

Single-cell Droplets Analyzer (SCDA) *Project description*

1. Introduction

The Single-cell Droplets Analyzer (SCDA) (Fig.1) workflow enables researchers to detect and classify droplets automatically and obtain the fluorescence intensity of each droplet in multiple fluorescence channels. The detection-based approach was performed using opencv-python, a package originally applied for computer vision. In order to obtain more accurate detection results, the detected droplets can be manually screened and modified. This workflow can help researchers process fluorescence images containing droplets more efficiently.



Fig.1 SCDA User Interface

2. Description of software

The SCDA software workflow (Fig.2) is realized as a shiny application with a user interface UI, which mainly consists of three parts (SCDA 1 of 3) droplets detection, (SCDA 2 of 3) droplets classification, (SCDA 3 of 3) Fluorescence Intensity Statistics.

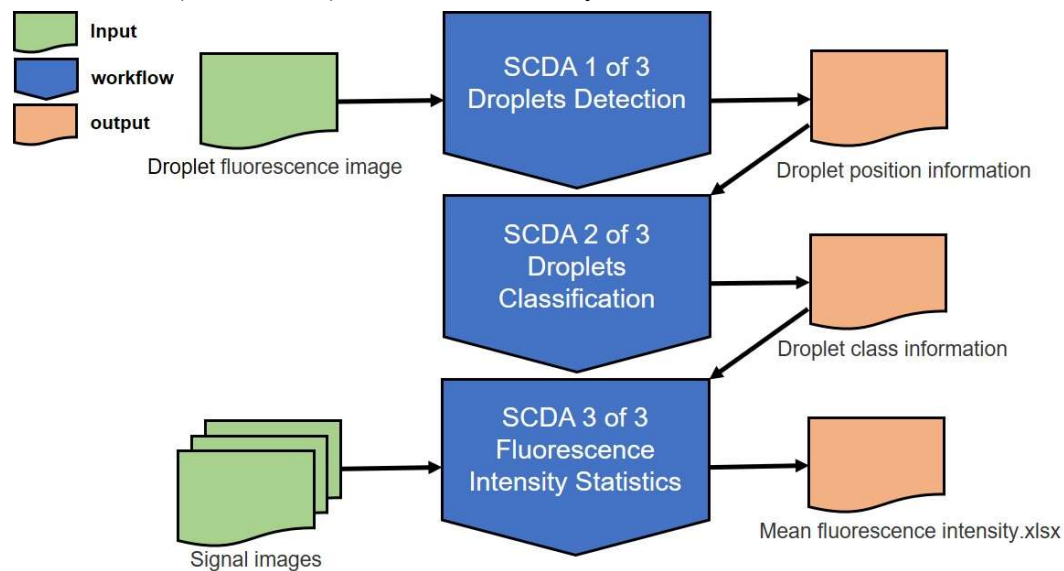


Fig.2 SCDA workflow.

2.1 Droplets Detection

SCDA 1 of 3 (Droplets Detection) consists of several steps leading to detection results. First, the

input image will be converted into an 8-bit grayscale image. Then the image will be normalized to the 0-255 range to increase contrast. Next, since the shape of droplets is round, after setting several parameters, the function `HoughCircles()` (for further details, see the [HoughCircles\(\)](#)) is used to detect the droplets. After the above steps, the center and radius of droplets can be obtained, and the results will be marked on the original image. Additionally, users can delete false-detected droplets by right-clicking the droplet area and drawing the round shape to supplement the missing Detection.

2.2 Droplets Classification

SCDA 2 of 3 (Droplets Classification) classifies each droplet based on the color of its cells in it. Four colors (red, green, blue, and yellow) and their combinations can be selected to perform classification. In order to determine the color composition of the cells in each droplet area, each droplet area will be converted to HSV color space and classified based on the brightness threshold and area threshold one by one. Finally, droplets will be marked with the colors corresponding to their internal cells.

2.3 Fluorescence Intensity Statistics

SCDA 3 of 3 (Fluorescence Intensity Statistics) statistics each droplet's fluorescence intensity based on the detection and classification results. After selecting the corresponding fluorescence images, the location of each droplet will be used to calculate the total and mean fluorescence intensity. In the end, the data is exported as a table.

