Detection of calbindin spheroids or foci or puncta

Usage: *Run it in FIJI (www.fiji.sc)

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Credit to Sébastien Tosi (IRB Barcelona) for SpotDetection algorithm: https://github.com/Neubias-WG5/W_SpotDetection-Dmap-IJ/blob/master/IJSpotDetection.ijm

Description:

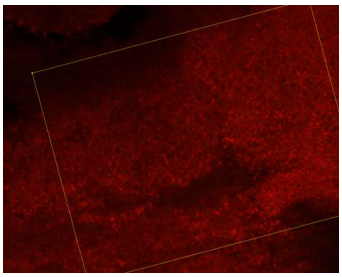
Code to use in a FIJI-macro for the batch detection/analysis of Calbindin spheroids.

First install PTBIOP and ImageScience plugings

- Open FIJI
- Go to Help < Update...
- Wait for the bar to complete and then press Manage update sites
- Scroll down and go to PTBIOP and IMAGESCIENCE, select them and then close window
- Finally press Apply changes in the ImageJ updater window already open
- Once it finishes the download, restart FIJI and open it again and try the macro

Open a single image in FIJI

```
open("C:/Users/mdp18pm/Desktop/FIJI Macro for detecting puncta-foci-spheroids/Picture3.tif");
```



Getting image information + Normalise the data name

```
title = getTitle();

run("Set Scale...", "distance=0 known=0 pixel=1 unit=pixel");
```

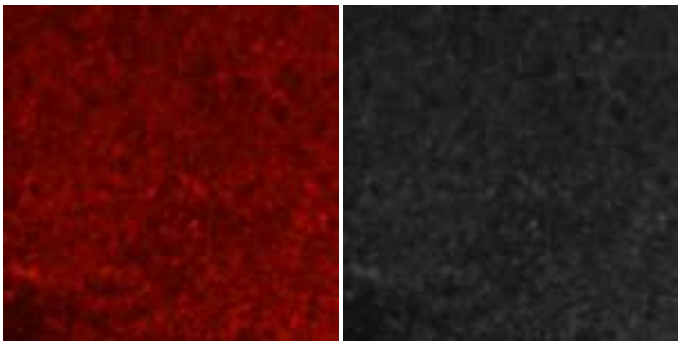
We now create a rectangle (region of interest- ROI); this can be later changed by the user for its size and location

The analysis will be performed only in the selected ROI

```
makeRectangle(211, 181, 150, 150);
setTool("rectangle");
```

Now the user can select a region of interest by moving the yellow square

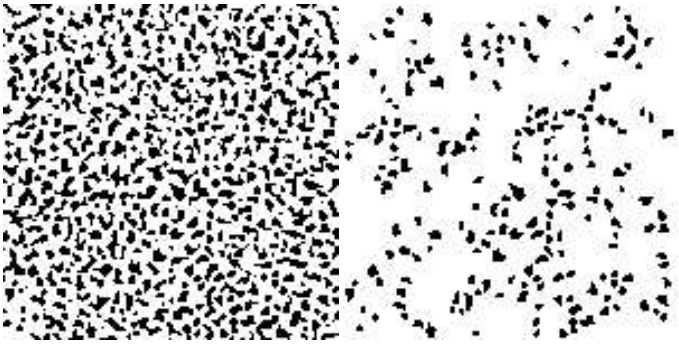
```
waitForUser("Please move ROI into a region of interest; in our case is Deep Cerebellar Nuclei (DCN). \n\nThen

run("Duplicate...", " ");
duplicated1 = getImageID();
run("Duplicate...", " ");
run("8-bit");
duplicated2 = getImageID();
```



