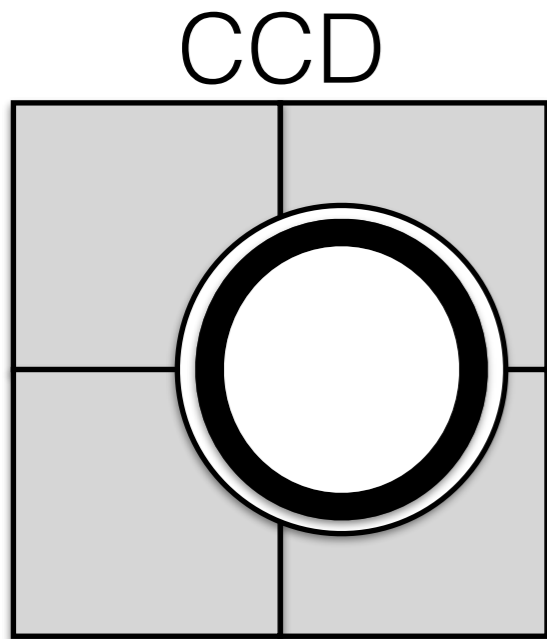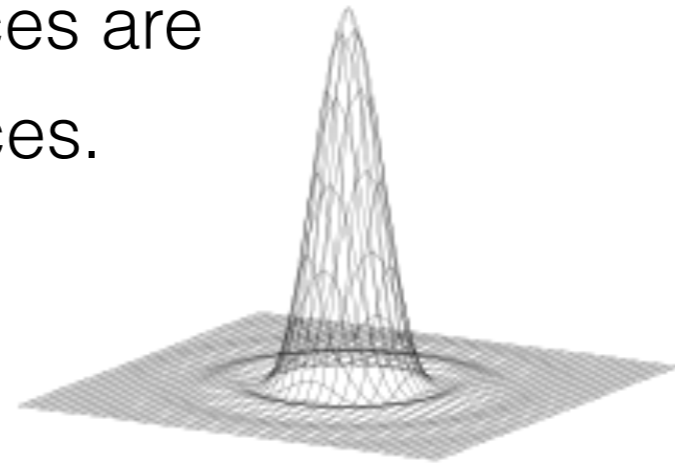# Single Molecule Localisation Microscopy and OMERO

Ian Munro

Imaging is fundamentally diffraction-limited but more information available by knowing all sources are fluorescent molecules - i.e. small point sources.
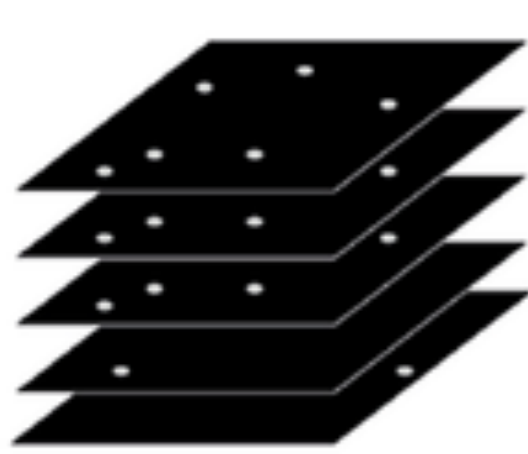
Airy disk Intensity plot.

CCD

The location of a single point-source can be determined to sub-pixel resolution.

Label sample with fluorophores, crucially ensure that most are switched off PALM, STORM etc