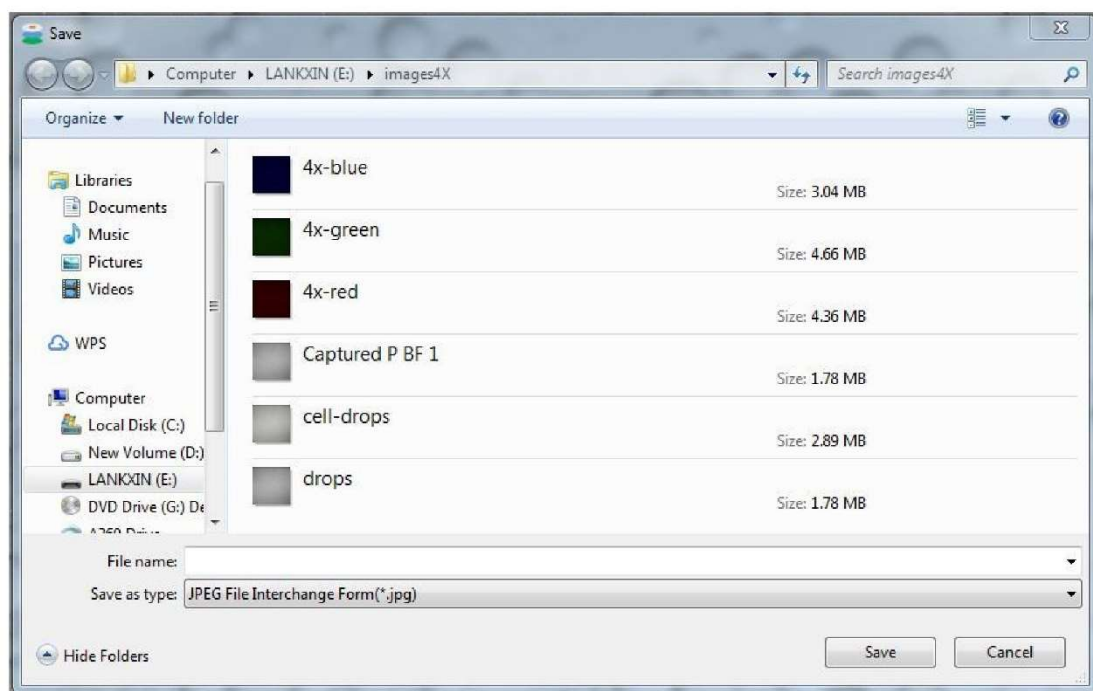
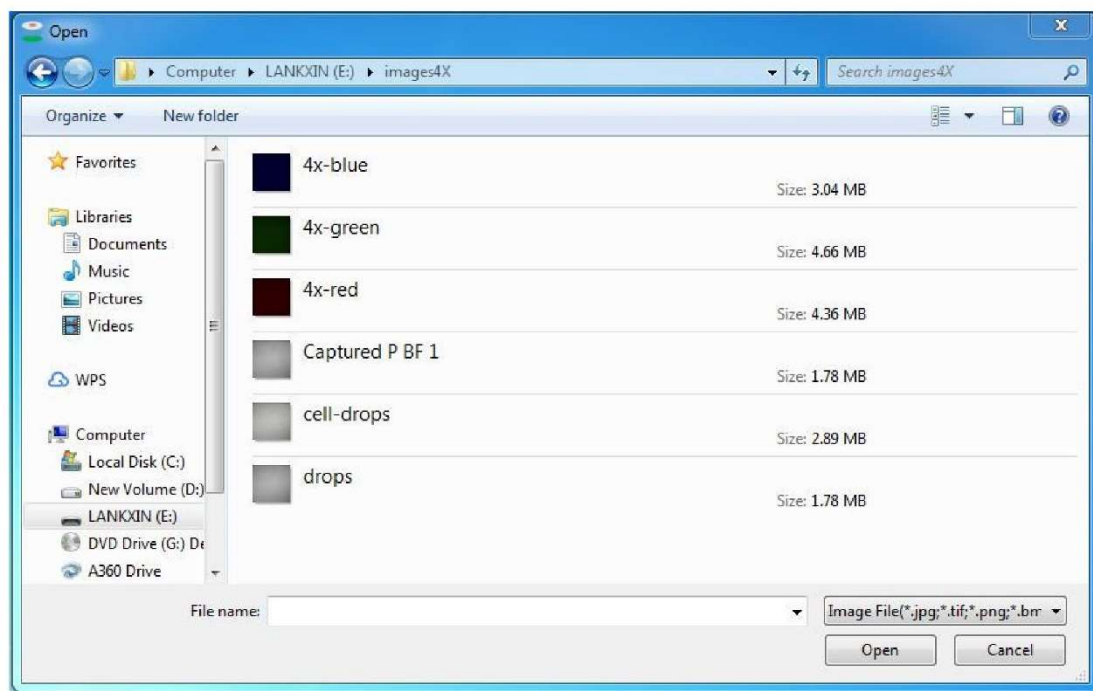
3. Analysis

