Validation process of the plugin NeuronZigzagJ

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In order to validate the plugin NeuronZigzagJ, we have undertaken two experimental studies. In the first experience, the aim was to investigate the role of the maximal projection (\Box) and the union (\cup) strategies (with the corresponding filter and thresholding methods). In the second experiment, a more systematic study was carried out to estimate the accuracy of our program with respect to the observations of some human experts. The files containing the images we have worked with are available at http://www.unirioja.es/cu/anromero/repository-of-images.html, in order to allow other researchers to reproduce our programming experiments.

1 First experiment

First of all, we have considered a series of actual neurophysiology images and we have applied the plugin using both the *maximal projection* and the *union* strategies (with the corresponding filter and thresholding methods). Concretely, we have applied our plugin starting from 1146 slices (coming from 12 GFP-Actin stacks of images, and 11 DiI stacks).

The results of the experimental study are collected in the table of Figure 1. The description of the columns in the table is as follows. Column 1 contains an identification number, and column 2 is the name of the file containing each stack. Column 3 indicates the number of slices in each stack. Data of column 4 are provided by a human, who inspected the relevant number of connected components (i.e., significant fragments of dendrites) in each stack; it is the column of quality control of the table. Then, each row is divided into two subrows, depending on the kind of operation used to compute the zigzag persistence: the union \cup or the maximal projection \sqcup (let us stress that each operation comes with its own filtering and thresholding methods, as explained before). Finally, the last column indicates the slices from which it is necessary start from to get some of the components (in brackets the number of connected components reached from each slice).

The conclusions that we can extract from these experimental data are the following.

1. An examination of table in Figure 1 shows the "right" slice to start from is not always located at the same place in the stack (in principle, our conjecture was that the intermediate slice would contain most of the relevant information); this justifies our decision of letting the user choose the initial slice.

- 2. In DiI images, in 40% of cases there is not a unique starting slice allowing recovering all the information (because dendrites can sprout at distant layers in the stack).
- 3. Our decision of treating differently GFP-Actin and DiI images is supported by the experiments, since in the former case in a 33% of cases no information is obtained with the union \cup operation, while in the latter case both operations behave similarly, and \cup is preferred because it is cheaper to be computed.
- 4. To reinforce the previous conclusion, it was checked (but we are not able to reflect it in the table), that the chosen combination filtering-operationthresholding produced a more accurate segmentation (other combinations were tested, but getting always worst results; in any case, let us recall that all these parameters can be fixed in the user interface, easing the reproduction of our computer experiments).
- 5. Information in the graphical and barcode outputs is complementary, without obtaining one as a by-product of the other one.

The last observation is specially interesting, because our preconception was that the barcode information would be simply a visual aid to a better understanding of the graphical output. This impression was biased because we underestimate the importance of the choosing of the starting slice. These experiments suggest a way of working with our plugin:

- 1. First, focus on the barcode diagram, looking for long bars and also considering when two longs bars are not intersecting in the same column (in this second case, it implies that no starting slice could produce all the relevant homological information).
- 2. Second, produce the corresponding graphical outputs starting from one or from several slices, determined by the barcode examination.

2 Second experiment

In a second experiment, we have selected 60 images (30 GFP images and 30 Dil images). They has been randomly divided into three blocks of 30 images, and each block has been assigned to a researcher to make a manual analysis. In that way, we got that some images were analyzed by more than one human, trying to measure the influence of subjective behaviour in the study. Two of the observers were biologists, and the third one a computer scientist (to also control in this way possible biases with respect to the initial training of the testers).

