

Progress-so-far on Single Molecule Localisation Microscopy (SMLM) and OMERO

Ian Munro

Brief introduction to OMERO

Can we standardise access to SMLM data?

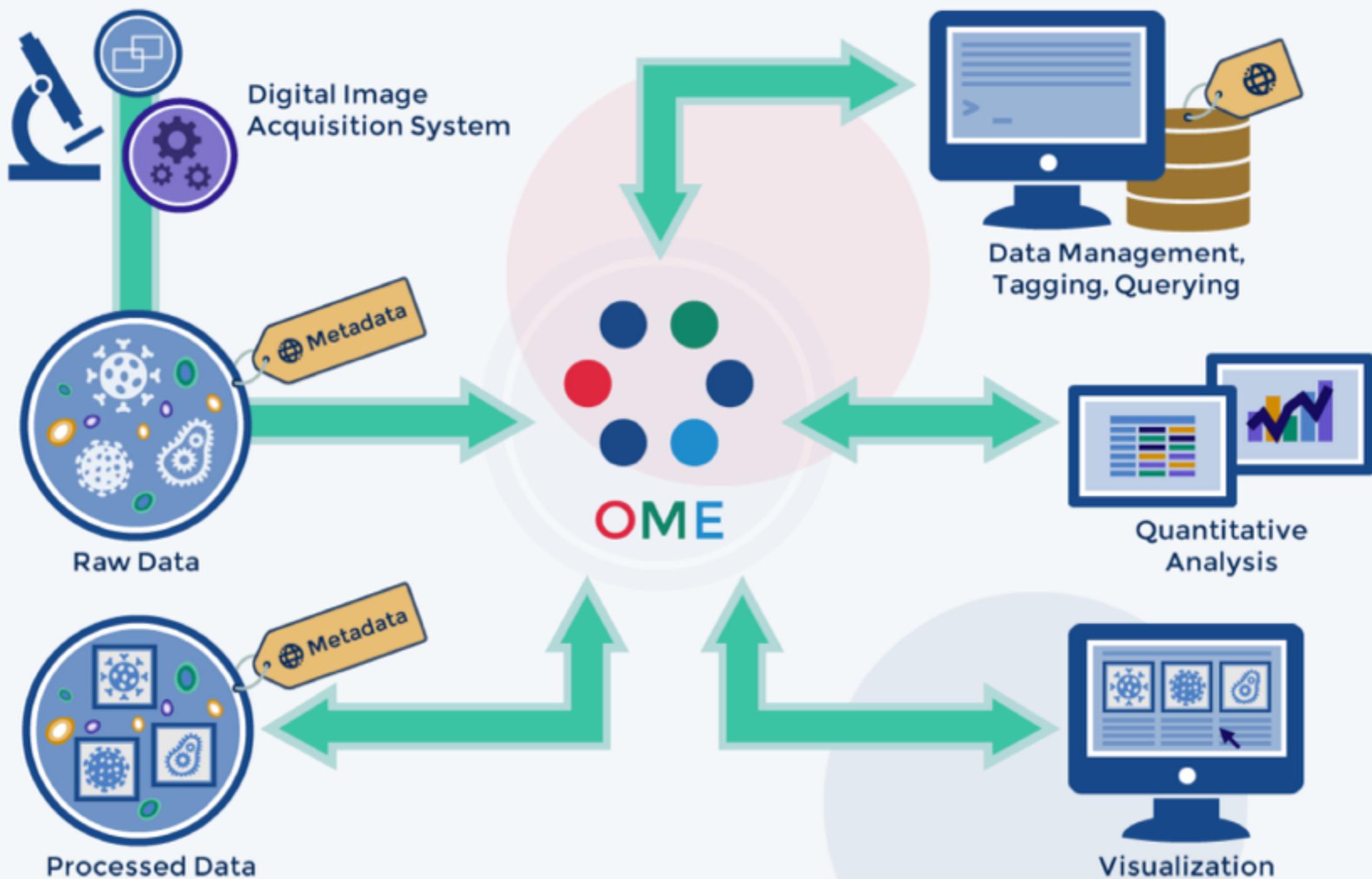
Reading an OMERO.table in ThunderSTORM

Reading an OMERO.table in PALMsiever.