3.1 Start the analysis

1. The software is realized as a shiny application (SCDA.exe) with a user interface in the SCDA folder.
2. SCDA shiny application (SCDA.exe) and droplet fluorescence images for testing are accessible from GitHub Download the entire repository and unzip it locally.
3. Run the SCDA.exe in the SCDA folder.

3.2 Data input and output

The fluorescence images to be read are determined(Open), and you can define where to store the output image(Save) or table(Export)-Fig.3.



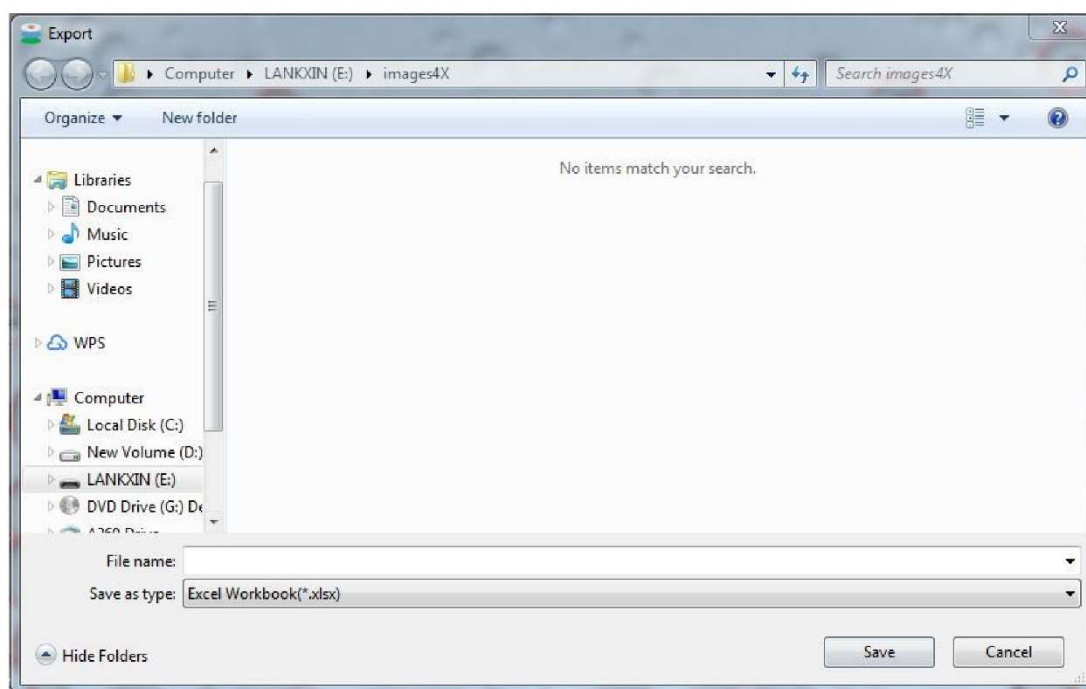


Fig.3 File operations menu.

3.3 SCDA 1 of 3 (Droplets Detection)

For the droplets detection step (Fig.4), the position of droplets can be obtained by adjusting four thresholds properly (see Fig.4).



Fig.4 Menu for SCDA 1 of 3.

3.4 SCDA 2 of 3 (Droplets Classification)

For the droplets classification step (Fig.5), the detected droplets would be classified by the color of cells in its.

