

Image Analysis with R: a Review

Stefan Rödiger, Hinrich Winther and Michał Burdukiewicz

Abstract Management, display, and processing of biological and medical imaging data is an important task in life sciences and medical research. R is a powerful cross-platform statistical computing environment. The aim of this mini-review is to give a brief overview about image processing software for the R statistical computing environment. When it comes to image analysis, R may appear to provide only few tools on the first sight. However, a systematic analysis of the existing packages shows that a huge potential for numerous applications.

Introduction

- Digital image processing?
- Where is it used?

For testing purposes it is possible to make use of public image repositories as listed by [Eliceiri et al. \(2012\)](#)

There are numerous software tools that have been made available for digital image acquisition and processing ([Wiesmann et al., 2015](#); [Chieco et al., 2013](#); [Eliceiri et al., 2012](#)). The scientific background of the users is deliberately broad. This includes biologists, biostatisticians, physicians and others, who fundamentally make use of the similar image analysis techniques. From discussion with peers we learned that knowledge of image processing is gained by self-study. In particular, learning of several programming languages may hamper the scientist to focus on their scientific aim. R ([R Core Team, 2012](#)) is *de facto* the *lingua franca* of statistical bioinformatics and therefore used in numerous research disciplines ([Rödiger et al., 2015a](#)). It is a powerful tool for statistical data analysis. It comes to no surprise that software packages for digital image processing have been implemented ([Frery and Perciano, 2013](#)). In this review, we give a current overview of the R ecosystem about which software packages exist and which deficits they may expose in comparison to other software packages. We aimed to aggregate information about R packages available on CRAN, Bioconductor ([Gentleman et al., 2004](#)), RForge or github.

Analysis of 2D and 3D digital images is a bridge technology which has been used to unravel quantitative and qualitative gene expression data (mRNA and protein level), cellular interactions and diagnostic data. In particular, immunofluorescence images were analyzed in relation to cell structures, tissues and organs ([Chieco et al., 2013](#); [Rödiger et al., 2013](#); [Schierack et al., 2014](#); [Willitzki et al., 2012](#)).

We performed two image processing case studies where we applied selected packages for immunofluorescence image analysis and RMI data.

Pixels and voxels are point samples in a grid pattern.

There are numerous software packages for the analysis of image data ([Wiesmann et al., 2015](#)). However, R is quite functional when it comes to digital image analysis.

Image processing capabilities of Cell-ID and data analysis by the statistical programming framework R for quantifying various cellular features (e.g., volume, total and subcellular fluorescence localization) from sets of microscope images of individual cells ([Bush et al., 2012](#))

Give me a title

General image processing and analysis ([Ljosa and Carpenter, 2009](#))

An 3D spatial distribution analysis of biomarkers such as 53BP1, phosphorylated ATM, and γ H2AX is important for an image-based modeling of dynamic redistribution of DNA damage into nuclear sub-domains ([Costes et al., 2007](#)). The biomolecules (proteins, RNA, DNA) distribution patterns within are complex. Patterns range from diffuse to punctate or microspeckled ([Shiels et al., 2007](#); [Willitzki et al., 2012](#)). However, they all work in a coordinated and controlled manner within the nucleus ([Shiels et al., 2007](#))

([Tabelow et al., 2012, 2014](#))

Murrel ([Murrell, 2011](#)) **mmand** ([Clayden, 2016](#))

CRAN provides well established packages. These are **jpeg**, **png** and **tiff** to read, write and display bitmap JPEG, PNG and TIFF images. The development of the **ripa** ([Perciano and Frery, 2014](#)) package was started in 2005 by Talita Perciano. This package can be used to process and analyse RGB, LAN (multispectral) and AVIRIS (hyperspectral) images. Recent advances of **ripa** make it a promising

tool for analysis of large datasets. The vast amount of image data is becoming more and more and essential part of Big Data analysis pipelines. R is among the frequently used for data mining and analysis. It comes to no surprise the commercial and non-commercial entities make heavy use of R (Chen et al., 2014). **EBImage** (Pau et al., 2010) is presumably the most comprehensive package and the foundation for many other R packages in the context of microscopy-based cellular assays (Gowen, 2015). This package offers tools to transform (e.g, rotate) the images, segment object (e.g., cells) and extract quantitative descriptors. The early version of **EBImage** used the Magick++ interface to the ImageMagick image processing library (Sklyar and Huber, 2006).