identification   localization

100 nm

Processing Pipeline

time

Localisation of individual fluorophores

Image reconstruction

	B	C	D	E	F	G	H	I
frame		x [nm]	y [nm]	sigma [nm]	intensity [phot	offset [photon	bkgstd [photo	uncertainty [nm]
1	1	565.093375	4593.70398	88.1144489	2483.86751	1412.56167	124.370762	15.9594856
2	1	573.706657	8297.65145	199.523849	11323.782	1525.4344	148.280179	21.3321043
3	1	217.465555	10944.9208	1389.86985	1544180.97	1.02E-05	114.442172	5.94143148
4	1	661.275016	9381.96477	250.985661	16498.9683	1513.18498	158.230368	24.7022221
5	1	701.321427	28382.9711	260.017835	25466.961	2888.42345	288.498803	31.2600562
6	1	766.41216	5817.39187	221.576255	10393.7079	1375.11842	124.085134	23.9908771
7	1	745.118959	6906.92549	136.898342	10765.3395	1364.05335	83.6962424	6.09309691
8	1	1024.74723	1138.376	218.832609	8076.67885	1281.38191	88.7788614	21.5957166
9	1	881.587431	3187.49	85.6219942	2274.78828	1393.02006	128.357936	16.9720139
10	1	852.019338	24940.0729	255.291653	16744.7161	2398.81091	192.958104	30.6754388
11	1	1094.26331	1505.77088	141.16995	4420.31356	1383.82375	114.475862	21.1494903
12	1	1071.07689	2071.2551	209.831912	7323.53536	1329.15487	84.6248055	20.8850501
13	1	977.582219	11449.6483	110.156044	4595.09291	1710.44667	184.776507	19.9608257
14	1	956.927567	20597.5277	241.869673	21656.3333	1867.0193	164.223938	18.15743
15	1	1062.40765	21902.5969	207.092753	20825.3127	1991.98731	243.307514	20.4743222
16	1	1109.84182	13384.1099	232.454032	25756.1089	1764.49654	292.58354	25.0612173
17	1	1227.07812	18286.5967	101.731158	2251.73423	1848.15508	103.418515	19.505932
18	1	1057.68035	23878.9277	1037.73066	1200230.1	0.03565667	172.270073	6.37073396

No standard file format. OMERO solution needed.

PALMsiever. Matlab

ThunderSTORM. Java, Imagej plugin

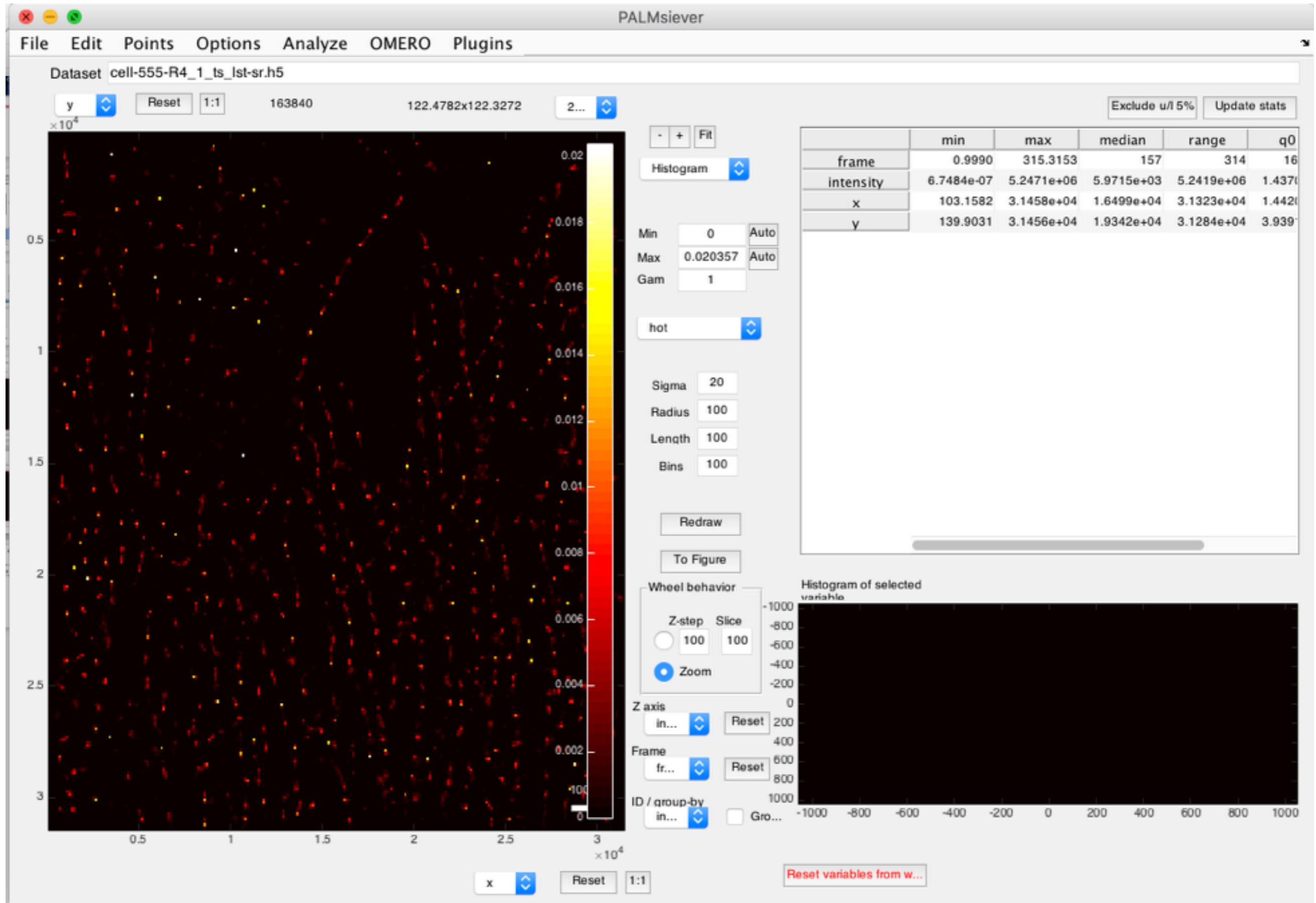# OMERO menu added to PALMsiever allows user to choose a dataset

