SOFTWARE TOOL ARTICLE

IncucyteDRC: An R package for the dose response analysis of live cell imaging data [version 1; peer review: 2 approved]

Philip J. Chapman, Dominic I. James, Amanda J. Watson, Gemma V. Hopkins, Ian D. Waddell, Donald J. Ogilvie

Drug Discovery Unit, Cancer Research UK Manchester Institute, Manchester, UK

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Abstract

We present *IncucyteDRC*, an R package for the analysis of data from live cell imaging cell proliferation experiments carried out on the Essen Biosciences IncuCyte ZOOM instrument. The package provides a simple workflow for summarising data into a form that can be used to calculate dose response curves and EC50 values for small molecule inhibitors. Data from different cell lines, or cell lines grown under different conditions, can be normalised as to their doubling time. A simple graphical web interface, implemented using shiny, is provided for the benefit of non-R users. The software is potentially useful to any research group studying the impact of small molecule inhibitors on cell proliferation using the IncuCyte ZOOM.

Keywords

R package , Drug discovery , Dose response curve , Live cell imaging , Oncology , Shiny

Open Peer Review

Approval Status

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1. **Guillaume Devailly**, University of Edinburgh, Edinburgh, UK

2. **Clara Alsinet-Armengol**, Wellcome Trust Sanger Institute, Hinxton, UK

Any reports and responses or comments on the article can be found at the end of the article.

This article is included in the **RPackage** gateway.

Corresponding author: Philip J. Chapman (phil.chapman@cruk.manchester.ac.uk)

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Introduction

Live cell imaging permits cell proliferation to be monitored in real time over a period of days or weeks, and the IncuCyte ZOOM from Essen Biosciences (<http://www.essenbioscience.com/essen-products/incucyte/>) is a platform used for such experiments. In oncology drug discovery the proliferation of cancer cell lines can be monitored in the presence of different small molecule inhibitors at different concentrations to gain insight as to the efficacy and mechanism of action of novel compounds. This approach provides a useful alternative to measuring cell proliferation as a phenotypic endpoint after a fixed period such as 3 days. It has the advantage of being able to visualise graphically and morphologically when treatments impact on proliferative capacity. Furthermore, vehicle dosed cells can be used to control for population doubling resulting in normalisation across many different cell lines. This may be particularly important when downstream effects of compound treatment require a round of replication before they are apparent.

The IncuCyte ZOOM comes with sophisticated software (<http://www.essenbioscience.com/essen-products/software/incucyte-base-software/>) for the analysis of imaging data and the calculation of metrics such as percent confluence, however we sought a more streamlined workflow for the analysis of data from cell-based compound screening assays. The result was the development of the R¹ package IncucyteDRC which permits quick and easy fitting of dose response curves and calculation of EC50 values, as well as the export of data to standard analysis packages such as GraphPad PRISM <http://www.graphpad.com/scientific-software/prism/> and Dotmatics Studies <http://www.dotmatics.com/products/studies/>. In addition, a graphical user interface was developed as part of the package using Shiny², as well as a novel algorithm for normalizing data by cell doubling time.

Methods

Workflow overview

A basic workflow for the IncucyteDRC package is outlined below:

1. Import data and plate map information
2. Create an IncucyteDRCSet object
3. Fit growth curves to the data
4. Calculate the growth curve values at a given 'cut time'
5. Carry out a dose response analysis using the extracted values

Optionally, the cut time can be calculated automatically for a required number of doublings.

Data import

The `importPlatemapXML` function imports .Platemap files generated by the IncuCyte ZOOM software to define the contents of a plate. The function can extract the following parameters: compound (description, concentration and units), growth condition (description), and cell type (description, passage and seeding density). These are converted into a `data.frame` object which is used as a basis for further analysis. Alternatively, the `importPlatemap` function can import a `data.frame` or a tab delimited text file but these must be in the correct format - see `example(importPlatemap)`.

