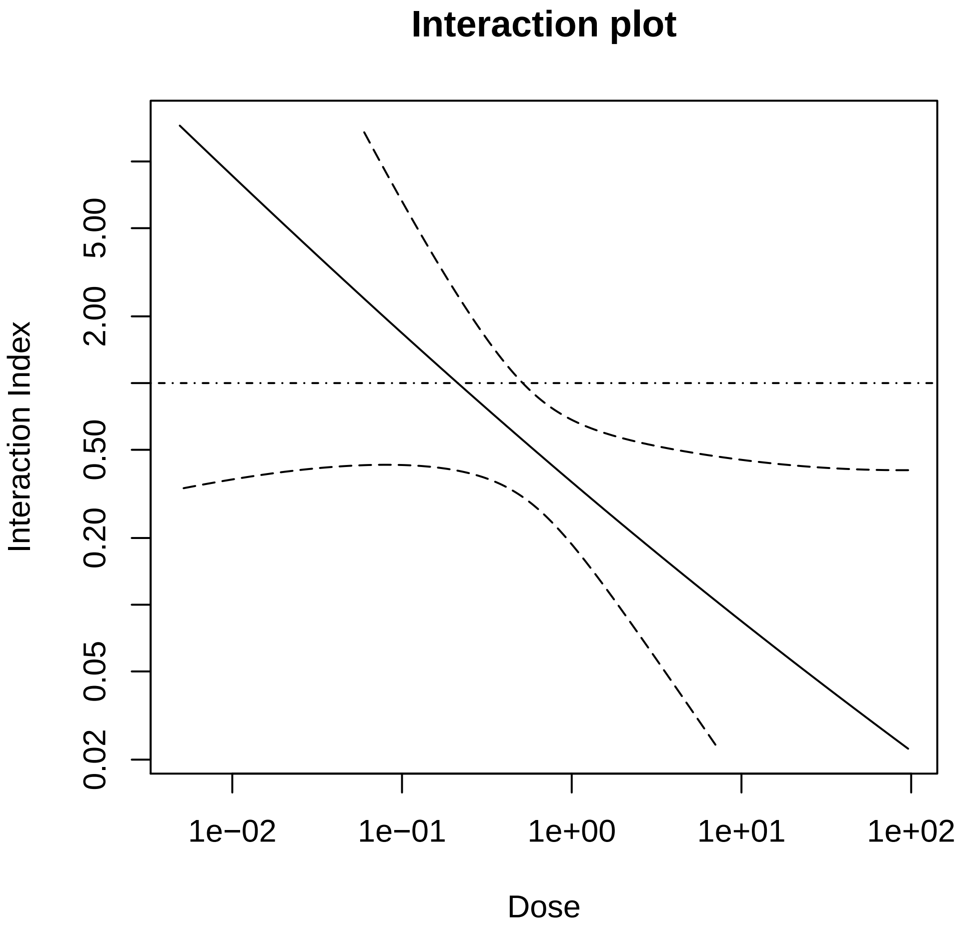
$$logit(E) = m(logd - logD_m)$$

The median effect plot is just plotting logit(E) versus log10 dose; The dose response curve is plotting E versus dose.