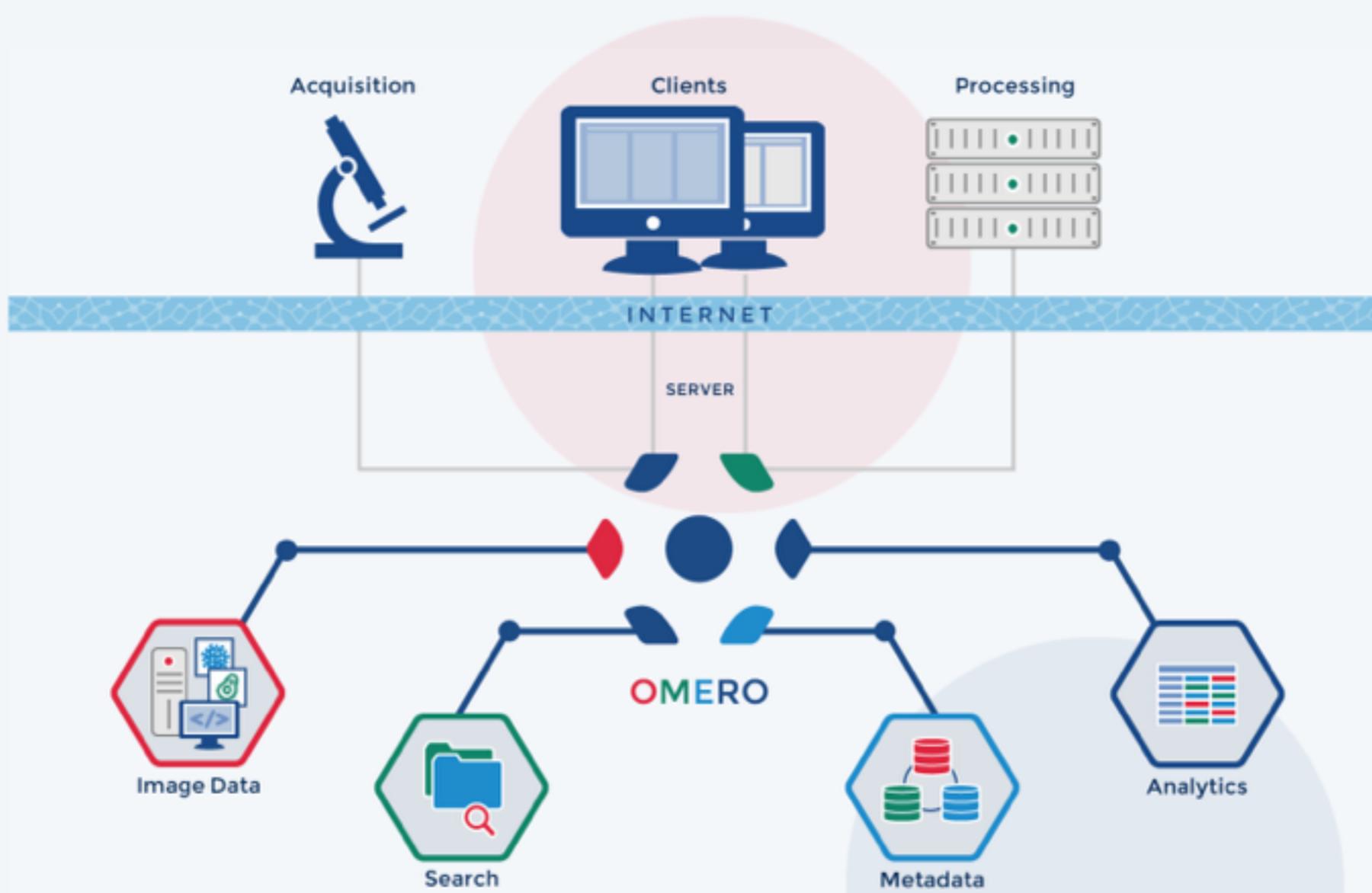
Questions.

