biopixR: Extracting Insights from Biological Images

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Summary

Imaging is crucial for data acquisition in biological laboratories and the broader field of life sciences. It provides essential insights into cells (Rödiger et al. 2018; Schneider et al. 2019), biomarkers (Vafajoo et al. 2018), stress responses (Korkmaz, Debelec Butuner, and Roggenbuck 2018), and gene expression (Brenner et al. 2000), which are necessary for diagnostics and predicting disease outcomes. Microbead technology is a highly promising field for gathering complex information in a straightforward, rapid, and flexible manner (Rödiger et al. 2014). These assays that use microbeads rely on visualization methods, such as microscopy (Ding et al. 2020). Therefore, it is necessary to have user-friendly image processing software to effectively analyze the data.

`biopixR` is an `R` package (R Core Team 2023a) that utilizes the `imager` (Barthelmé and Tschumperlé 2019) and `magick` (Ooms 2024) packages to perform various image processing tasks, such as preprocessing, object counting, feature extraction, and filtering. It is designed for medium-throughput analysis and generates log files to track the analytical process in detail. Core functionality of `biopixR` was used in a recent study (Geithe et al. 2024) to perform quality control on microbeads. `biopixR` can extract various types of information, including *object size*, *quantity*, *shape* and *intensity*. The package includes an algorithm that fills gaps between lines and reconnects loose ends, making complex images accessible for later analysis.

Statement of need

Extracting valuable information from complex biological images in a quick, reliable, and straightforward manner presents a significant challenge. A critical aspect of this process is the quantification of objects within the images.

Quantification is crucial for deriving biological insights from experiments, as demonstrated in bead-assays. This method is highly promising due to its extensive multiplexing abilities, high-throughput capacity, shortened analysis time, and minimal sample requirements (Rödiger et al. 2014). Detection methods for microbeads include flow cytometry, microfluidics, and image-based techniques (Choi et al. 2019). A user-friendly tool integrated into R could be highly beneficial for comprehensive analysis, particularly when combined with statistical analysis and visualization techniques. Advancements in smart device imaging and chip development for bead-assays are improving the use of microbeads in point-of-care testing (POCT) and disease diagnosis (Dinter et al. 2019; Zhang et al. 2019). This progress underscores the need for direct extraction of biological information from images, eliminating the dependence on complex laboratory equipment. Additionally, software capable of extracting attributes like fluorescence intensity, size, and shape enables the encoding of beads (Zhang et al. 2019). This capability makes it possible to differentiate between populations, thus significantly enhancing the scope for multiplexing.

Bead-based ePCR assays, like other bead-based assays, are often analyzed using fluorescence activated cell sorting (FACS) (Fraser et al. 2015). However, this software could enable the analysis of bead-based ePCR through imaging techniques, as droplets can be imaged and altered to appear distinct by closing gaps between

their contours. The package could be applied in various domains, including wastewater assessment, to investigate the presence of microplastics in water (Ding et al. 2020). It could also be used in microbead-based drug delivery systems to facilitate real-time detection and localization (Bannerman and Wan 2016).

Therefore, the `biopixR` package for `R` is needed, as it automates the evaluation process and enables medium-throughput analysis directly from images, simplifying analytical procedures and opening up new experimental possibilities.

Software engineering

`biopixR` (0.2.4, LGLP-3.0license) is an `R` package (S3 class systen). The choice of `R` as a programming language for image processing is based on its open-source framework, advanced packages such as `imager` (Barthelmé and Tschumperlé 2019) and `magick` (Ooms 2024), and its strong support for reproducible research (Xie 2019). These features collectively provide a sophisticated environment for image analysis and editing, with the added advantage of community-driven improvements (Chambers 2008). `R`'s integration of analysis with documentation ensures methodological precision and transparency in scientific research, making it a preferred choice for complex image processing tasks. The `biopixR` package underwent quality control through unit testing using the `testthat` package (Wickham 2011), ensuring its reliability.

