

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.

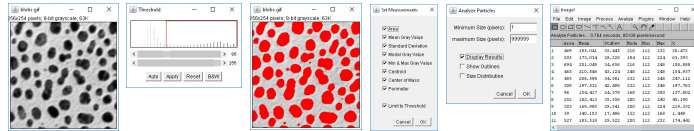


Figure 1: The tested workflow includes thresholding, setting measurements and particle analysis.

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 observation 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
									file-all- 20081016_ Dresden	file-all- 20090116_ Luxembourg	file-all- 20100614_H eidelberg	file-all- 20110307_M adison		file-win64- 20130715	file-win64- 20140902	file-win64- 20151222		file-win64- 20170530	file-win64- 20180904
										file-all- 20090429_ Dresden					file-win64- 20141125				
										file-all- 20091014_ Plzen									

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.

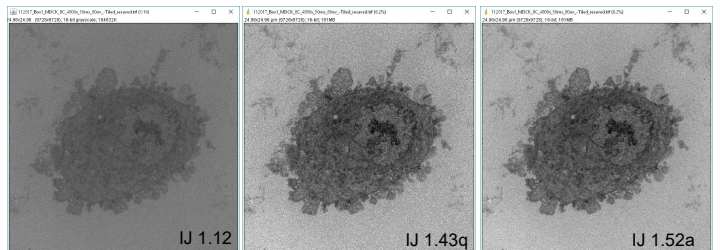


Figure 2: Visualisation of images changed twice: In 2003, reading the TIF header for the displayed grey value range was introduced. In 2013 visualisation of images displayed less than 100% was improved [4].

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

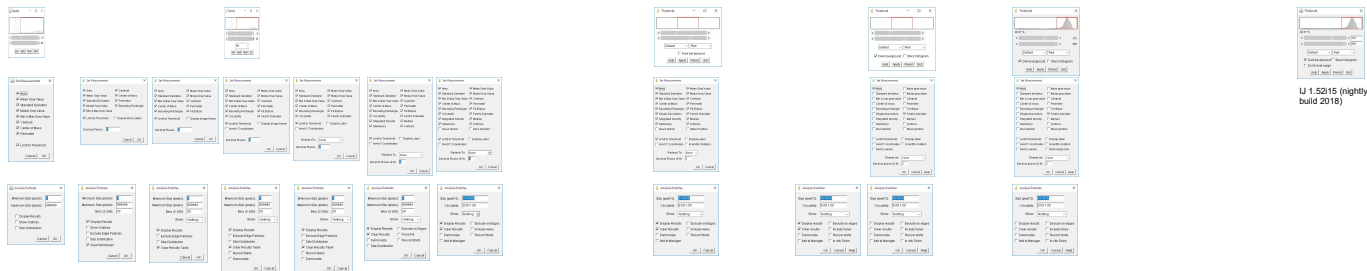


Figure 3: The changing user interface of thresholding, measurements and particle analysis dialogs suggests underlying code changes over the years.

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

Count	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00
Area	363.40	363.40	363.40	363.40	363.40	363.40	363.40	363.40	363.40	363.40	363.40	363.40	363.40	363.40	363.40	363.40	363.40	363.40	363.40
Perimeter	102.47	102.47	102.47	102.47	102.47	102.47	102.47	102.47	102.47	102.47	102.47	102.47	102.47	102.47	102.47	102.47	102.47	102.47	102.47
Mean	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Std	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00
Min	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Max	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Area*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Perimeter*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Mean*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Std*	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00
Min*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Max*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Area*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Perimeter*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Mean*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Std*	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00
Min*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Max*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Area*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Perimeter*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Mean*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Std*	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00
Min*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Max*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Area*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Perimeter*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Mean*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Std*	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00
Min*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Max*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Area*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Perimeter*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Mean*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Std*	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00
Min*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Max*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Area*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Perimeter*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Mean*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Std*	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00
Min*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Max*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Area*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Perimeter*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Mean*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Std*	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00
Min*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Max*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Area*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Perimeter*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Mean*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Std*	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00
Min*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Max*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Area*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Perimeter*	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Mean*	100.00	100.00	100.00	100.00	100.00	100.00	100												