Actin-GFP	Title Image	No of Slices	Main Components	Operation	Slices which show components
1	Actin01	4	1	U	None
1	71ctill01	т	1		all(1)
2	Actip02	6	1	U	None
2	Actilio2	0	1		5,6(1)
2	Actip03	4	9	U	3,4(2)
3	Actino5	4	2		3,4(2)
4	A atip 04	F	1	U	4,5(1)
4	ACUII04	5	1		all(1)
Б.	A atip 05	0	1	U	all(1)
5	Actino5	0	1		all(1)
6	Actin06	4	9	U	all(2)
0	Actilioo	4	2		all(2)
7	Actip07	4	1	U	2(1)
'	Actilio7	4	1		all(1)
0	A atin 08	F	1	U	all(1)
0	Actillos	5	1		all(1)
0	A atip00	2	1	U	all(1)
9	Actin09	3	1	Ū	all(1)
10	Astin 10	F	1	U	None
10	Actin10	9	1	Ŭ	1(1)
11	A	F	1	U	all(1)
11	Actin11	5	1	Ŭ	all(1)
10	A	-	9	U	4,5(2)
12	Actin12	9	2	Ŭ	4,5(2)
DiI	Title Image	No of Slices	Main Components	Operation	Slices which show components
1	DaMagaCa	26	9	U	7-12(1)/14-23(1)
T	131123203	20	2		7-12(1)/14-23(1)
0	DaMagaCr	26	2	U	4-14(1)/17-26(1)
2	101/25205			Ū	4-14(1)/17-26(1)
2	R3PbR1N1C3	40	9	U	1-9(2)/14(1)
э	Nor DRINICS	40	9	Ū	1-8(2)/None
4	D2DbD1N1C4	50	9	U	4-9(2)/11-23(1)/24-31(1)/35-59(1)
4	K3PDKINIC4	59	J	Ŭ	4-11(2)/18-23(1)/24-31(1)/35-59(1)
٣	DADI DINIG	50	0	U	11,12(3)
Э	R5FDRINIC5	- 59	9	Ū	11,12,13(3)
C	D9DLD1N1CC	50	1	U	18-43(1)
0	K3PDRINIC0	- 59	1	Ŭ	18-43(1)
7	D9DLD1N1C7	50	0	U	36-44(2)
1	NOF DRINIC /	- 59	2	Ū	39-47(2)
0	D2DLD1N1C9	50	4	U	17-18(3)/ 30-33(1)
0	R3PbR1N1C8	59	4	Ū	16-17(3)/27-32(1)
0	Digode	44	2	Ū	36(2)
9	L129C5			Ū	35,36(2)
10	Digogo	10	1	Ū	11-34(1)
10	R158U2	40	1	Ŭ	11-34(1)
11 I	D100C4	44	2	U	28-38(2)
	R1S8C4			Ŭ	27(2)

Figure 1: Results of first experiment.

For each image, the human observer had to annotate the number of crossings, the number of connected components, and also the exact location of each crossing and of each dendrite. In addition, a free text box was included where the observer could write some comments about his/her interpretation (quality of the image, ambiguities and so on). Figures 2 and 3 include a summary of the results of the three observers. We also include in Figures 4, 5 and 6 the individual studies.

The result of the crossover study was clear: there wasn't any relevant discrepancy among the interpretations of the three observers, when looking at a same image. Even the free comments were quite similar in each case. This increases the reliability of the aggregated results obtained in the final table.

The success of the plugin was remarkable with respect to GFP images (90% of hits) and reasonable with respect to Dil images (77.6% of hits). Here *hit* means that the plugin found the same results than the human observers, up to some small ambiguity present in the image. If we consider *full hits*, that is to say, exact equality with respect to the four measured features (number of crossing, number of dendrites, and location of both), the figures are 86% for GFP images, and 66% for Dil images. The poorer performance for Dil images is explained because each image has around 45 slices; then the human eye didn't perceive all the intricacies contained in the image.