The `importIncucyteData` function imports data generated by the Incucyte Zoom software, as described in detail in the package vignette entitled 'Exporting From Incucyte Zoom Software'. The output is a `IncucyteDRCPlateData` S3 object.

Creating an IncucyteDRCSet object

The `IncucyteDRCSet` object is at the centre of the package workflow. Subsequent functions add to and operate on the data contained within the object. It can be initiated using the `makeIncucyteDRCSet` function and at its simplest combines a plate map data frame generated by `importPlatemapXML` function with an `IncucyteDRCPlateData` object created by the `importIncucyteData` function. In addition, a cut time can be specified, and a single row metadata `data.frame` provided.

A key attribute of an `IncucyteDRCSet` is that all data share a common control growth curve. Thus it is appropriate to have an `IncucyteDRCSet` object containing multiple compounds on a common cell line background, but if the cell line background changes then one object is needed per condition.

The `splitIncucyteDRCPlateData` function is a convenience function that splits up an `IncucyteDRCPlateData` object into a list of `IncucyteDRCSet` objects if necessary, or a single `IncucyteDRCSet` object if not. It also automatically populates the metadata element. Therefore it is sensible to use this function, rather than the `makeIncucyteDRCSet` function, in a standard workflow.

Fit growth curves

The first step in the analysis process is to fit growth curves to the data using the base R `loess` function. This is done using the related functions `fitGrowthCurvesGrouped` and `fitGrowthCurvesIndividual`. The former combines replicates to create a single growth curve for a given sample and compound concentration, whilst the latter fits a growth curve for every single well on a plate. The `IncucyteDRCSet` object is updated with four `dplyr`³ data frames (`tbl_df` objects) containing the models themselves and the fitted data for plotting. Refer to the `dplyr`³ package documentation for more information on how models and other objects can be stored in data frames.

Once the growth curves have been fitted, they can be visualised using the `plotIncucyteDRCSet` function (Figure 1).

Calculate growth curve values at a cut time

Although the growth curves themselves can give some insight into the effect that different compounds have on the growth of the cell line, to analyse this formally the growth parameter can be determined at a specific 'cut time' using the `calculateDRCData` function. This data is stored in the `IncucyteDRCSet` object and can subsequently be exported to various formats using the `exportDRCDataToDataFrame`, `exportDRCDataToPRISM` and `exportDRCDataToDotmatics` functions. These data can then form the basis of a dose response analysis to generate an EC50 value.

Figure 1. Visualisation of individual growth curves for the data in an `IncucyteDRCSet` object: x-axis is elapsed time in hours, y-axis is the growth value (% confluence in this case). The cut time is represented as a blue dashed vertical line.

Carry out a dose response analysis

It is advisable to export the dose response data and fit the curves in specialist software such as GraphPad PRISM or Dotmatics Studies, but for convenience there are also functions that utilise the `drc` package⁴ to provide an end-to-end workflow. The `fitDoseResponseCurve` function generates models for each compound in the `IncucyteDRCSet` (using the `drc::LL.4` function) and `calculateEC50` predicts an EC50 value for each model (using the `drc::EC50` function). The `exportEC50Data` function generates a `data.frame` containing the values stored in the `IncucyteDRCSet` object, whilst the dose response curves can be visualised with the `plotDoseResponseCurve` function.

Calculation of the cut time

In the previous examples, a cut time has been manually provided following an inspection of the growth curves. Typically the cut time should be as long as possible to allow differences to become pronounced, but still during the exponential phase of cell growth before the cells become confluent. However, sometimes it might be desirable to determine the cut time for a given number of doublings, so that the EC50 for fast and slow growing cell lines can be normalised.