The Open Microscopy Environment (OME)

Interoperability

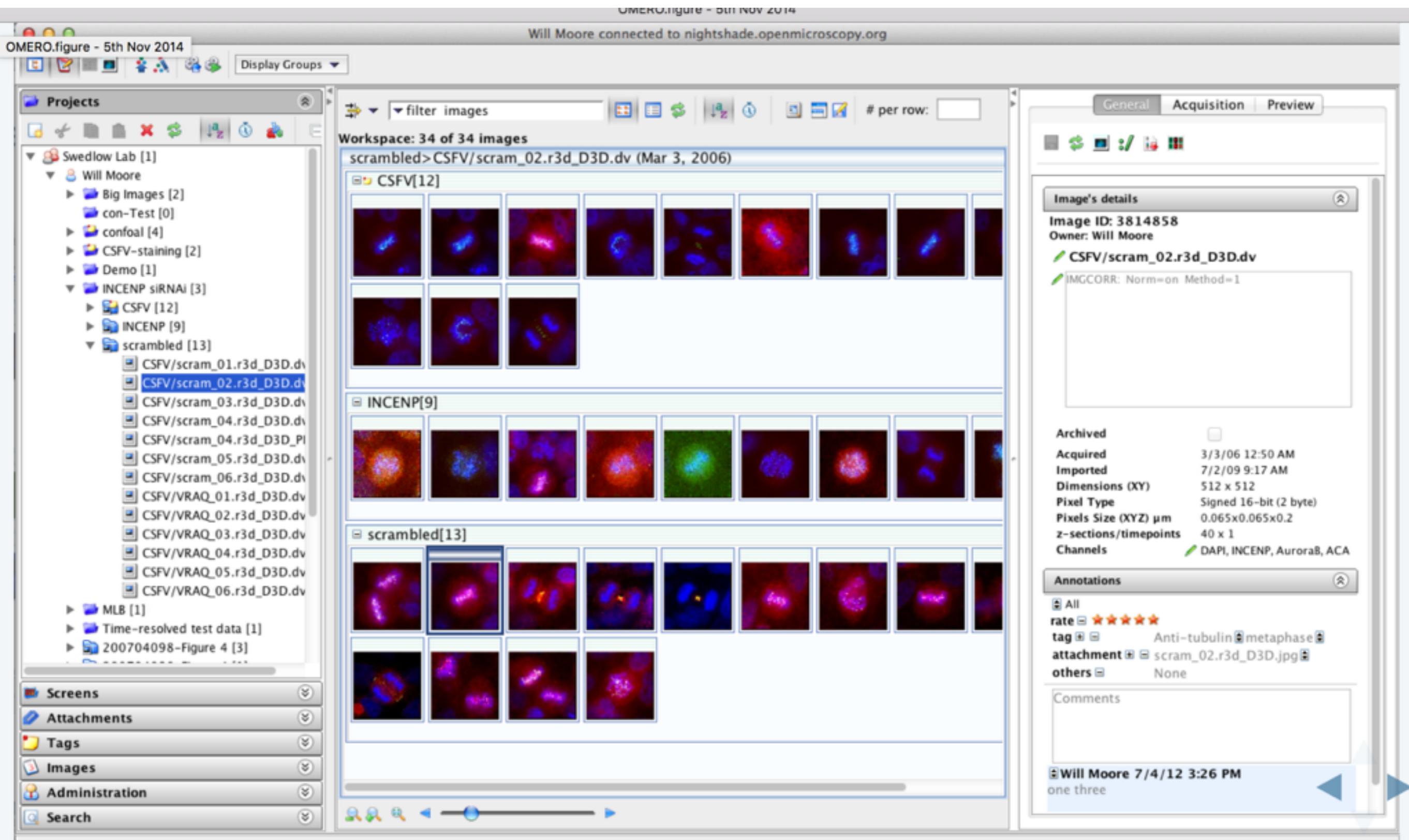


OMERO is a server with clients



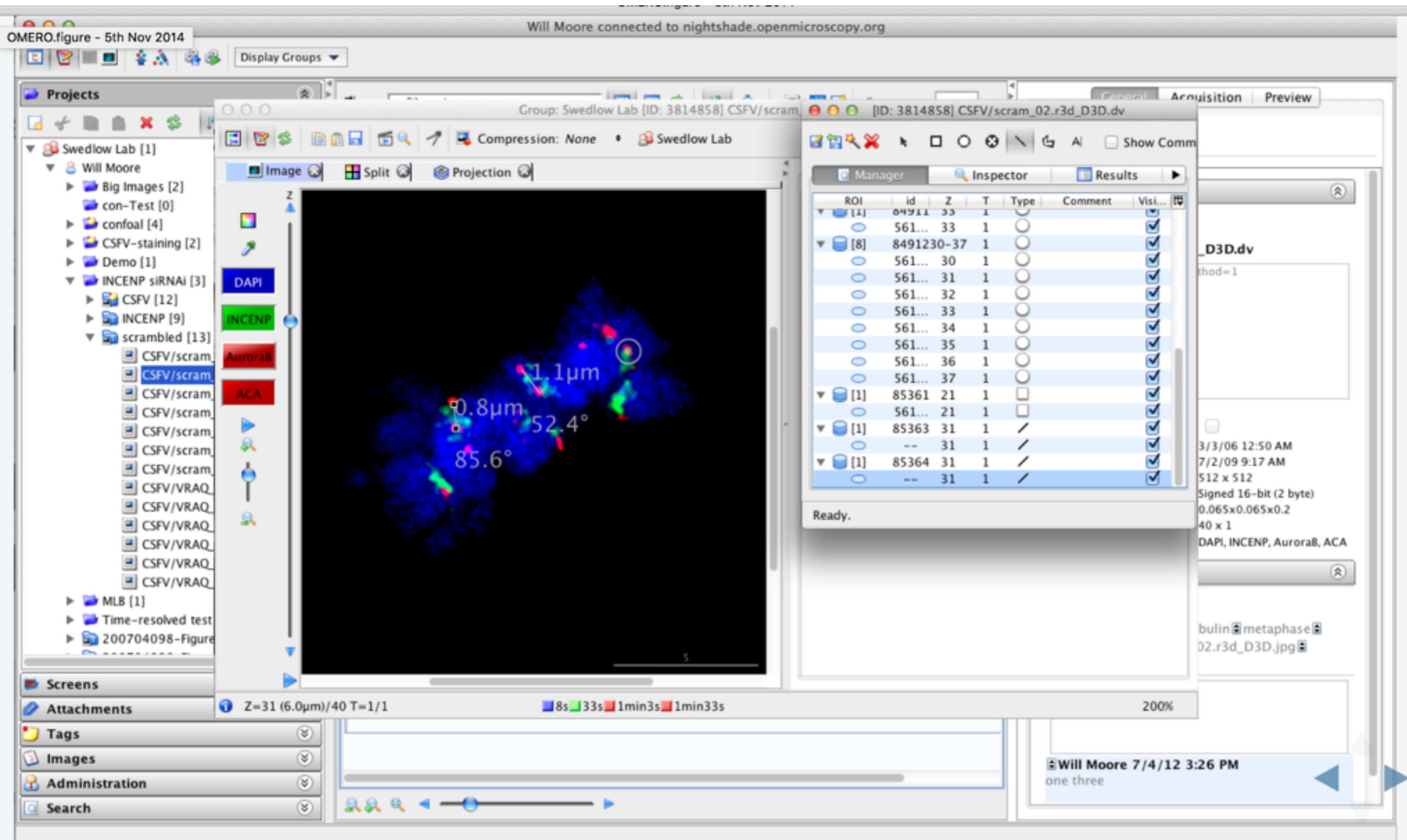
<http://www.openmicroscopy.org/site>

The OMERO.insight Java client

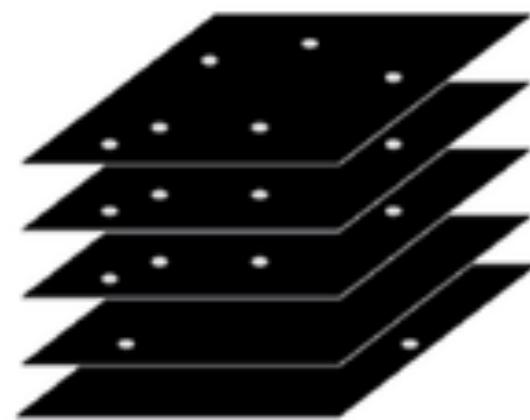


<https://cisbic.bioinformatics.ic.ac.uk/omero/webclient/?show=plate-1751>

OMERO.insight, Image Viewer & ROI tool



SMLM Processing Pipeline



Localisation of individual fluorophores

Image reconstruction

B	C	D	E	F	G	H	I
frame	x [nm]	y [nm]	sigma [nm]	intensity [photons]	offset [photons]	bkgstd [photons]	uncertainty [nm]
1	565.093375	4593.70398	88.1144489	2483.86751	1412.56167	124.370762	15.9594856
2	573.706657	8297.65145	199.523849	11323.782	1525.4344	148.280179	21.3321043
3	217.465555	10944.9208	1389.86985	1544180.97	1.02E-05	114.442172	5.94143148
4	661.275016	9381.96477	250.985661	16498.9683	1513.18498	158.230368	24.7022221
5	701.321427	28382.9711	260.017835	25466.961	2888.42345	288.498803	31.2600562
6	766.41216	5817.39187	221.576255	10393.7079	1375.11842	124.085134	23.9908771
