Check for updates

SOFTWARE TOOL ARTICLE

The Focal Adhesion Analysis Server: a web tool for analyzing focal adhesion dynamics [version 1; peer review: 2 approved]

Matthew E Berginski¹, Shawn M Gomez¹⁻³¹UNC/NCSU Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599-7575, USA²UNC Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599-7575, USA³UNC Department of Computer Science, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599-7575, USA

V1 First published: 04 Mar 2013, 2:68
<https://doi.org/10.12688/f1000research.2-68.v1>
Latest published: 04 Mar 2013, 2:68
<https://doi.org/10.12688/f1000research.2-68.v1>

Abstract

The Focal Adhesion Analysis Server (FAAS) is a web-based implementation of a set of computer vision algorithms designed to quantify the behavior of focal adhesions in cells imaged in 2D cultures. The input consists of one or more images of a labeled focal adhesion protein. The outputs of the system include a range of static and dynamic measurements for the adhesions present in each image as well as how these properties change over time. The user is able to adjust several parameters important for proper focal adhesion identification. This system provides a straightforward tool for the global, unbiased assessment of focal adhesion behavior common in optical microscopy studies. The webserver is available at: <http://faas.bme.unc.edu/>.

Keywords

Focal Adhesion Analysis Server, web too, focal adhesion, dynamics

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

Open Peer Review

Approval Status

	1	2
version 1
04 Mar 2013	view	view

1. **Jonathan Jones**, Northwestern University Medical School, Chicago, IL, USA

2. **Kenneth Yamada** , National Institutes of Health, Bethesda, MD, USA

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

Corresponding author: Shawn M Gomez (smgomez@unc.edu)

Competing interests: No competing interests were disclosed.

Grant information: Funding was provided to SMG by the University Cancer Research Fund (<http://unclineberger.org/ucrf>).
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2013 Berginski ME and Gomez SM. This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Berginski ME and Gomez SM. **The Focal Adhesion Analysis Server: a web tool for analyzing focal adhesion dynamics [version 1; peer review: 2 approved]** F1000Research 2013, 2:68 <https://doi.org/10.12688/f1000research.2-68.v1>

First published: 04 Mar 2013, 2:68 <https://doi.org/10.12688/f1000research.2-68.v1>

Introduction

The quantitative analysis of focal adhesion (FA) structures in motile cells commonly relies on the use of fluorescently tagged protein components and time-lapse fluorescence microscopy. Traditionally, the resulting images are analyzed using NIH ImageJ¹ or related tools, but we have recently developed a set of computer-vision algorithms designed to automate many of these analysis steps. These core methods have been documented in a prior publication² and made available as an open source download; however, they require substantial expertise with the command line interface for their use. With the focal adhesion analysis server (FAAS), we have created a web application that allows users to submit time-lapse fluorescence image sets of FA proteins and have these images automatically analyzed.

The methods implemented by the analysis system have been previously used in several studies to investigate the quantitative properties of FAs in cells under various conditions. For example, adhesion static and dynamic properties were quantified with fluorescently labeled FAK, vinculin and paxillin³⁻⁵. Global, whole-cell changes to adhesion and cytoskeletal architecture when the Arp 2/3 complex is disabled have also been characterized⁴. By integrating these image analysis methods into a straightforward web application, we hope to make them more broadly accessible to the cell-imaging community.

Features

The primary interface is a set of webpages that allow a user to upload a stacked tiff set of images for processing. After the images are uploaded to the server, the processing pipeline is run, and the results are returned as a downloadable zip file. This results file contains all the intermediate processing steps as well as a set of visualizations. These visualizations show which regions of the cell were identified as adhesions and how the tracking algorithm followed single adhesions through time (Figure 1).

FA properties and visualizations

The analysis pipeline extracts and quantifies a wide range of properties. FA properties characterized in each individual image include adhesion area, marker protein intensity and the lengths of the major and minor axes. In addition to these static properties, the system also collects dynamic adhesion properties, which are quantified by recording the changes in individual adhesions between frames in the image stack. Dynamic properties currently include the FA assembly and disassembly rates⁶ and the focal adhesion alignment index⁴. All of these results are saved in CSV format, which is suitable for import into statistical or graphing software. For users only interested in static results derived from individual images, as in an analysis of a set of fixed-cell images, all the other dynamic properties can be safely ignored.

The user is also provided with two types of visualizations that show either the entire field of view or single adhesions over time. The visualization of the entire field of view is produced for every image in the submitted image set and outlines each adhesion with a unique color (Figure 1). This visualization can be used to verify that the adhesions were correctly detected, segmented and tracked.

The second visualization type shows a single adhesion segmented and tracked through time (Figure 1).

Provided that adhesions are present for at least 10 sequential images, this visualization allows the user to compare an individual FA's properties with the appearance of the adhesion in the original image data. This suite of automatically extracted properties and visualizations enables the user to minimize the amount of laborious manual analysis normally required to quantify FA image sets.

