

# biopixR: Extracting Insights from Biological Images

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## Summary

**biopixR** is an R package for the analysis of bioimage data. The **biopixR** package contains 15 functions for various tasks including image import, segmentation, clustering, parameter optimization, and line gap mitigation. Among these is an optimized edge detection algorithm based on the Canny algorithm. Users can employ object detection and filtering within a pipeline function. Both Canny edge detection and thresholding can be parameterized via an interactive Tcl/Tk user interface. Additionally, the package includes a function for clustering objects within an image based on their shape features using Self-Organizing Maps (SOM). For circular objects, threshold and smoothing parameters for object detection can be automatically calculated using Gaussian process regression models (Kriging models).

Additionally, **biopixR** provides unique datasets of microbead images and microbeads in water-oil emulsions. Designed for medium-throughput analysis, it generates detailed log files to track the analytical process. The core functionality of **biopixR** was recently utilized in a 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. It 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.

## Statement of need

Imaging plays a pivotal role in data acquisition within biological laboratories and the broader life sciences. It offers crucial 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 vital for diagnostics and disease prediction. Microbead technology, known for its rapid and flexible data collection, relies heavily on imaging techniques like microscopy (Rödiger et al. 2014; Ding et al. 2020). The challenge lies in the fast and accurate extraction of valuable information from complex biological images, particularly in the quantification of objects within these images.

The quantification of biological image data is a fundamental aspect of deriving insights into biological processes. This is exemplified by bead-assays, which offer extensive multiplexing abilities, high-throughput capacity, reduced analysis time, and minimal sample requirements (Rödiger et al. 2014). Methods for detecting microbeads include flow cytometry, microfluidics, and image-based techniques (Choi et al. 2019). A user-friendly tool integrated into R could enhance analysis by combining statistical analysis and visualization techniques. The advancement of smart device imaging and chip development is facilitating the utilization of microbeads in point-of-care testing (POCT) and disease diagnosis (Dinter et al. 2019; Zhang et al. 2019). This progress underscores the necessity for the development of efficient methods for extracting biological information from images, thereby eliminating the necessity for the use of complex laboratory equipment. Software that extracts attributes such as fluorescence intensity, size, and shape allows for bead encoding and differentiation between populations, thereby enhancing multiplexing capabilities (Zhang et al. 2019).

Bead-based ePCR assays, which are typically analyzed using fluorescence-activated cell sorting (FACS) (Fraser et al. 2015), could potentially benefit from the use of imaging techniques. As this software is capable of analyzing bead-based ePCR by modifying droplet images to appear distinct. The aforementioned applications extend to the assessment of wastewater for the detection of microplastics (Ding et al. 2020), the real-time localization in microbead-based drug delivery systems (Bannerman and Wan 2016), and other fields of life science, such as cell biology.

Consequently, the **biopixR** package for R is a fundamental tool. The automated evaluation process enables medium-throughput analysis directly from images, simplifying analytical procedures and expanding experimental possibilities.

## Software engineering

**biopixR** (0.2.4, LGPL-3.0license) is an R (R Core Team 2023a) package (S3 class system). 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.

## Installation

The **biopixR** package can be installed from the Comprehensive R Archive Network (CRAN) or GitHub, providing users with stable and developmental versions respectively.

To install the stable version of **biopixR** from CRAN, execute the following command in R:

```
install.packages("biopixR")
```

This command will download and install the latest stable release of the package, ensuring compatibility and reliability.

For users interested in the latest features and ongoing development, the developmental version of **biopixR** is available on GitHub. To install this version, it is first necessary to install the **devtools** package if it is not already present in your R environment:

```
install.package("devtools")
devtools::install_github("Brauckhoff/biopixR")
```

This command enables the installation of the development build from the GitHub repository. This provides access to the most recent features and updates that may not yet be available in the CRAN release.