7	745.118959	6906.92549	136.898342	10765.3395	1364.05335	83.6962424	6.09309691
8	1024.74723	1138.376	218.832609	8076.67885	1281.38191	88.7788614	21.5957166
9	881.587431	3187.49	85.6219942	2274.78828	1393.02006	128.357936	16.9720139
0	852.019338	24940.0729	255.291653	16744.7161	2398.81091	192.958104	30.6754388
1	1094.26331	1505.77088	141.16995	4420.31356	1383.82375	114.475862	21.1494903
2	1071.07689	2071.2551	209.831912	7323.53536	1329.15487	84.6248055	20.8850501
3	977.582219	11449.6483	110.156044	4595.09291	1710.44667	184.776507	19.9608257
4	956.927567	20597.5277	241.869673	21656.3333	1867.0193	164.223938	18.15743
5	1062.40765	21902.5969	207.092753	20825.3127	1991.98731	243.307514	20.4743222
6	1109.84182	13384.1099	232.454032	25756.1089	1764.49654	292.58354	25.0612173
7	1227.07812	18286.5967	101.731158	2251.73423	1848.15508	103.418515	19.505932
8	1057.68035	23878.9277	1037.73066	1200230.1	0.03565667	172.270073	6.37073396

No standard file format.
OMERO solution?

PALMsiever. Matlab

ThunderSTORM. Java, Imagej plugin

OMERO.tables