User adjustable parameters

Several of the parameters used to analyze the images can be specified when an image set is submitted for analysis. The most important of these is the threshold used to identify the regions of the image that qualify as FAs versus background. The appropriate threshold

Figure 1. Sample input images and output visualization from the focal adhesion processing pipeline. The results section shows examples from the visualizations produced by the pipeline. In the top example, the entire cell is shown, with an individual adhesion outlined and tracked through time. The bottom three examples show single adhesions, outlined in green, with other nearby adhesions outlined in blue.

will vary depending on the type of cell imaged and the imaging conditions. To make setting this parameter easier, we have added a feature where a single image can be submitted, segmented using various thresholds and then immediately returned to the user for visual inspection of the results obtained when the threshold is varied. The user also has the option to turn off the default watershed-based segmentation that is used to split adjacent FAs and modify the minimum or maximum FA size accepted by the system. Finally, the time between images can also be specified to ensure that the calculation of the rates of assembly or disassembly are made in the correct units.

Users have the option of providing an email address when an image set is submitted. If an email address is provided, notification of the completion of the image processing pipeline, along with a link to download the results, is sent. The system can also be used without an email address, but the user must return to the web interface to check on the status of the processing. The processing time is dependent on the number of images in the set and how many adhesions are detected during processing. Using typical input data, we tested the system throughput and found that the average processing and analysis time per image, under full load, is 13 seconds. Because the system can handle four image sets at once, we expect experimental throughput to be acceptable for everyday usage.

Conclusion

The Focal Adhesion Analysis Server provides an automated image processing pipeline in an easy-to-use web-based application.

A wide range of FA properties are automatically collected from the image sets submitted, and the results are returned in CSV formatted files. Users have the option to adjust the parameters used to process their image sets to suit their specific imaging conditions and cell types of interest.

Author contributions

MEB and SMG conceived the study, MEB coded the web application, MEB and SMG wrote the manuscript. All authors read and approved the manuscript.

Competing interests

No competing interests were disclosed.

Grant information

Funding was provided to SMG by the University Cancer Research Fund (External link: <http://unclineberger.org/ucrf>).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

We would like to thank the initial users of the beta version of the FAAS website, who provided helpful feedback concerning the website interface and results.

References

- Schneider CA, Rasband WS, Eliceiri KW: **NIH Image to ImageJ: 25 years of image analysis.** *Nat Methods*. 2012; **9**(7): 671–675.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Berginski ME, Vitriol EA, Hahn KM, *et al.*: **High-resolution quantification of focal adhesion spatiotemporal dynamics in living cells.** *PLoS One*. 2011; **6**(7): e22025.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Shen K, Tolbert CE, Guilluy C, *et al.*: **The vinculin c-terminal hairpin mediates f-actin bundle formation, focal adhesion, and cell mechanical properties.** *J Biol Chem*. 2011; **286**(52): 45103–45115.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Wu C, Asokan SB, Berginski ME, *et al.*: **Arp2/3 is critical for lamellipodia and response to extracellular matrix cues but is dispensable for chemotaxis.** *Cell*. 2012; **148**(5): 973–987.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chen Z, Lessey E, Berginski ME, *et al.*: **Gleevec, an abl family inhibitor, produces a profound change in cell shape and migration.** *PLoS One*. 2013; **8**(1): e52233.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Webb DJ, Donais K, Whitmore LA, *et al.*: **Fak-src signalling through paxillin, erk and mck regulates adhesion disassembly.** *Nat Cell Biol*. 2004; **6**(2): 154–161.
[PubMed Abstract](#) | [Publisher Full Text](#)

Open Peer Review

Current Peer Review Status:

Version 1

Reviewer Report 07 March 2013

<https://doi.org/10.5256/f1000research.1236.r814>

© 2013 Yamada K. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Kenneth Yamada

Laboratory of Cell & Developmental Biology, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA

This automated web-based analysis system provides a helpful system for considerably simplifying analyses of dynamic focal adhesions of cells in 2D cell culture. Although two examples of the analysis of single adhesions are clear, the example at the bottom left of Figure 1 seems to show appearance of a rather large, complex, multi-pronged cell adhesion that may be a composite of several smaller focal adhesions – it may be useful to discuss this conceptual issue briefly. After further validation and use by other colleagues, this analysis server may become a valuable shared resource for the field.

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 05 March 2013

<https://doi.org/10.5256/f1000research.1236.r810>

© 2013 Jones J. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Jonathan Jones

Department of Cell and Molecular Biology, Northwestern University Medical School, Chicago, IL, USA

The authors have generated a valuable tool for analysing adhesion site dynamics. The article is

clear, logical and focused. This should be a useful resource for the field.

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.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research