## Functions

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

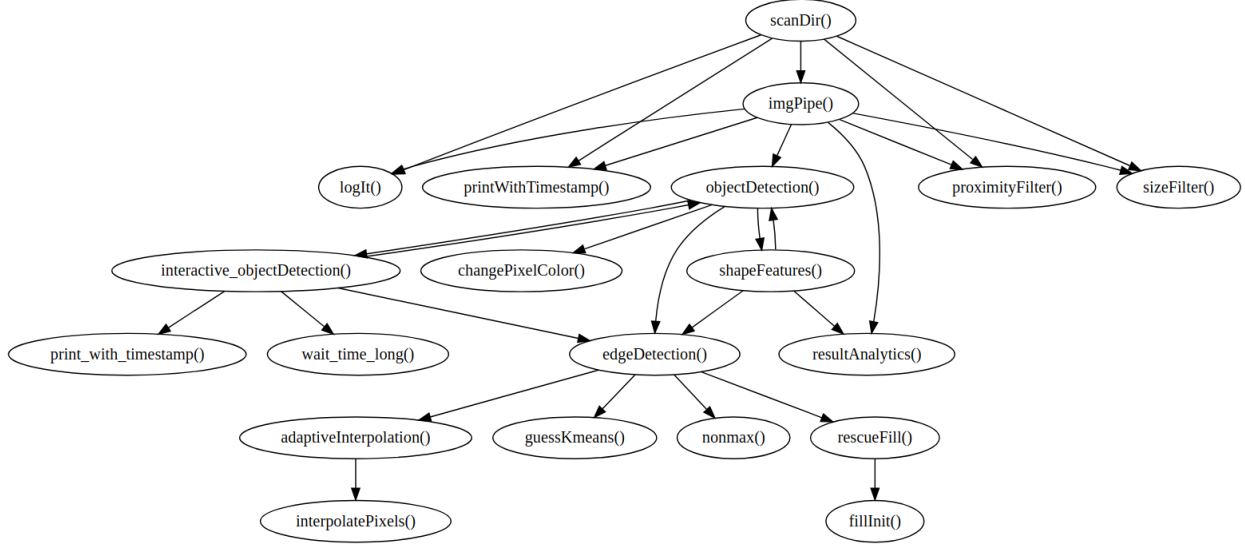


Figure 1: Dependency graph of the functions present in the **biopixR** package. Showing the levels of complexity by showing the descendants and ancestors of the **imgPipe()** function. The figure was created using the **foodwebR** package from Appleton-Fox (2022) (package version 0.1.1, RStudio 2023.09.0+463,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

particles to prevent inaccurate results, with the goal of improving the analysis of microparticles in diverse scientific disciplines.

The core function of **biopixR** is the **imgPipe()** function, which can perform the object detection and apply individual filter. Moreover, it processes three image channels simultaneously to work with false color images. The embedding of this function in the dependency network and the interaction between all other functions is shown in Figure 1.

The **shapeFeatures()** function is 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()**, joins 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) (Figure 2) 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 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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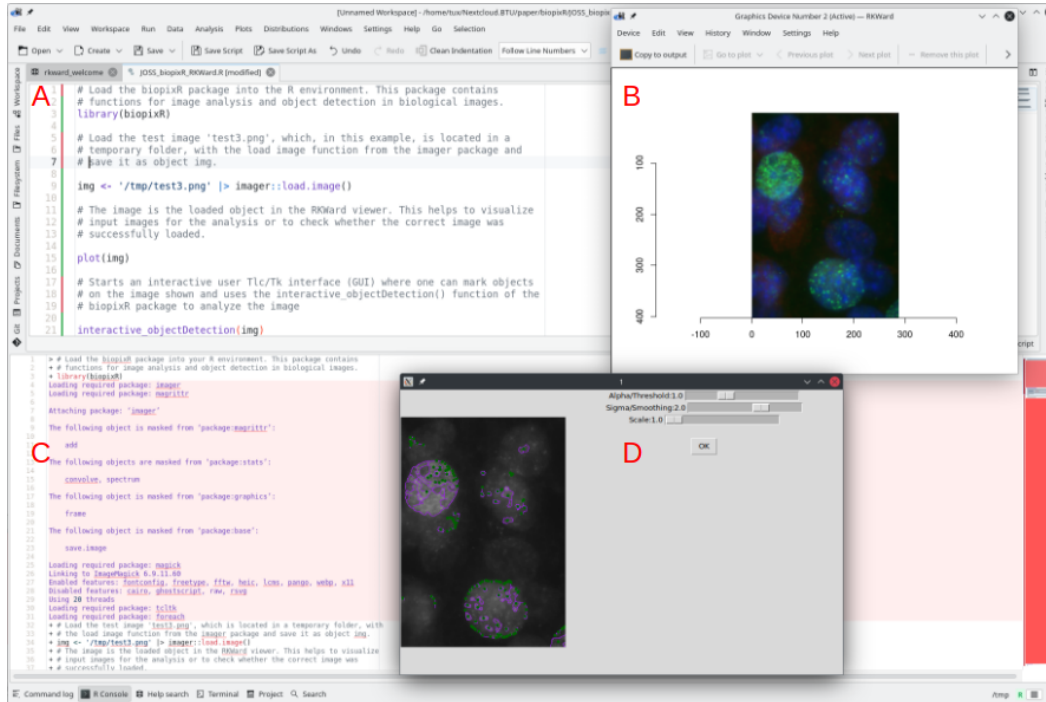


Figure 2: Graphical User Interface for interactive parameter selection. The function `interactive_objectDetection()` provides a simple interface with sliders to adjust threshold, smoothing, and scale. It highlights object contours in purple and centers in green for easy visualization. A) In this example, 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.

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