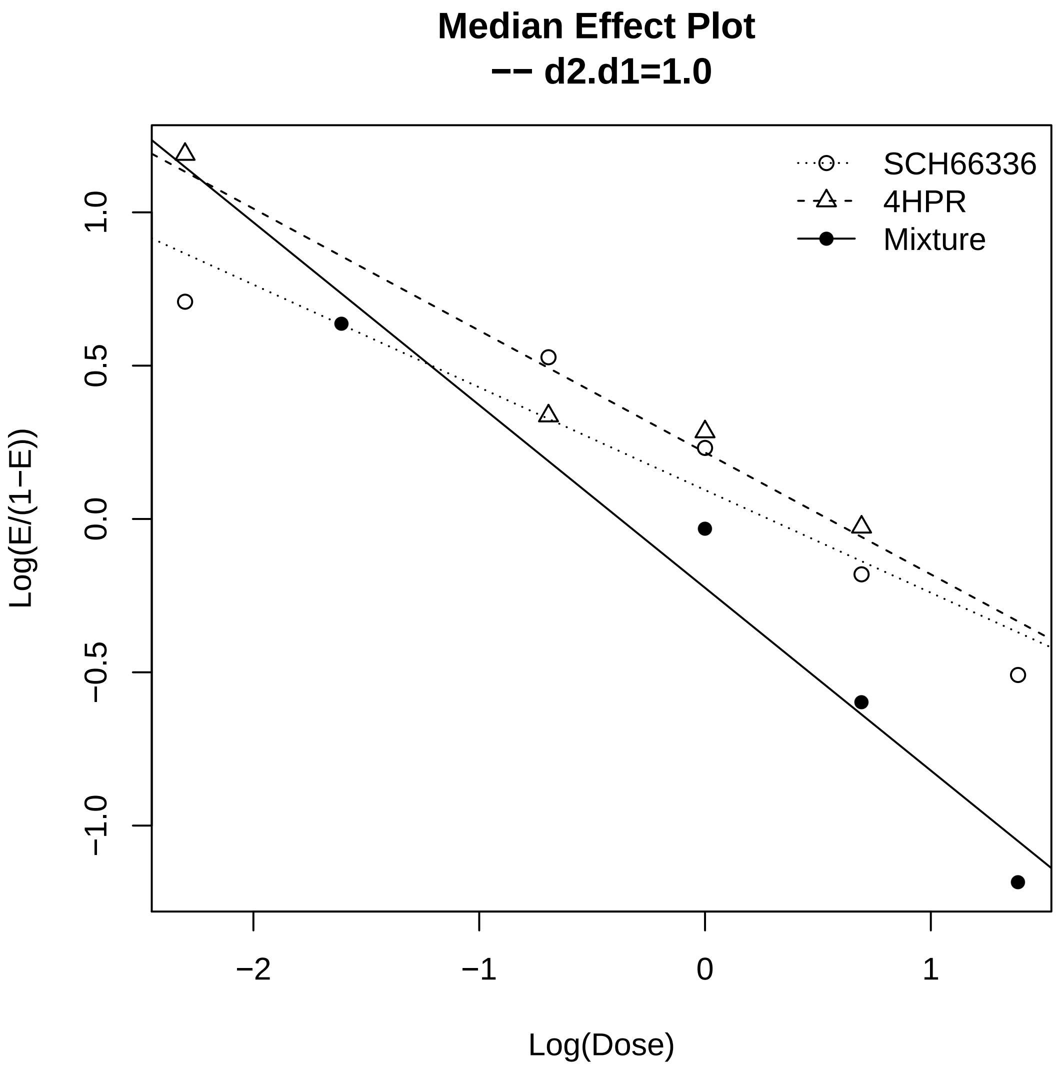
```
# IAI vs response
plotIAI(fit_fixedRay, type = "IAI", mode = "response")
```



```r
# IAI versus dose
plotIAI(fit_fixedRay, type = "IAI", mode = "dose")
```



```
# median effect
plotIAI(fit_fixedRay, type = "medianEffect")
```