Then choose a Table

Super resolved image

OMERO menu added to ThunderSTORM allows user to choose a dataset
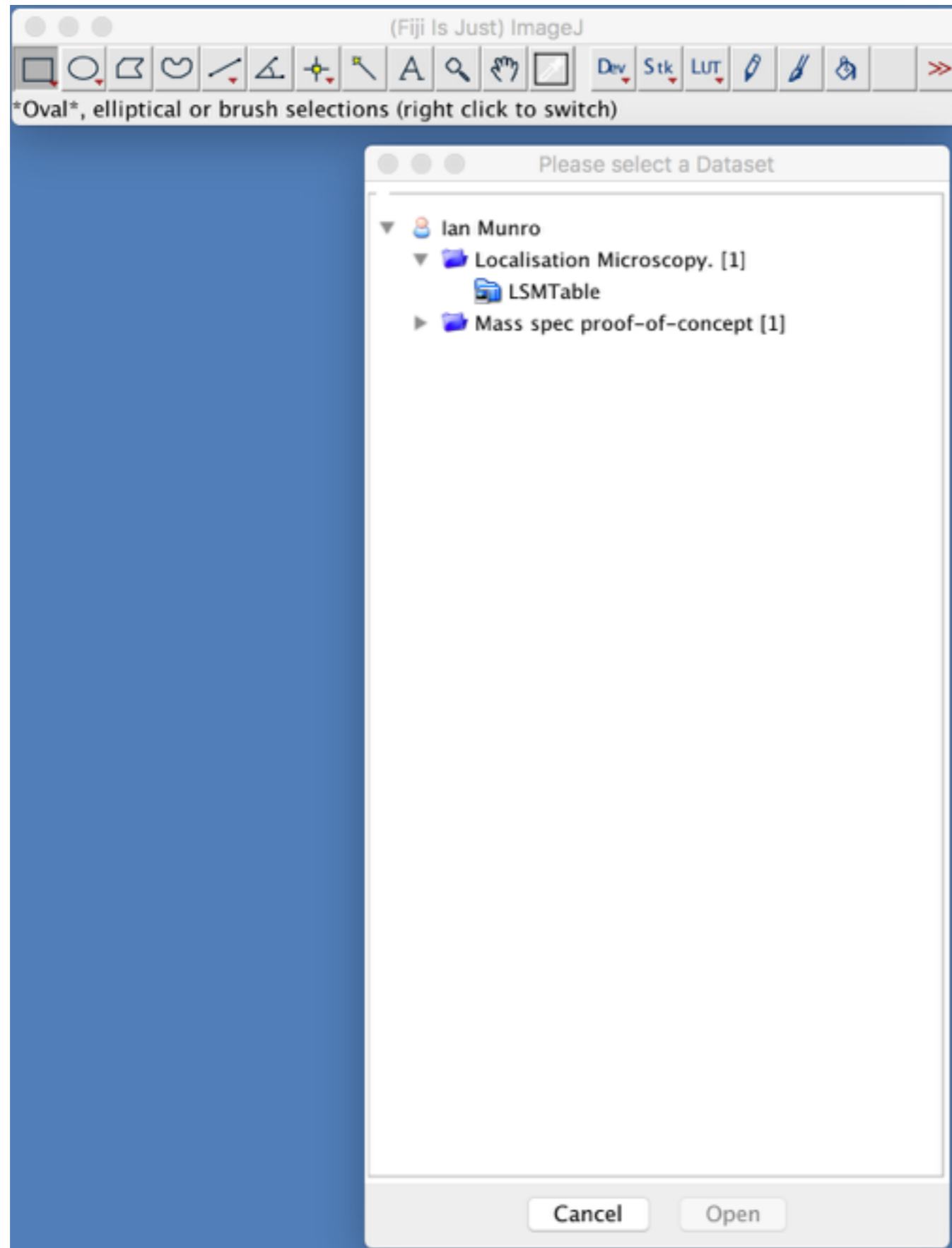
N.B. same UI as PALMsiever