Functions

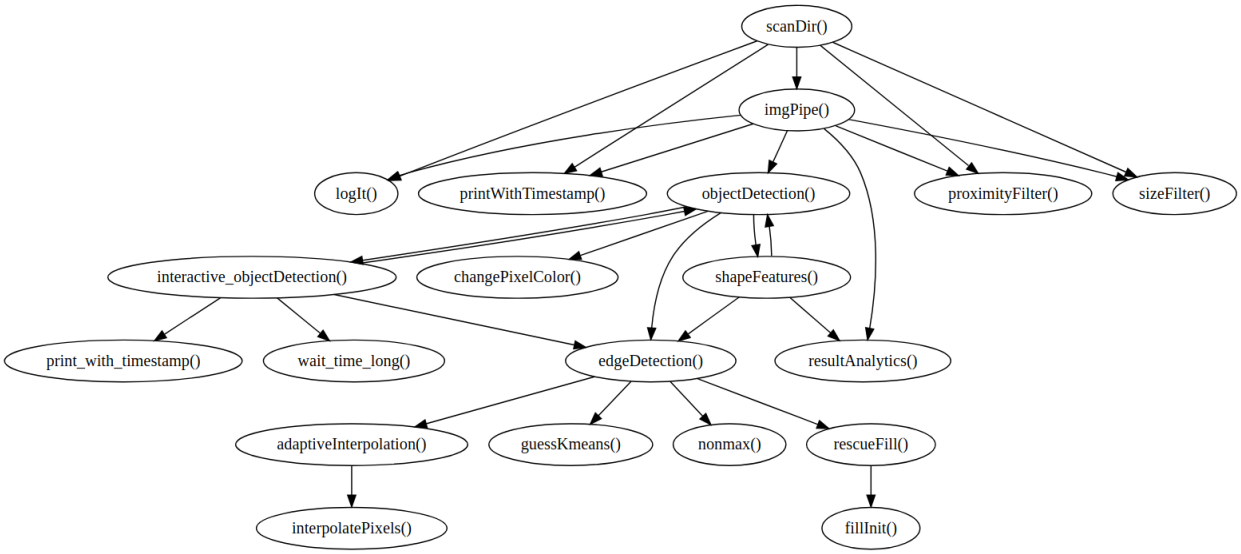

Figure 1: Dependency graph of the functions present in the `biopixR` package. Showing the levels of complexity by showing the descendants and ancestors of the `imgPipe()` function. The figure was created using the `foodwebr` package from Appleton-Fox (2022) (package version 0.1.1, RStudio 2023.09.0+463, R 4.3.2 on Linux, Ubuntu 22.04.3 LTS).

The `biopixR` package in R is intended for analyzing bioimage data, with a specific focus on the analysis and characterization of bead microparticles. The package provides tools for image preprocessing, segmentation, feature extraction, and visualization. It supports automation for medium-throughput analysis, utilizing algorithms to identify spherical objects, extract their features, and implement interactive tools for threshold and smoothing factor selection. Furthermore, it offers features for removing clumped or closely positioned particles to prevent inaccurate results, with the goal of improving the analysis of microparticles in diverse scientific disciplines.

In addition to the `shapeFeatures()` function, capable of extracting shape-related information from detected objects and grouping them using the SOM (Self-Organizing Map) algorithm (Wehrens and Buydens 2007), there's also the `imgPipe()` function. This latter function serves as a comprehensive pipeline for image analysis, offering a variety of selectable functions:

- `importImage()`, a wrapper import function combining the `imager` (Barthelmé and Tschumperlé 2019) and `magick` (Ooms 2024) packages.
- `edgeDetection()`, a combination of a Canny edge detector and gap filling (Barthelmé and Tschumperlé 2019),
- `objectDetection()`, detects objects in an image by identifying their coordinates,
- `sizeFilter()`, eliminates objects that exceed or fall below a certain size threshold,
- `proximityFilter()`, filters objects that are in close proximity to each other,
- `resultAnalytics()`, summarizes the extracted features in a clear and concise manner.

The `biopixR` package includes functions for analyzing entire directories, allowing for medium-throughput analysis. Making feature extraction and image clustering easily accessible:

- `haralickCluster()`, extracts Haralick features and cluster using PAM (Partitioning Around Medoids) (Haralick, Shanmugam, and Dinstein 1973; Carlson 2018; Maechler et al. 2023),
- `scanDir()`, utilizing the pipeline for whole directory analysis (under development).

The `fillLineGaps()` algorithm, along with helper functions:

- `interpolatePixels()`, calculates the coordinates required to connect two given points,
- `adaptiveInterpolation()`, searches a given radius surrounding a line end for contours and connects them,

addresses the issue of discontinuous edges by iteratively scanning for line ends within the image and reconnecting them to adjacent contours.

Examples demonstrating the use of Brauckhoff, Rödiger, and Kieffer (2024) for image analysis tasks can be found in the package's vignette.