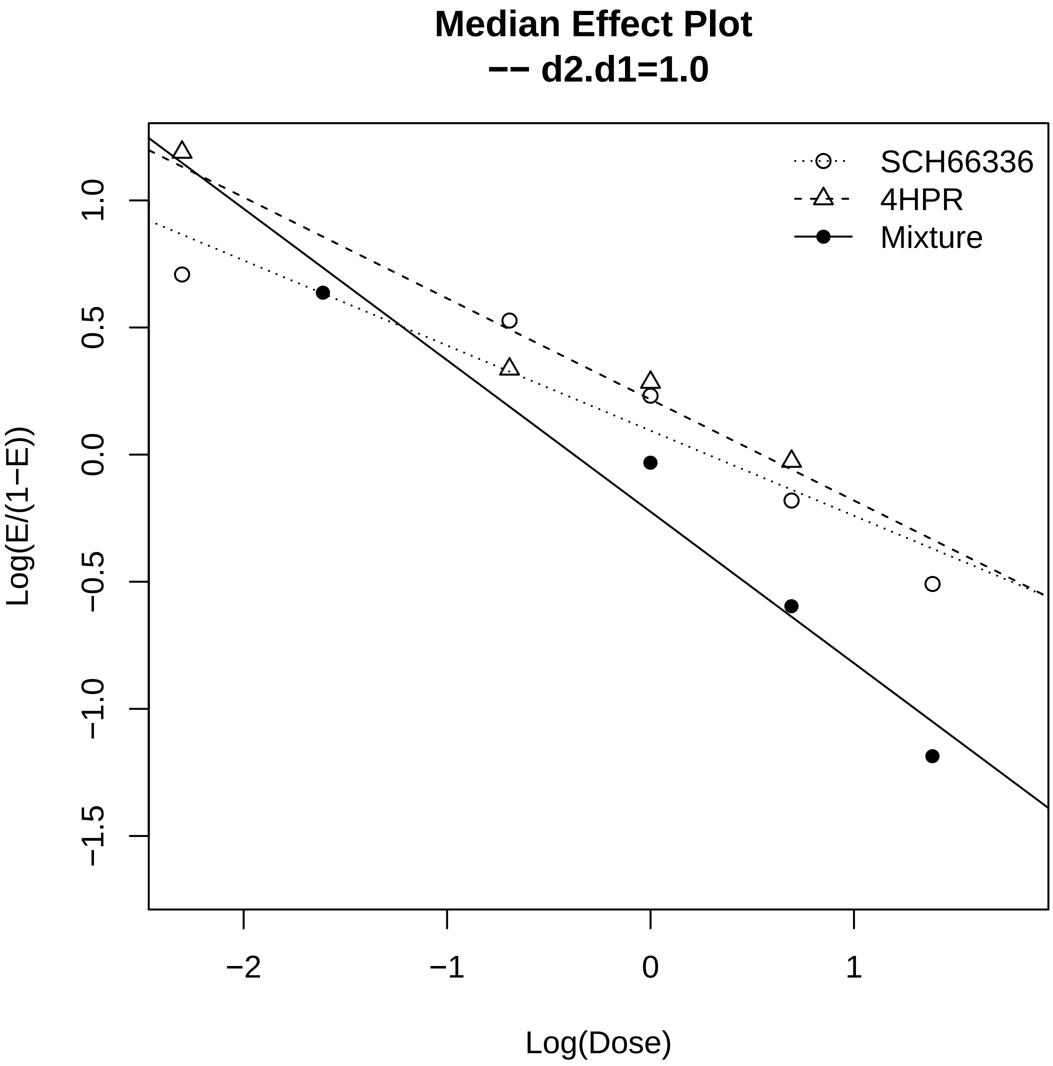


In [1], there is an example data (nl22B2) using grid design. Here we examine the estimate of IAI at different fixed ratios.