Dev | Stk | LUT

Averaged shifted histograms (33.3%)

31.6x31.6 µm (1975x1975); 32-bit; 15MB

ThunderSTORM: results

id	frame	x [nm]	y [nm]	sigm...	intensity [p...	offset [phot...	bkgstd [pho...	uncertainty ...
1	1	565.093	4593.704	88.1...	2483.868	1412.562	124.371	15.959
2	1	573.707	8297.651	199....	11323.782	1525.434	148.28	21.332
3	1	217.466	10944...	138....	1544180....	0	114.442	5.941
4	1	661.275	9381.965	250....	16498.968	1513.185	158.23	24.702
5	1	701.321	28382...	260....	25466.961	2888.423	288.499	31.26
6	1	766.412	5817.392	221....	10393.708	1375.118	124.085	23.991
7	1	745.119	6906.925	136....	10765.34	1364.053	83.696	6.093
8	1	1024.747	1138.376	218....	8076.679	1281.382	88.779	21.596
9	1	881.587	3187.49	85.6...	2274.788	1393.02	128.358	16.972
10	1	852.019	24940...	255....	16744.716	2398.811	192.958	30.675
11	1	1094.263	1505.771	141....	4420.314	1383.824	114.476	21.149
12	1	1071.077	2071.255	209....	7323.535	1329.155	84.625	20.885
13	1	977.582	11449...	110....	4595.093	1710.447	184.777	19.961
14	1	956.928	20597...	241....	21656.333	1867.019	164.224	18.157
15	1	1062.408	21902...	207....	20825.313	1991.987	243.308	20.474
16	1	1109.842	13384.11	232....	25756.109	1764.497	292.584	25.061
17	1	1227.078	18286...	101....	2251.734	1848.155	103.419	19.506
18	1	1057.68	23878...	103...	1200230....	0.036	172.27	6.371
19	1	839.877	24729...	696....	342541.072	1209.982	210.231	12.188
20	1	995.251	27383...	321....	45661.183	2400.402	246.672	22.848
21	1	1173.474	29757...	148....	12365.043	3367.921	275.07	20.174
22	1	1319.443	3813.837	197....	8038.714	1369.541	125.725	24.919
23	1	1022.327	16089...	288....	32631.079	1678.843	244.207	25.365
24	1	1427.049	4693.293	122....	3664.23	1363.273	87.142	14.81
25	1	1389.917	30529...	196....	25647.709	3288.581	381.012	23.373
26	1	1569.894	9435.143	94.4...	1685.959	1415.374	97.112	21.071
27	1	1473.579	19662...	105....	3651.187	1927.468	202.548	25.28
28	1	1509.785	28806...	231....	44794.374	2992.554	345.576	16.925
29	1	1611.35	25776.75	181....	10916.883	2272.657	189.583	23.311
30	1	1848.565	14349...	193....	12898.527	1722.652	144.925	17.273
31	1	1835.828	6934.69	252....	12914.542	1292.136	135.874	27.512
32	1	1900.169	23536...	153	10618.288	2068.133	206.464	18.816

Filter | Density filter | Remove duplicates | Merging | Drift correction | ▶

...ter:

Apply | Restrict to ROI

...-processing history: –

Reset

Preview | Defaults | Plot histogram | Visualization | Import | Expo...

Future work

UI improvements.
UI was to-hand and easy but too many Imagej-OMERO interfaces already.
Clean disconnect on closing Fiji needed.
Currently Imagej OMERO login separate from ThunderSTORM.
Select Table in a single step rather that as a 2-step process.
More extensive OMERO interface required for PALMsiever?

Other File formats.
Handling in Bio-Formats required?
possible to re-use ThunderSTORM import code to generate OMERO Tables?