Graphical User Interface:

In this section, we aim to provide a concise outlook of the `biopixR` package, emphasizing its broader applicability beyond microbead detection. The `biopixR` package is adaptable and can be utilized in any research field where the identification of distinct objects in images can be achieved through the use of a Canny edge detector or thresholding. This encompasses research areas such as foci detection, microplastic, and plant seeds. The automation methods employed in `biopixR` are predicated on the assumption of circular objects, rendering it particularly well-suited for the detection, quantification, and extraction of useful information from circular objects within images. Another integrated tool is an interactive function that assists the user in selecting the optimal input for their analysis. The function `interactive_objectDetection()` initiates a graphical user interface (GUI) that utilizes the Tcl/Tk framework (R Core Team 2023b), enabling users to adjust the threshold and smoothing settings of the image.

Current status and outlook

The `biopixR` package was first released on CRAN in March 2024. To ensure code quality, we employed various methodologies, including Continuous Integration (CI), unit testing (Wickham 2011), adherence to naming conventions (Bååth 2012), and the application of style guidelines (Wickham 2019). Although the

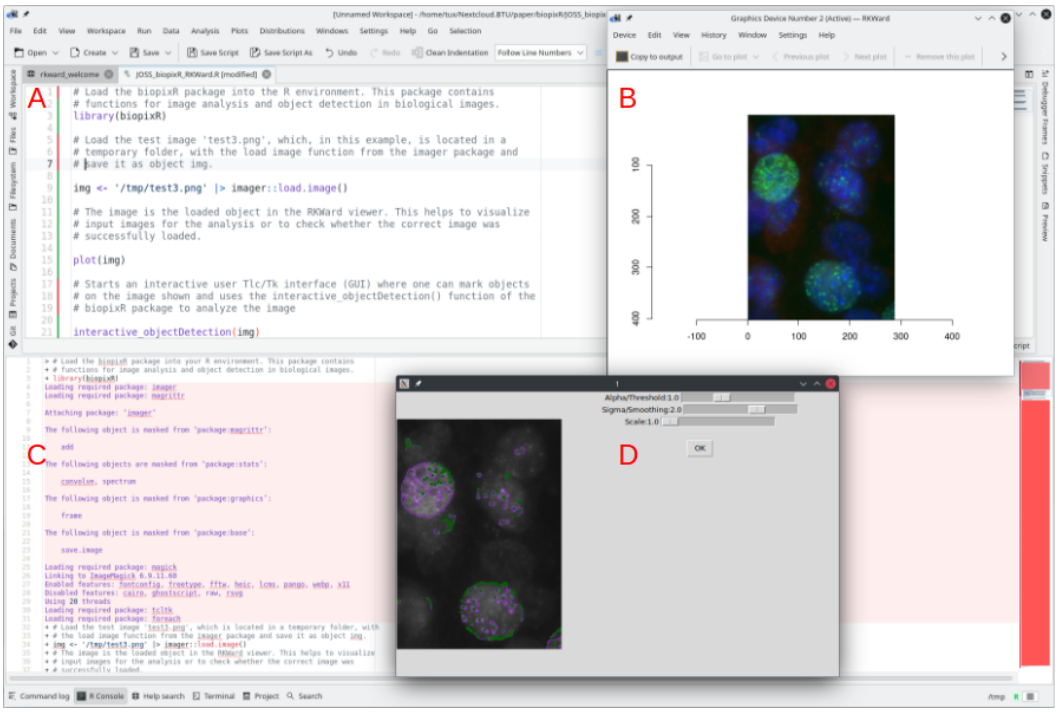

Figure 2: Graphical User Interface for interactive parameter selection. The function `interactive_objectDetection()` provides a simple interface with sliders to adjust threshold, smoothing, and scale. It highlights object contours in purple and centers in green for easy visualization. A) In this example, the GUI was used in RKWard (0.7.5z+0.7.6+devel3, Linux, TUXEDO OS 2, (Rödiger et al. 2012)). With fewer commands, an image can be imported and analyzed. B) The `plot()` function displays the false-color image as a preview. In this figure, cells with DNA damage (similar to Rödiger et al. (2018)) are visible. C) Loading the biopixR package in the R console shows additional information such as loaded libraries and the number of CPU threads (n = 20, parallel processing). D) The rendering process is displayed on the console, including timestamps and current state.

package is relatively new, we are working to expand its features and evaluate its applicability using empirical research data from diverse sources. In addition, future developments will involve expanding its capabilities to identify DNA damage, particularly in the form of foci.

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