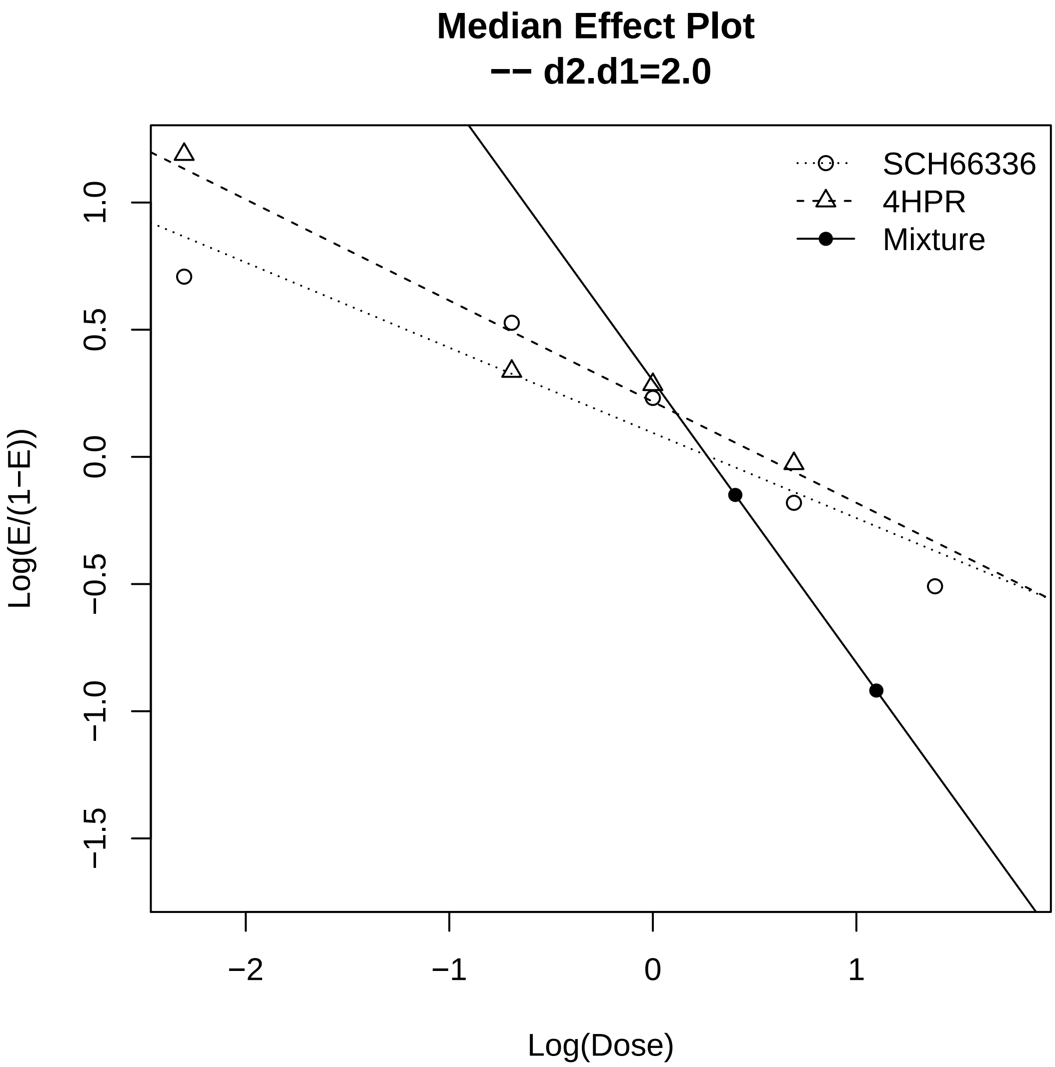
```
data(nl22B2)
fit_allPoss_1 <- fitIAI(d1 = nl22B2$schd, d2 = nl22B2$hpr,
    e = nl22B2$y1, name1 = "SCH66336", name2 = "4HPR",
    d2.d1.force = 1)

## Warning:  This is not a ray design; computation is done with method
derived from fixed ratio!

fit_allPoss_2 <- fitIAI(d1 = nl22B2$schd, d2 = nl22B2$hpr,
    e = nl22B2$y1, name1 = "SCH66336", name2 = "4HPR",
    d2.d1.force = 2)

## Warning:  This is not a ray design; computation is done with method
derived from fixed ratio!
```

From the median effect plot, we can find that there are 4 data points for drug mixtures at fixed ratio of 1 while only 2 data points are available at fixed ratio of 2.

```
# median effect
plotIAI(fit_allPoss_1, type = "medianEffect")
```