Let's start our Connected Components Analysis

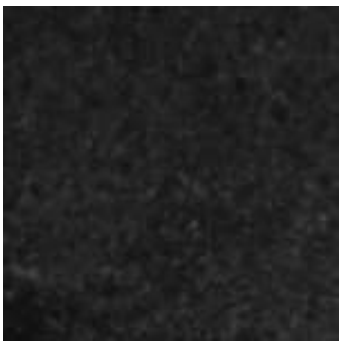
We will use FeatureJ Laplacian filter. You will see a first image with a first detection and then a second image in which restrictions are applied (i.e. size= 5-350 or circularity = 0.7-1)

```
selectImage(duplicated2);

run("FeatureJ Laplacian", "compute smoothing=1");
        setOption("BlackBackground", false);
        run("Convert to Mask");
        run("Watershed");
        run("Analyze Particles...",
        "size=5-350 pixel  circularity=0.70-1.00 show=Masks display exclude clear add");

run("Clear Results");

selectImage(duplicated2);

roiManager("Show All without labels");
n=roiManager("count");
```



Exclude all of those ROIs below a certain mean threshold value (i.e. 41)

```
for(i=n-1;i>0;i=i-1) {
        roiManager("Select", i);
        run("Measure");
        if(getResult("Mean",nResults-1)<41){
                roiManager("Delete");
        };
};

roiManager("Show None");
roiManager("Show All");
run("Clear Results");
roiManager("Deselect");
roiManager("Measure");
```



Label	Area	Mean	Min	Max	X	Y	XM	YM	Perim.	Feret	IntDen	RawIn
Picture3-2.tif:0001-0003	9	30.444	29	31	25.611	3.167	25.613	3.168	10.485	4.472	274	274
Picture3-2.tif:0005-0008	21	41.238	36	48	89.690	7.643	89.730	7.667	17.556	7.211	866	866
Picture3-2.tif:0017-0016	17	44.824	38	56	123.971	16.559	124.013	16.605	15.314	6.403	762	762
Picture3-2.tif:0022-0021	21	42.429	38	49	128.500	21.738	128.480	21.776	18.971	8.602	891	891
Picture3-2.tif:0026-0022	16	41.562	39	45	120.312	22.500	120.317	22.511	15.071	6.325	665	665
Picture3-2.tif:0028-0024	17	42.176	37	48	38.324	25.029	38.292	25.036	15.314	5.831	717	717
Picture3-2.tif:0041-0037	15	43.200	39	46	117.700	37.100	117.721	37.086	13.899	5.831	648	648
Picture3-2.tif:0055-0051	14	42.071	39	47	115.571	51.643	115.597	51.651	14.142	5.385	589	589
Picture3-2.tif:0065-0056	10	44.500	39	50	41.300	56.500	41.282	56.493	11.071	4.472	445	445
Picture3-2.tif:0067-0057	15	41.667	36	47	83.300	56.767	83.265	56.754	14.728	6.708	625	625
Picture3-2.tif:0070-0057	8	42.250	38	48	78.375	57.625	78.417	57.627	9.071	4.243	338	338
Picture3-2.tif:0078-0064	23	42.087	36	49	44.717	64.239	44.711	64.248	20.142	8.602	968	968
Picture3-2.tif:0079-0064	28	44.821	40	54	51.643	64.679	51.641	64.682	20.971	8.062	1255	1255
Picture3-2.tif:0090-0071	9	43.333	38	51	111.167	71.722	111.156	71.733	10.485	4.472	390	390