Title Image	Tester 1	Tester 2	Tester 3	Conclusion
DiI01	OK	OK	OK	1
DiI02	OK		OK	1
DiI03			OK	1
DiI04	It doesn't fit all			0
DiI05	It doesn't fit all	There are more dendrites		0
		in the PI because they are		
		in some slice with low intensity		
DiI06	OK			1
DiI07	It counts one dendrite as 2	OK		1
	but is just a ramification			
DiI08	There are too many dendrites	There are more dendrites		0
	superpossed, it is very	in the PI because they are in		
	difficult even by eye	some slice with low intensity		
DiI09	There is one piece that corresponds			0
	to a dendrite after the crossing but it			
	is recognized as part of the other one			
DiI10	OK	OK	OK	1
DiI11			The plugin finds one dendrite	1
			that the human doesn't	
DiI12	OK		The plugin finds one dendrite	1
			that the human doesn't	
DiI13	OK	OK		1
DiI14	Very difficult to say as there are a			0
	lot and they all cross (not even sure			
	that there are 18) The program counts			
	a lot of them as the same in white			
Dil15			OK	1
Dil16	OK			1
Dil17	OK	OK		1
Dil18		It doesn't recognize one dendrite		0
Dil19		OK		1
Dil20		OK		1
Dil21		OK		1
Dil22			OK	1
Dil23		There are more dendrites		0
		in the PI because they are in		
Dilat		some slice with low intensity	01/	
D1124			OK	1
Dil25			OK	1
D1126			The plugin detects a crossing that	1
			seems to be real although it is not	
D:107			seen in the maximal projection	1
D:127			UK	1
D1128			I ne plugin detects a crossing that	1
			seems to be real although it is not	
D:190			Seen in the maximal projection	1
Di129				1
01130			UK, It is an axon	1
			Total	23

Figure 2: Summary of second experiment - DiI images.

$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	Title Image	Tester 1	Tester 2	Tester 3	Conclusion
	GFP01	OK, but a piece of dendrite	Loses half dendrite		1
GFP02 OK 1 GFP03 OK 1 GFP04 OK 0K 1 GFP05 They all came from the same soma, so it finds them persistent from there but they are 3 different dendrites OK 1 GFP06 OK OK 1 GFP07 OK OK 1 GFP08 OK OK 1 GFP09 It has desconnected one dendrite (it has a disconction point) OK 1 GFP10 It loses the smallest dendrite OK 1 GFP11 OK, one of them is noise 0K 1 GFP13 OK (this image has a soma, and it's recognized too) 1 GFP14 OK OK 1 GFP15 OK OK 1 GFP14 OK OK 1 GFP15 OK OK 1 GFP14 OK OK 1 GFP15 OK OK 1 GFP14 OK OK 1 GF		from one of them is missing	of one of them		
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		in the plugging result			
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$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	GFP03	OK			1
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GFP29 OK OK 1 GFP30 OK 1 1 Total 27 27	GFP28		OK		1
GFP30 OK 1 Total 27	GFP29		OK	OK	1
Total 27	GFP30		OK		1
				Total	27

Figure 3: Summary of second experiment - GFP images.

	Kind	l of stained	of stained Original image(OI) Pro		Processed	image(PI)	Comparative OI vs. PI	
							Dendrites which	Crossings which
Title Image	DiI	GFP	Dendrites	Crossings	Dendrites	Crossings	coincide	coincide
DiI01	1		2	1	2	1	2	1
DiI02	1		2	0	2	0	2	0
DiI04	1		5	3	3	3	2	3
DiI05	1		4	4	3	2	3	2
DiI06	1		3	0	3	0	3	0
DiI07	1		4	1	5	3	4	2
DiI08	1		10	3	8	2	8	2
DiI09	1		2	1	2	1	2	1
DiI10	1		2	1	2	1	2	1
DiI12	1		1	0	1	0	1	0
DiI13	1		2	0	2	0	2	0
DiI14	1		18	5	10	2	10	2
DiI16	1		2	0	2	0	2	0
DiI17	1		1	0	1	0	1	0
GFP01		1	2	0	2	0	2	0
GFP03		1	1	0	1	0	1	0
GFP04		1	2	0	2	0	2	0
GFP05		1	3	1	1	0	1	0
GFP06		1	1	0	1	0	1	0
GFP07		1	1	0	1	0	1	0
GFP09		1	1	0	2	0	1	0
GFP10		1	2	0	2	0	1	0
GFP12		1	1	0	2	0	1	0
GFP14		1	2	0	2	0	2	0
GFP16		1	2	0	1	0	1	0
GFP17		1	1	0	1	0	1	0
GFP18		1	2	1	1	0	1	0
GFP19		1	2	0	2	0	2	0
GFP20		1	1	0	1	0	1	0

Figure 4: Results of second experiment - first observer.

