

A study on long-term reproducibility of image analysis results on ImageJ and Fiji



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Introduction

Reproducibility is a key requirement for reliable scientific results. While open source and open data is prevalent in the field of BioImage Analysis, using open research software poses some additional challenges: Will the software run on future operating systems? Will a given piece of analysis software deliver the same results also a decade from now? To assess an insight to the status-quo, we performed a retrospective study.



Figure 1: The tested workflow includes thresholding, setting measurements and particle analysis

Materials & Methods

For our study we used ImageJ [1] and Fiji [2] versions listed in Table 1 from the past 18 and 10 years, respectively. All compared versions were run on a recent 64 bit Windows 10. ImageJ versions which came without a launcher, were supplemented by the ImageJ launcher provided on the ImageJ download site [3].

Initially, visual inspection of images from electron microscopy (EM) was compared over the years. Furthermore, a simple analysis workflow consisting of thresholding followed by particle analysis was executed to compare the versions. The workflow is visualised in Figure 1. The workflow was executed once measuring all available image based features on blobs.tif. Afterwards, all versions were tested again with a different image to check independence of the observersion from the dataset. In order to make the analysis comparable, it was necessary to manually set an upper threshold to the maximum in early ImageJ versions until 2004. Later versions of ImageJ and Fiji did so by default.

2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 IJ 1.12 IJ 1.22d IJ 1.26t IJ 1.30v IJ 1.31v IJ 1.34s IJ 1.37v IJ 1.38x IJ 1.39u IJ 1.42q IJ 1.43q IJ 1.44p IJ 1.46r IJ 1.47v IJ 1.48v IJ 1.49r IJ 1.50i IJ 1.51r IJ 1.52a

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Table 1: Full list of tested ImageJ and Fiji versions

Results

Visual inspection of images changed twice over the years as shown in Figure 2 potentially causing differences in manual outlining of objects. Furthermore, the user interface of ImageJ revealed changes over all the years shown in Figure 3. Thus, the code base behind the changing interface was also changed. Quantitative analysis revealed one major change in results appeared in ImageJ in the year 2004 as shown in Table 2. This might be related to one of the numerous changes in the thresholding tool made according to the release notes [4].

Conclusions

Even though high reproducibility was observed in general, it is recommended to archive ImageJ/Fiji executables together with the analysed research data. Furthermore, it is emphasized that proper release note documentation actually allows tracing down roots of software changes over time and thus, such documentation is worth to maintain. These recommendations might be generalized to any kind of research software.

IJ 1.12 IJ 1.43a IJ 1.52a

Figure 2: Visualisation of images changed twice: In 2003, reading the TIF header for the displayed grey value range was introduced. In 2013 visualisation of images displayed less than 100% was improved [4].

We also would like to encourage users and software developers to debate on these challenges in automated and computerized science.

2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018



Figure 3: The changing user interface of thresholding, measurements and particle analysis dialogs suggests underlying code changes over the years.

2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 69.00 393.46 963.42 39.06 188.41 88.00 218.90 123.18 133.76 113.06 71.36 71.36 71.36 118.01 20.48 22.10 24.87 17.16 72.33 64.00 347.55 187.73 20.47 203.38 128.00 129.00 120.29 127.42 120.29 127.42 120.29 127.42 120.29 120.29 120.20 127.42 120.20 10.20 120.2 69.00 393.40 163.42 39.00 188.4 29.00 218.90 129.10 130.7 129.10 130.5 129.10 130.5 129.10 69.00 202.46 163.42 39.05 180.41 80.00 210.90 129.18 130.74 129.15 130.65 71.36 71.36 119.61 20.48 22.10 69.00 393.46 163.42 39.06 188.41 88.00 218.90 129.18 130.74 129.15 130.68 71.36 119.01 20.48 22.10 24.87 17.16 72.33 0.68 64.00 347.53 187.53 28.47 203.30 128.00 230.30 129.00 230.30 120.42 120.42 120.42 120.42 120.42 120.42 120.42 120.42 120.42 145.50 82.03 24.87 15.50 82.03 24.87 15.50 82.03 24.87 15.50 82.03 24.87 15.50 82.03 24.87 15.50 82.03 15.50 82.03 15.50 1 64.00 547.55 187.73 187.73 128.00 120.00 120.20 64.00 167.75 167.75 167.75 128.77 128.77 128.00 120.20 130.29 127.44 130.29 120.22 130.22 127.24 130.29 120.25 64.00 947.55 187.73 187.73 128.47 128.67 128.67 120.20 130.29 120.48 130.29 120.48 130.29 120.48 130.29 120.58 64400 54755 2847 20138 19775 20138 12000 10000 120 64000 347353 18773 20138 12000 12023 12020 20138 12022 20138 12022 20138 12022 20138 12022 20138 12022 20138 12022 20138 12022 20138 12022 20138 12022 20138 12022 20138 12022 20138 12022 20138 12022 20138 12022 1202 347.52 197.72 20.43 20.3 30 120.0 31 120.0 32 127.44 130.22 127.44 130.22 127.44 130.22 127.44 130.22 127.44 130.32 110.64 19.34 21.67 23.97 16.55 0.82 24.87 24.87 24.87 24.97 24. 047.55 107.73 20.38 120.00 220.38 120.02 120.29 127.42 120.58 127.42 120.58 127.42 120.58 120.58 120.59 120.59 120.59 120.55 0100 01100 01107 0117 0117 0117 0117 0120 0110 0120 347 52 187.7 28.43 203.38 128.00 220.38 127.44 130.25 127.44 130.25 127.44 130.25 127.44 130.25 127.44 130.25 127.58 146.64 19.34 21.61 23.50 146.50 82.00 0.73 203.38 120.02 120.38 120.02 127.42 67.90 120.58 150.55 120.58 150.55 120.58 150.56 150.56 150.50 120.50 130.2 127.4 130.2 127.4 67.90 120.51 116.64 19.34 21.61 21.91 16.50 82.02 123.8 131.11 85.31 16.97 1.62 0.71 0.93 85.31 16.97 1.62 0.71 85.31 16.97 1.63 0.71

Table 2: Analysis summary from the Thresholding - Particle Analysis workflow: All fields contain the average values, except the "Count" column. Value changes from year to year are highlighted. The results marked with a * were observerd in a single Fiji version, namely Fiji-win64-20141125. Fiji versiosn before and after that release resulted in the same values while this particular version delivered different values. No such outlier was detected in the 2014 ImageJ version

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for biological-image analysis, Nature methods 9(7): 676-682 [3] ImageJ download website https://imagej.nih.gov/ij/download/ accessed 2018-08-24

[4] Release notes https://imagej.nih.gov/ij/all-notes.html accessed on 2018-11-17

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