Picture3-2.tif:0091-0074	14	42.143	37	49	45.643	74.000	45.637	74.085	14.485	6.083	590	590
Picture3-2.tif:0095-0074	12	41.583	35	48	90.583	74.667	90.566	74.646	12.485	5.385	499	499
Picture3-2.tif:0096-0075	17	43.412	39	48	123.500	75.324	123.463	75.324	15.314	6.325	738	738
Picture3-2.tif:0097-0076	13	42.923	40	47	22.423	75.731	22.412	75.742	13.314	5.385	558	558
Picture3-2.tif:0098-0077	7	42.429	38	46	15.500	77.786	15.476	77.776	9.071	3.606	297	297
Picture3-2.tif:0099-0078	10	41.200	37	48	60.600	78.600	60.568	78.614	12.485	5.831	412	412
Picture3-2.tif:0100-0080	12	41.250	36	47	95.000	80.000	95.013	80.005	11.314	4.472	495	495
Picture3-2.tif:0102-0081	14	43.714	41	49	37.500	81.786	37.515	81.783	13.899	5.831	612	612
Picture3-2.tif:0106-0085	22	43.955	37	50	99.818	84.773	99.830	84.792	18.728	8.246	967	967
Picture3-2.tif:0109-0088	19	45.895	40	52	135.605	87.711	135.601	87.758	17.314	7.280	872	872
Picture3-2.tif:0111-0090	15	42.867	34	52	118.900	89.700	118.869	89.596	15.314	6.708	643	643
Picture3-2.tif:0117-0094	15	56.333	45	69	142.767	94.567	142.782	94.631	13.314	5.831	845	845
Picture3-2.tif:0120-0097	18	49.167	38	64	84.833	97.833	84.906	97.834	15.314	7.071	885	885
Picture3-2.tif:0124-0101	17	41.706	38	46	23.794	100.971	23.807	100.957	15.314	6.325	709	709
Picture3-2.tif:0125-0100	15	42.000	36	48	71.700	100.567	71.690	100.586	13.899	5.831	630	630
Picture3-2.tif:0126-0100	14	48.643	43	56	137.214	100.071	137.227	100.067	13.314	5.831	681	681
Picture3-2.tif:0127-0100	15	44.467	38	57	143.633	100.233	143.593	100.128	14.485	5.657	667	667
Picture3-2.tif:0129-0103	19	41.158	36	47	41.342	103.132	41.318	103.105	15.899	6.708	782	782

Picture3-2.tif:0130-0104	18	42.000	36	48	57.167	104.111	57.185	104.048	15.314	6.325	756	756
Picture3-2.tif:0132-0103	16	43.438	35	52	87.000	102.812	86.981	102.769	15.314	7.211	695	695
Picture3-2.tif:0137-0108	8	41.125	36	45	121.000	108.000	121.035	108.005	9.657	4.472	329	329
Picture3-2.tif:0145-0113	10	41.600	39	44	33.000	113.300	33.017	113.315	11.899	5.657	416	416
Picture3-2.tif:0146-0112	11	42.273	39	46	142.773	112.500	142.786	112.513	12.485	5.385	465	465
Picture3-2.tif:0147-0114	16	43.625	34	54	89.000	113.938	89.125	113.890	15.314	6.708	698	698
Picture3-2.tif:0148-0116	11	44.455	40	48	143.773	116.500	143.809	116.494	12.485	5.385	489	489
Picture3-2.tif:0149-0119	20	57.350	43	69	13.350	119.300	13.340	119.381	16.728	6.325	1147	1147
Picture3-2.tif:0151-0120	10	43.500	39	46	102.000	120.500	101.974	120.491	10.485	5.000	435	435
Picture3-2.tif:0152-0120	8	42.250	39	47	121.625	120.625	121.618	120.633	9.071	4.243	338	338
Picture3-2.tif:0153-0122	14	45.857	43	50	96.500	122.143	96.492	122.156	13.314	5.831	642	642
Picture3-2.tif:0156-0123	11	41.545	39	45	57.591	123.136	57.585	123.126	11.071	5.000	457	457
Picture3-2.tif:0158-0122	9	41.000	36	46	86.833	122.611	86.871	122.627	9.899	4.472	369	369
Picture3-2.tif:0159-0124	11	42.455	36	48	16.409	124.136	16.399	124.059	11.071	5.000	467	467
Picture3-2.tif:0160-0123	10	42.300	39	46	28.400	123.300	28.389	123.285	12.485	5.831	423	423
Picture3-2.tif:0161-0124	11	44.545	41	50	145.045	124.409	145.067	124.422	13.314	5.657	490	490
Picture3-2.tif:0163-0126	16	46.625	39	55	64.250	125.500	64.267	125.536	15.899	6.708	746	746
Picture3-2.tif:0164-	10	44.700	42	48	121.500	124.700	121.500	124.697	11.071	4.472	447	447