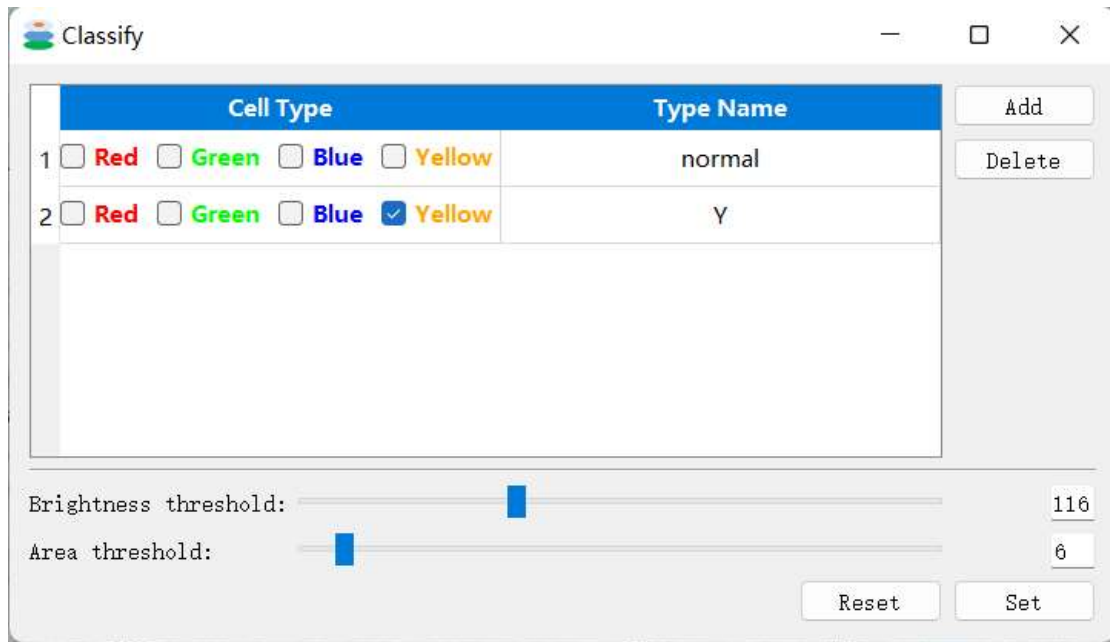


Fig.5 Menu for SCDA 2 of 3.

3.5 SCDA 3 of 3 (Fluorescence Intensity Statistics)

For the fluorescence intensity statistics step (Fig.6), the position, type, and fluorescence intensity information of droplets will be combined to calculate fluorescence intensity, and the fluorescence images added can be converted to grayscale or split by channels as required.

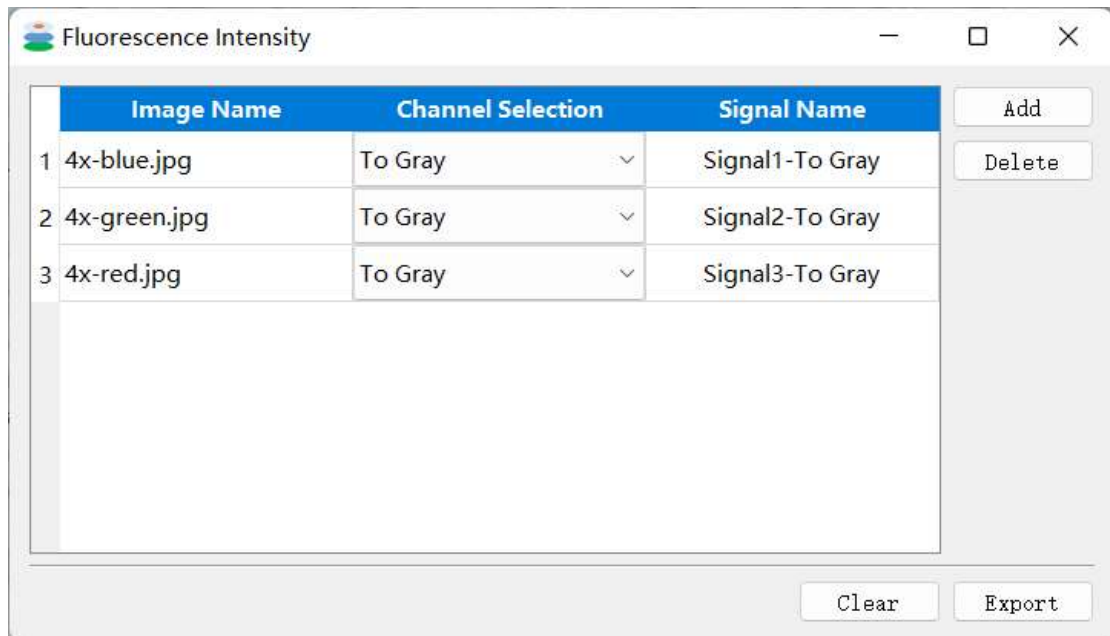


Fig.6 Menu for SCDA 3 of 3.