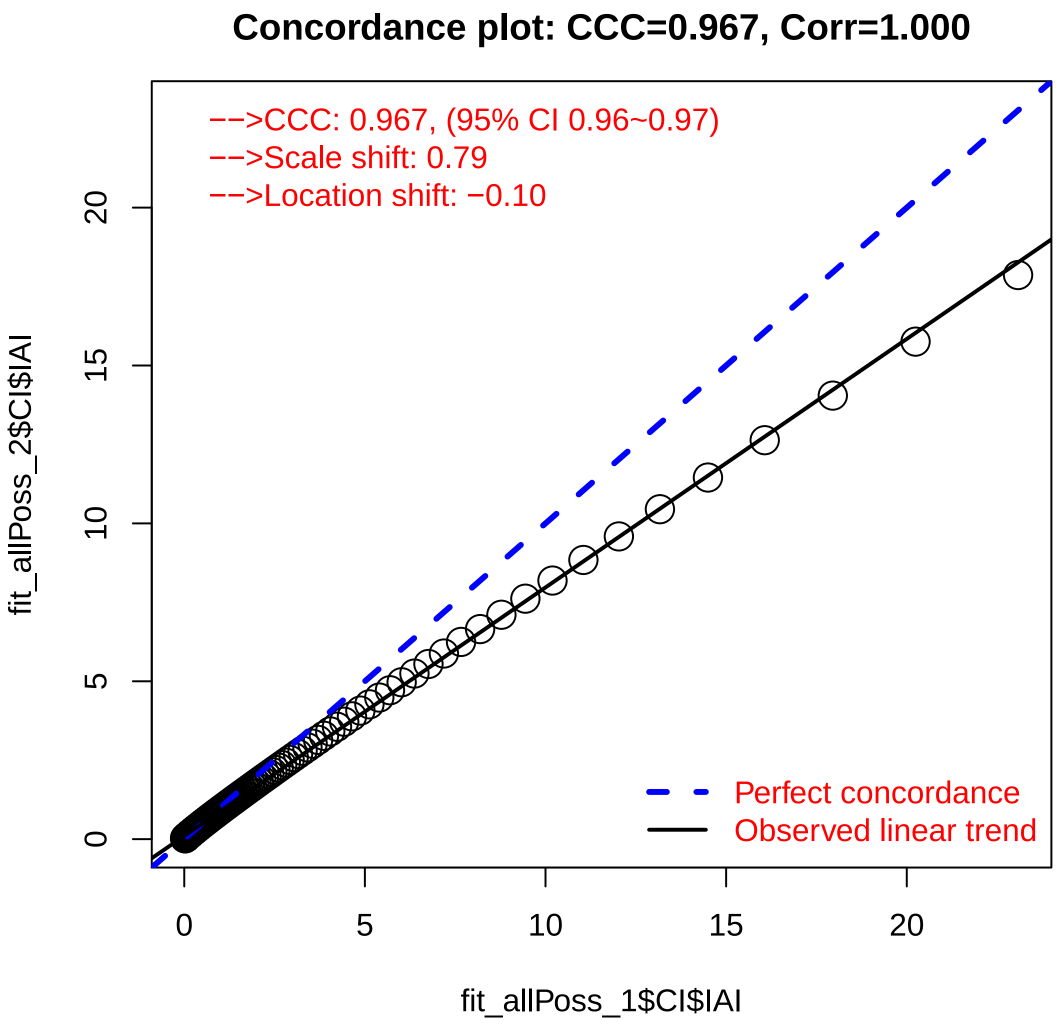


```
plotIAI(fit_allPoss_2, type = "medianEffect")
```



Below we compare IAI estimated from the two scenarios.

```
plotCCC(fit_allPoss_1$CI$IAI, fit_allPoss_2$CI$IAI)
```



```
##       ccc  s_shift  l_shift   ccc_lo   ccc_hi       Cb     corr   mean_x
##   0.96701  0.78723 -0.09956  0.96324  0.97040  0.96739  0.99961  2.00115
##    mean_y     sd_x     sd_y
##   1.67744  3.67464  2.89279
```

9 File Location and Session Info

```
getwd()

## [1] "/data/bioinfo2/ptong1/Projects/Coombes/IC50Package/Package/for_vignettes"

sessionInfo()
```

```
## R version 3.1.0 (2014-04-10)
## Platform: x86_64-unknown-linux-gnu (64-bit)
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8        LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] tools     splines   stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] drexplorer_0.1.0   fgui_1.0-5         plyr_1.8.1         stringr_0.6.2
##  [5] RColorBrewer_1.0-5 scales_0.2.4       calibrate_1.7.2    epiR_0.9-58
##  [9] survival_2.37-7    DoseFinding_0.9-11 mvtnorm_1.0-0      lattice_0.20-29
## [13] drc_2.3-96         plotrix_3.5-7      magic_1.5-6        abind_1.4-0
## [17] MASS_7.3-33        gtools_3.4.1       car_2.0-21         knitr_1.6
##
## loaded via a namespace (and not attached):
## [1] colorspace_1.2-4 evaluate_0.5.5   formatR_1.0      grid_3.1.0
## [5] highr_0.3        munsell_0.4.2    nnet_7.3-8       Rcpp_0.11.2
## [9] tcltk_3.1.0
```

References

[1] Lee, J Jack and Kong, Maiying and Ayers, Gregory D and Lotan, Reuben Mapping and quantifying mammalian transcriptomes by rna-seq. *Journal of biopharmaceutical statistics*, 17(3):461–480, 2007.

[2] Lee, J Jack and Kong, Maiying Confidence intervals of interaction index for assessing multiple drug interaction. *Statistics in biopharmaceutical research*, 1(1):4–17, 2009.

[3] Chou, Ting-Chao and Talalay, Paul Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Advances in enzyme regulation*, 22:27–55, 1984.