The `calculateCutTimeForIDRCSet` function can be run on an `IncucyteDRCSet` object to determine the optimal cut time

from the control (vehicle treated) growth curves given a set of parameters. These include `baseline_time` (the timepoint to take as a baseline for calculating doublings), `no_doublings` (the number of doublings required) and `max_val` (the maximum allowable growth curve value). The algorithm works out the growth curve value at the baseline time and what value would correspond to a given number of doublings from this point, then returns the timepoint at which this value is achieved. If this value exceeds the maximum value, then the time at which the maximum value is returned instead, along with the actual number of doublings achieved.

In detail, the growth curve is truncated by removing its non-exponential phase along with any part exceeding the user defined maximum (usually 80%). The non-exponential phase is defined as the part of the curve after the timepoint at which the $n=30$ moving average of the second differences of the growth values reaches its minimum. This corresponds to the time at which the rate of cell growth is slowing most rapidly. The growth value required to reach the desired number of doublings from a user defined baseline time was calculated (using the `predict` function on the loess model object), and the elapsed time to reach this is returned as the cut time. If the required growth value is not reached on the truncated growth curve, then the maximum growth value achieved on the truncated curve is returned instead along with the actual number of doublings that occurred.

Figure 2. Visualisation of an individual dose response curve for a compound with the EC50 represented as a red dashed vertical line. The y-axis is the growth value (percent confluence in this case) whilst the x-axis is the micromolar compound concentration.

Figure 3. Control growth curve value (upper plot) and moving average of second differences (lower plot) used to calculate the cut time with elapsed time in hours on the x-axis. The black dashed lines represent user-defined parameters whilst the blue dashed lines are calculated by the algorithm. The blue vertical dashed line is the cut time.

Operation

The IncucyteDRC R package is available on CRAN and is built on top of R version 3.2.3¹ and Shiny 0.13.2² and has no special R dependencies beyond packages that are available on CRAN and so should work on any operating system supported by R. A full list of R package dependencies is present in the Description file of the package.

The package can be installed from CRAN:

```
install.packages('IncucyteDRC')
```

The latest development version can be installed in an R session from GitHub using the devtools⁵ package:

```
devtools::install_github('chapmandu2/IncucyteDRC')
```

Having successfully installed the IncucyteDRC package, it can be loaded using `library('IncucyteDRC')` and a detailed vignette is available which can be viewed by typing

`browseVignettes('IncucyteDRC')`. Help and examples are available for functions within the package e.g.

```
?importIncucyteData
```

```
example(importIncucyteData)
```

Finally, a Shiny app² provides a graphical user interface for the functionality within the package. This can be launched from within R using `shinyVisApp()` whilst details for how to set up the IncucyteDRC package as a Shiny app on a Shiny server is included in the vignette.

Use cases

The package vignette contains detailed use cases using two example datasets. These can be loaded by running the examples for the `importPlatemapXML` and `importIncucyteData` functions:

```
example('importPlatemapXML')
```

```
example('importIncucyteData')
```

Figure 4. Screenshot of the Shiny web application that allows non-R users to run the IncucyteDRC workflow.

Summary

The IncucyteDRC R package provides a simple, reproducible workflow for the dose response analysis of live cell imaging data from the IncuCyte Zoom instrument. To facilitate its use by non-R users, a comprehensive and user-friendly graphical interface is provided as a Shiny² web application. The software is freely available on CRAN and is potentially useful to any research group studying the impact of small molecule inhibitors on cell proliferation.

Software availability

1. Software available from: <https://cran.r-project.org/web/packages/IncucyteDRC/>
2. Latest source code: <https://github.com/chapmandu2/IncucyteDRC/>
3. Archived source code as at time of publication: <http://dx.doi.org/10.5281/zenodo.512606>
4. License: GPL-2

Author contributions

PJC conceived and wrote the software and wrote the manuscript. DJ, MW, GH designed and executed experiments, analysed data, and provided feedback on functionality. DJ, IW, DO conceived the cell doubling normalisation concept. All authors helped review the manuscript.

Competing interests

No competing interests were disclosed.