A recent package addition to this field of research is **imager** (Barthelme and Cecchi, 2016).

General image processing and analysis

This section may contain a figure such as Figure 1.

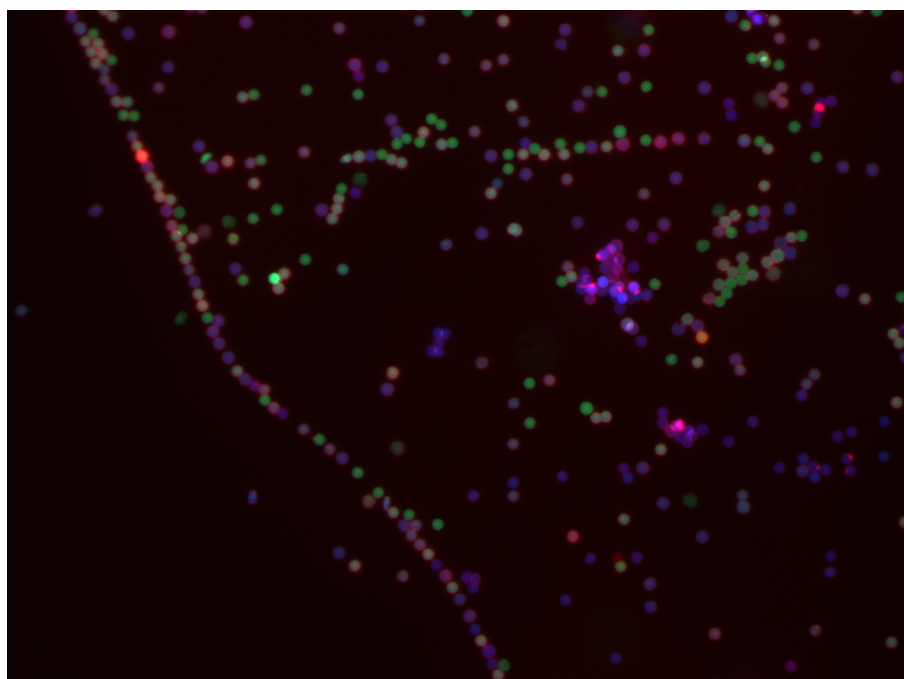


Figure 1: Image of beads.

segmentation

(Holmes et al., 2009)

adimpro is a package for manipulation of digital images and the Propagation Separation approach for smoothing digital images (Polzehl and Tabelow, 2007a). For example, image analysis is used for the detection and quantification of cell patterns and array technologies like microarrays and bead-based assays (Rödiger et al., 2013; Willitzki et al., 2012, 2013; Dunning et al., 2006). Several software packages have been developed. **imageJ** belongs to the most used and cited tools. When it comes to R numerous packages exist, which can be readily integrated in the analysis routines (Frery and Perciano, 2013).

The accuracy of image segmentation is a critical step in a computer-aided diagnosis systems. The recognition of mitotic cells and the classification of fluorescent patterns is heavily dependent on this. Immunofluorescent images of cell, such as Hep-2, exhibit a high variability due a wide range of staining patterns and intensity levels (FIGURES OF CELLS), the presence of mitotic cells and artifacts. The later may be caused by uneven illumination and photo-bleaching effects (Tonti et al., 2015).

Intensity inhomogeneity (bias field) is a common artefact in magnetic resonance (MR) images, which hinders successful automatic segmentation. (Ivanovska et al., 2016)

Applications

Examples

Statistical analysis of functional magnetic resonance imaging data

Statistical analysis of functional magnetic resonance imaging (fMRI) data is a non-invasive neuroimaging technique. R has a impressive number of packages for such analysis (Tabelow et al., 2011).

largely used in clinical routine and advanced brain research

AnalyzeFMRI (Marchini, 2002; Bordier et al., 2011) and **fmri** (Polzehl and Tabelow, 2007b) and are packages for the analysis of Magnetic Resonance Imaging (MRI) and functional Magnetic Resonance Imaging (fMRI) data, respectively.

PET (Philipsen, 2010)

Others include **dcmriS4** (Dunning et al., 2006; Frery and Perciano, 2013).