	Kind of stained O		Original image(OI)		Processed image(PI)		Comparative OI vs. PI	
							Dendrites which	Crossings which
Title Image	DiI	GFP	Dendrites	Crossings	Dendrites	Crossings	coincide	coincide
DiI01	1		2	1	2	1	2	1
DiI05	1		2	1	4	2	2	1
DiI07	1		3	0	3	0	3	0
DiI08	1		4	0	6	0	6	0
DiI10	1		2	1	2	1	2	1
DiI13	1		2	0	2	0	2	0
DiI17	1		2	0	2	0	2	0
DiI18	1		3	0	2	0	2	0
DiI19	1		1	0	1	0	1	0
DiI20	1		2	0	2	0	2	0
DiI21	1		2	0	2	0	2	0
DiI23	1		1	0	2	0	1	0
GFP01		1	2	0	2	0	2	0
GFP04		1	2	0	2	0	2	0
GFP06		1	1	0	1	0	1	0
GFP08		1	1	0	1	0	1	0
GFP09		1	1	0	1	0	1	0
GFP11		1	1	0	1	0	1	0
GFP13		1	2	0	2	0	2	0
GFP14		1	2	0	2	0	2	0
GFP15		1	2	0	2	0	2	0
GFP16		1	2	0	2	0	2	0
GFP19		1	1	0	1	0	1	0
GFP21		1	1	0	1	0	1	0
GFP23		1	2	0	2	0	2	0
GFP26		1	1	0	2	0	1	0
GFP28		1	2	0	2	0	2	0
GFP29		1	3	0	3	0	3	0
GFP30		1	1	0	1	0	1	0

Figure 5: Results of second experiment - second observer.

	Kind	of stained	Original image(OI)		Processed image(PI)		Comparative OI vs. PI	
							Dendrites which	Crossings which
Title Image	DiI	GFP	Dendrites	Crossings	Dendrites	Crossings	coincide	coincide
DiI01	1		3	1	3	1	3	1
DiI02	1		2	0	2	0	2	0
DiI03	1		3	1	3	1	3	1
DiI10	1		2	1	2	1	2	1
DiI11	1		1	0	1	0	1	0
DiI12	1		1	0	2	1	1	0
DiI15	1		1	0	1	0	1	0
DiI22	1		2	0	2	0	2	0
DiI24	1		2	0	2	0	2	0
DiI25	1		1	0	1	0	1	0
DiI26	1		4	0	5	1	4	0
DiI27	1		2	0	2	0	2	0
DiI28	1		2	0	3	0	2	0
DiI29	1		2	0	2	0	2	0
DiI30	1		2	1	3	1	2	1
GFP02		1	3	0	3	0	3	0
GFP05		1	3	0	3	0	3	0
GFP06		1	1	0	1	0	1	0
GFP07		1	3	1	3	1	3	1
GFP09		1	1	0	2	0	1	0
GFP10		1	2	0	2	0	2	0
GFP14		1	2	0	2	0	2	0
GFP16		1	3	0	3	0	3	0
GFP17		1	2	0	1	0	1	0
GFP19		1	1	0	1	0	1	0
GFP22		1	1	0	1	0	1	0
GFP24		1	2	0	2	0	2	0
GFP25		1	1	0	1	0	1	0
GFP27		1	1	0	1	0	1	0
GFP29		1	3	0	3	0	3	0

Figure 6: Results of second experiment - third observer.