4. Results

4.1 Result of SCDA 1 of 3 (Droplets Detection)

SCDA 1 of 3 (Droplets Detection) flags most of the droplets in the image. As shown in Fig.7, the red dotted circle marks the automatically detected droplets, and the green dotted circle marks the droplets selected manually.

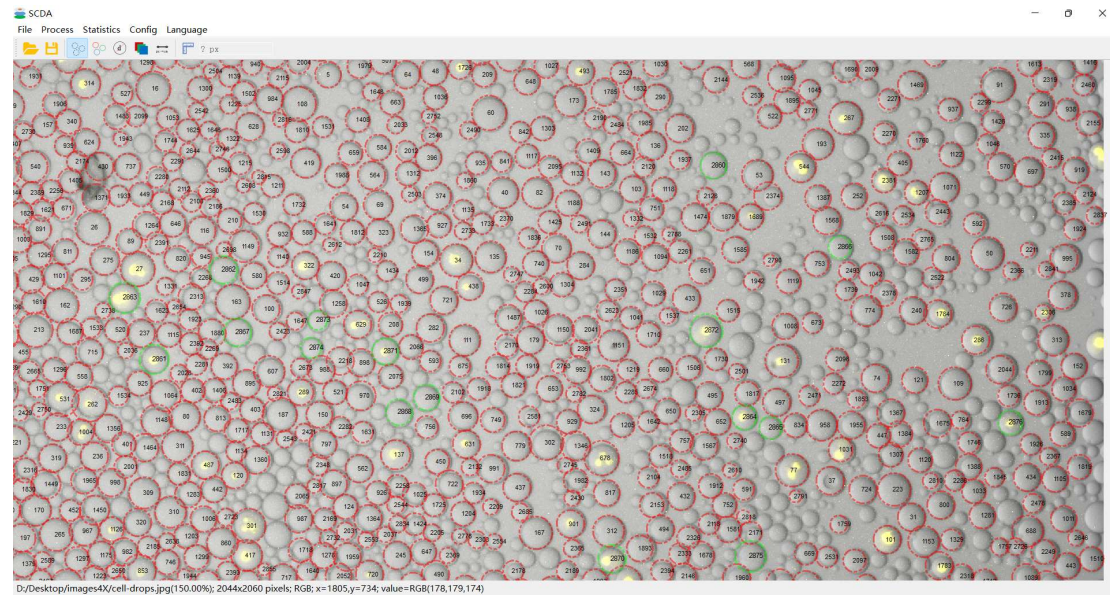


Fig.7 Result for SCDA 1 of 3.

4.2 Result of SCDA 2 of 3 (Droplets Classification)

SCDA 2 of 3 (Droplets Classification) flags the droplets furtherly. Droplets without cells are marked with blue circles, and the others are marked with circles of corresponding colors.

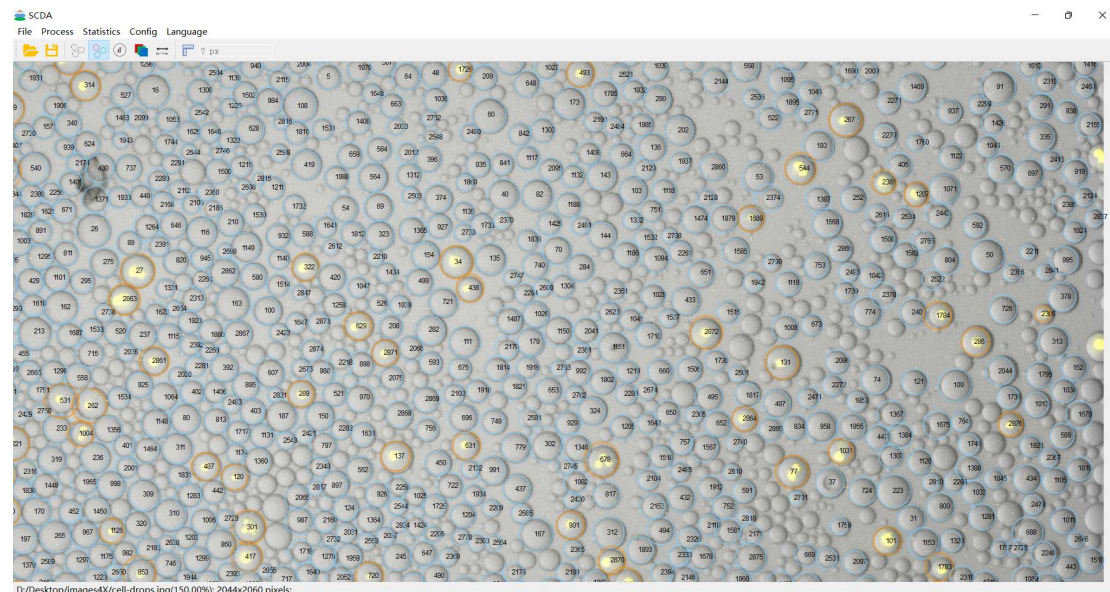


Fig.8 Result for SCDA 2 of 3.