0125												
Picture3-2.tif:0166-0126	9	43.556	39	49	88.500	126.167	88.505	126.143	10.485	5.000	392	392
Picture3-2.tif:0167-0127	17	45.294	40	53	111.912	127.618	111.940	127.669	17.314	6.708	770	770
Picture3-2.tif:0168-0128	11	47.091	42	52	125.136	128.045	125.139	128.064	11.314	4.472	518	518
Picture3-2.tif:0169-0130	18	41.778	31	56	3.056	129.389	3.039	129.327	16.485	6.708	752	752
Picture3-2.tif:0171-0128	7	41.857	38	49	60.500	128.786	60.497	128.794	9.071	3.606	293	293
Picture3-2.tif:0173-0130	18	45.611	38	55	80.556	130.611	80.558	130.681	15.899	7.071	821	821
Picture3-2.tif:0177-0131	8	41.625	37	45	101.625	131.625	101.656	131.620	9.071	4.243	333	333
Picture3-2.tif:0179-0132	18	47.500	41	57	143.167	132.167	143.184	132.135	17.314	7.211	855	855
Picture3-2.tif:0180-0134	16	44.875	38	49	135.000	134.688	134.947	134.680	13.899	5.831	718	718
Picture3-2.tif:0182-0135	8	42.000	37	46	87.750	135.125	87.780	135.137	10.485	4.472	336	336
Picture3-2.tif:0183-0136	19	47.895	42	55	103.921	136.447	103.947	136.454	16.142	6.708	910	910
Picture3-2.tif:0184-0135	7	42.000	40	44	122.500	135.500	122.517	135.520	8.485	4.243	294	294
Picture3-2.tif:0187-0137	13	46.615	40	54	117.269	137.269	117.309	137.297	12.485	5.657	606	606
Picture3-2.tif:0192-0140	9	47.667	44	52	143.611	139.833	143.628	139.836	10.485	4.472	429	429
Picture3-2.tif:0193-0141	22	44.318	39	55	109.500	141.682	109.373	141.723	18.728	7.280	975	975
Picture3-2.tif:0194-0142	10	42.700	36	53	86.500	142.000	86.500	142.034	10.485	5.000	427	427
Picture3-2.tif:0195-0143	20	53.000	43	63	103.150	143.450	103.130	143.450	18.728	7.616	1060	1060
Picture3-	7	43.143	37	48	86.786	147.071	86.811	147.020	10.485	4.472	302	302

2.tif:0199-0147												
Picture3-2.tif:0200-0147	12	45.250	40	54	98.583	147.167	98.634	147.187	11.899	5.000	543	543
Picture3-2.tif:0201-0147	6	42.167	38	46	59.500	147.000	59.555	146.994	7.657	3.606	253	253

Save the results

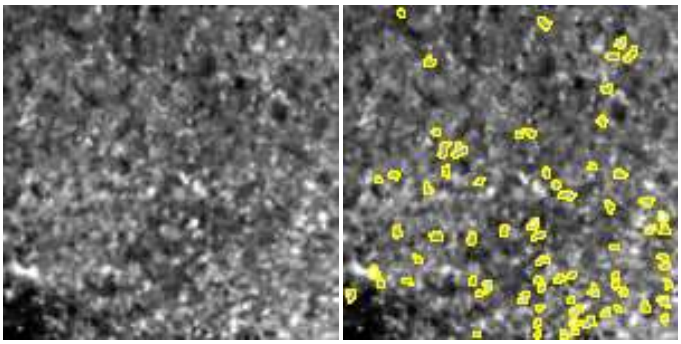
Now we save the results.

```
saveAs("results", "C:/Users/mdp18pm/Desktop/FIJI Macro for detecting puncta-foci-spheroids/results.xls");
```

Save the image containing the detected spheroids

- you can open it once the macro is finished

```
selectImage(duplicated2);
roiManager("Show None");
roiManager("Show All without labels");

run("Enhance Contrast", "saturated=0.35");
run("Enhance Contrast", "saturated=0.35");
run("Enhance Contrast", "saturated=0.35");
run("Enhance Contrast", "saturated=0.35");

run("Flatten");
saveAs("Tiff", "C:/Users/mdp18pm/Desktop/FIJI Macro for detecting puncta-foci-spheroids/Spheroids.tif");
```



Save the detected spheroids (ROIs)

- so you will be able to upload them anytime from the ROI manager and they will not be lost

```
roiManager("Save", "C:/Users/mdp18pm/Desktop/FIJI Macro for detecting puncta-foci-spheroids/RoiSet.zip");
```