Grant information

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Clara Alsinet-Armengol

Wellcome Trust Sanger Institute, Hinxton, UK

The authors have developed an R package to visualise drug response data and make some basic calculations from experiments performed on the Incucyte ZOOM system. The functions are easy to use and, interestingly, the useful IncucyteDRCSet object has been designed so that it can progressively integrate all data generated. Both the article and the R documentation are clearly explained.

I would just make one suggestion on the manuscript. One of the features of the software is that it allows for normalization of the drug response by cell doubling time. A brief description of its relevance could be added.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 03 August 2016

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Guillaume Devailly

Department of Developmental Biology, The Roslin Institute, University of Edinburgh, Edinburgh, UK

P. Chapman *et al.* developed an R package to analyse data obtained from the Incucyte instrument,

and specifically growth curve responses to various treatment. While one could interpret Incucyte data without this package, or without any programming skills at all, there is no doubt that Incucyte users knowing R, or intensive Incucyte users wishing to automatize their analysis, will greatly benefit from using it. Overall the package is working well, is easily available through the popular CRAN repository, is well documented, with notably nice vignette, a video tutorial and a companion shiny app. The present manuscript present this package appropriately.

I have no major concern with it, and only small comments and suggestions for improvement. About the manuscript:

1. Package availability on CRAN could be mentioned in the abstract.
2. Authors could detail the reasons why they advise against using their package for carrying dose response analysis, in favour of alternative software.
3. Page 2: "A key attribute of an IncucyteDRCSet is that all data share a common control growth curve." I was a bit confused at first as I interpreted this as the non-support of replicates for the negative control. It could be mentioned that the control growth curve can (and should) come from multiple replicated control wells.
4. Since the public shiny app is now available, it could be mentioned in the manuscript, and even in the abstract, if the authors are willing to support it for a few years.

About the package (I tested only the CRAN version IncucyteDRC_0.5.4, what follows might not apply to future version, or to even to the github version.):

1. The importPlatemap and importPlatemapXML functions has a default value "DMSO" as a control_cpd parameter. I feel this is a terrible idea as users might at first not be aware of this crucial parameter. Not everyone use DMSO as a control, and some silly users might even have named it "dms0" in their incucyte data. Notably it is not used (left as default) in the first usage of the importPlatemapXML function in the "Overview of the IncucyteDRC Package" vignette. I would advise to not use any default value for this parameter and throw an error if it is missing.
2. The importIncucyteData helps mentioned that you wanted to add little more details about it, you might want to have a look ("NEED MORE DOCUMENTATION HERE ABOUT HOW TO DO EXPORT!!").
3. The help pages for functions might benefit form a "suggest/see also" section (i.e. linking back the various import and export functions).
4. The IncucyteDRCSetList object is quite useless at the moment. Could you overload the fitGrowthCurvesGrouped, fitGrowthCurvesIndividual, calculateCutTimeForIDRCSet, calculateDRCData, fitDoseResponseCurve, and calculateEC50 function so that when provided with an IncucyteDRCSetList object, they return an IncucyteDRCSetList object in which each IncucyteDRCSet object has been modified? That would be a bit more convenient for the user than the apply method you suggest in the vignette.
5. I got an error with the local version of the shiny app when testing it with the example 2 files (Error in match.arg: 'arg' must be NULL or a character vector). Since it does not affect the shinyapps.io version, I guess it might be already fixed in github, or it might be a local issue.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Comments on this article

Version 1

Author Response 22 Jul 2016

Phil Chapman, Cancer Research UK Manchester Institute, Manchester, UK

Please note that there is now a hosted version of the shiny app that avoids the need to install the R package.

https://cruk-mi-ddu.shinyapps.io/IncucyteDRC_app2/

Troubleshooting advice on installing the R package is available on the IncucyteDRC GitHub page:

<https://github.com/chapmandu2/IncucyteDRC/wiki/Installation-Issues>

Competing Interests: No competing interests were disclosed.

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