4.3 Result of SCDA 3 of 3

Single-cell Droplets Analyzer – SCDA

If the analysis is finished, the results are exported as a table (Fig.9). This table contains all calculated droplets class information (Type Name), position information (Centre(px), Radius(px), Area(px)) and fluorescence intensity information (Total Intensity, Mean Intensity).

A	B	C	D	E	F	G	H	I
1	Type Name	Centre(px)	Radius(px)	Area(px)	Total Intensity(Signal1-To Gray)	Mean Intensity(Signal1-To Gray)	Total Intensity(Signal2-To Gray)	Mean Intensity(Signal2-To Gray)
2	normal	(1138, 684)	20	1255	7425	5.916334661	33203	26.4565737
3	normal	(948, 602)	18	1007	5968	5.926514399	25870	25.6901686
4	normal	(1184, 1380)	20	1255	7450	5.93625498	35606	28.3713147
5	normal	(620, 762)	19	1127	6603	5.85891748	31159	27.6477373
6	normal	(1148, 720)	15	707	4410	6.237623762	19555	27.6591230
7	normal	(296, 344)	19	1127	6038	5.357886513	26875	23.8464951
8	normal	(932, 1328)	19	1127	6633	5.865538823	31157	27.6459627
9	normal	(648, 1594)	20	1255	7461	5.94501992	34185	27.2390438
10	Y	(76, 1272)	20	1255	6925	5.517928287	44492	35.4517928
11	normal	(258, 612)	20	1255	6907	5.503585657	33476	26.6741035
12	normal	(588, 412)	16	795	4401	5.535849057	19283	24.2553459
13	Y	(848, 700)	18	1007	6121	6.078450844	30222	30.0119165
14	normal	(252, 874)	20	1255	7375	5.876494024	36628	29.1856573
15	normal	(956, 494)	20	1255	7173	5.715537849	32502	25.8980079
16	Y	(68, 1064)	20	1255	6986	5.566533865	37629	29.9832669
17	normal	(946, 736)	20	1255	7409	5.903585657	35317	28.1410358
18	normal	(994, 1718)	20	1255	7349	5.850776892	33607	26.8262948
19	normal	(92, 896)	20	1255	6978	5.560159363	33030	26.318725
20	Y	(264, 1264)	20	1255	7408	5.902788845	42041	33.4988047
21	Y	(1856, 1328)	19	1127	6667	5.915705413	37729	33.4773735
22	normal	(1344, 620)	18	1007	5974	5.932472691	27821	27.6276067
23	normal	(402, 1498)	20	1255	7531	6.000796813	36422	29.0215139
24	Y	(832, 1594)	20	1255	7574	6.035059761	45212	36.0254980
25	normal	(314, 1030)	18	1007	5681	5.641509434	25561	25.3833167
26	normal	(1144, 570)	16	795	4722	5.939622642	21822	27.1974842
27	normal	(876, 896)	20	1255	7610	6.04374502	36651	29.2039840
28	Y	(928, 944)	20	1255	7942	6.328286853	44136	35.1681274
29	Y	(688, 1486)	19	1127	6803	6.036379769	34261	30.4001774
30	normal	(268, 950)	16	795	4647	5.845283019	21603	27.1735849
31	normal	(284, 894)	16	795	4615	5.895031447	22156	27.8691823
32	normal	(1822, 1220)	16	795	4549	5.722012579	20413	25.6787295
33	normal	(1294, 454)	15	707	4195	5.933521924	18779	26.5615275
34	normal	(478, 644)	20	1255	7348	5.85498008	34496	27.4868525

Fig.9 Result for SCDA 3 of 3 (Fluorescence Intensity Statistics).

5. Software

The software has been implemented in Python with corresponding Python packages.

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If SOPRA workflow is used to generate data, please cite:

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