Analysis of fluorescence image data

CRImage package (Failmezger et al., 2012) for tumor image analysis

gitter, for quantification of colony sizes from plate images (Wagih and Parts, 2014)

Analysis of microbead assays

(Rödiger et al., 2014; Rödiger et al., 2013)

Detailed installation instructions are available at

<http://bioconductor.org/packages/EBImage/>

Install the EBImage from Bioconductor

source("http://bioconductor.org/biocLite.R")

biocLite("EBImage")

Load the EBImage package

require(EBImage)

Graphical User Interface for Digital Image Analysis

There exist also R graphical user interfaces (Rödiger et al., 2012) which can be used for image processing. Bio7 is an integrated development environment based on the Eclipse Rich Client Platform (RCP). The main purpose of this tool is the modeling and analysis of ecological systems. However, Bio7 is not restricted to this discipline. The application contains GUIs and plugins for simulation and analysis tasks. Interestingly, one of these plugins is an adaption image application ImageJ and another is available for a bidirectional Java connection to R. This means that data can be transferred from and to ImageJ and R.

Performance

Michał, would you like to take this section?

Requirements for recent research include the rapid processing of massive amounts of image data (Mega to Tera byte scale) that modern technologies (e.g., microscopes, MRI scanner) produce nowadays. Preferably, affordable personal desktop computers should be usable. R has several disadvantages when it comes to memory management and GPU and CPU usage ...

Summary

Many scientist are using R. However, it appears that only few make use of the image analysis tools currently available. We would like to raise awareness for the fact that R provides sophisticated

Package	Main function	Comment	Source
adimpro	×	×	×
AnalyzeFMRI	×	×	×
CRImage	×	×	Bioconductor
dcmriS4	×	×	CRAN
EBImage	fancy stuff	well maintained	Bioconductor
gitter	×	based on EBImage	CRAN
imager	×	×	CRAN
jpeg	×	×	CRAN
PET	×	×	CRAN
png	×	×	CRAN
ripa	×	×	×
tiff	×	×	CRAN
<i>videoTools</i>	video and image analysis	-	RForge

Table 1: R packages. *videoTools*: <http://www.rforge.net/videoTools/files/>.

packages for digital image analysis. The central advantage is that all analysis is conducted within the same environment across most platforms. Added values for the user are that there is less need to learn a new programming languages and that all analysis can be performed in a consistent and cross-platform environment. Table 1 gives an overview of R packages currently available. We found that functions from the packages can be easily combined to conduct manipulations and analysis (e.g., object transformations, measurements, object counting, gray level statistics, binarization) at advanced levels. The organization of such functions in customized packages is straightforward even for more complex analysis functions.

(Rödiger et al., 2015b) or combined assays of microbeads and cells (Scholz et al., 2015; Grossmann et al., 2016)

Automated image acquisition systems enable microscopy experiments that generate large image datasets. Therefore it is important to have robust, objective and quantitative image analysis systems. Since most image analysis systems are developed for a specific type of experiment, cell type or imaging technology it is challenging to find one tool that fits all needs by manual adaption of all parameters for a specific image analysis task. Pattern recognition is a useful tool in this context. This can be achieved by trained algorithms as described by Shamir et al. (2010).

While evaluation the packages we ended up with experiences regarding the installation and maintenance. Basically all installation were well instructed. For example, the installation of sophisticated packages like **EBImage** is well documented. However, many packages depend on a number of third-party packages. This applies also to **EBImage** which requires tiff and fftwtools. Similar applies to **imager** which required the installation of fftw3 for computing Fast Fourier Transforms.

Others and we recommend to make use of packages for reproducible research (Rödiger et al., 2015a).

There exist several GUI technologies for R which make it possible to integrate R code into an easy to master point-and-click interface.

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Stefan Rödiger (corresponding author)
orcid.org/0000-0002-1441-6512
Faculty of Natural Sciences
Brandenburg University of Technology Cottbus–Senftenberg
Senftenberg
Germany
Stefan.Roediger@b-tu.de

Hinrich Winther
Affiliation
Address
Country
author@work

Michał Burdukiewicz
University of Wrocław
Faculty of Biotechnology
Department of Genomics
Wrocław
Poland michalburdukiewicz@gmail.com