HDF5 backed datastore for managing tabular data in OMERO.

Store data alongside your images in OMERO.

Data can be fetched, either in bulk, or element-by-element, through the OMERO Application Program Interface (API) for analysis.

Split the problem of accessing localisation data into two parts

1) Create OMERO.tables from Localisation data files for some subset of formats.

2) Provide tools with the ability to read OMERO.tables.

SMLM Data in OMERO

Ian Munro connected to demo.openmicroscopy.org

Search ...

Display Groups

Projects

Experimental Formats [1]

- Ian Munro
- Localisation Microscopy. [1]
 - LSMTable [2]
 - cell-555-R4_1.png
 - cell-555-R4_1_MMStack_Pos0.ome.tif
- Mass spec proof-of-concept [1]
- Orphaned images

Screens

Attachments

Tags

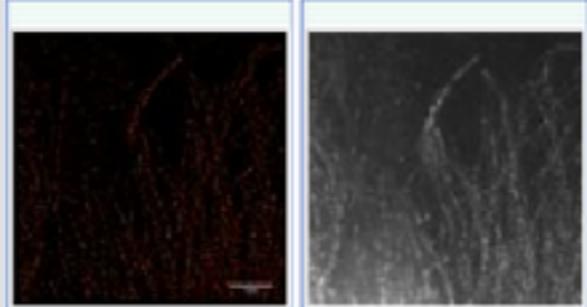
Images

Administration

Search

filter images

Workspace: 2 of 2 images



General Acquisition Preview

LSMTable

Dataset ID: 5079
Owner: Ian Munro [Show all](#)

Dataset Details

Add Description

Creation Date: 2016-01-05 17:08:01

Tags

Key-Value Pairs

Attachments

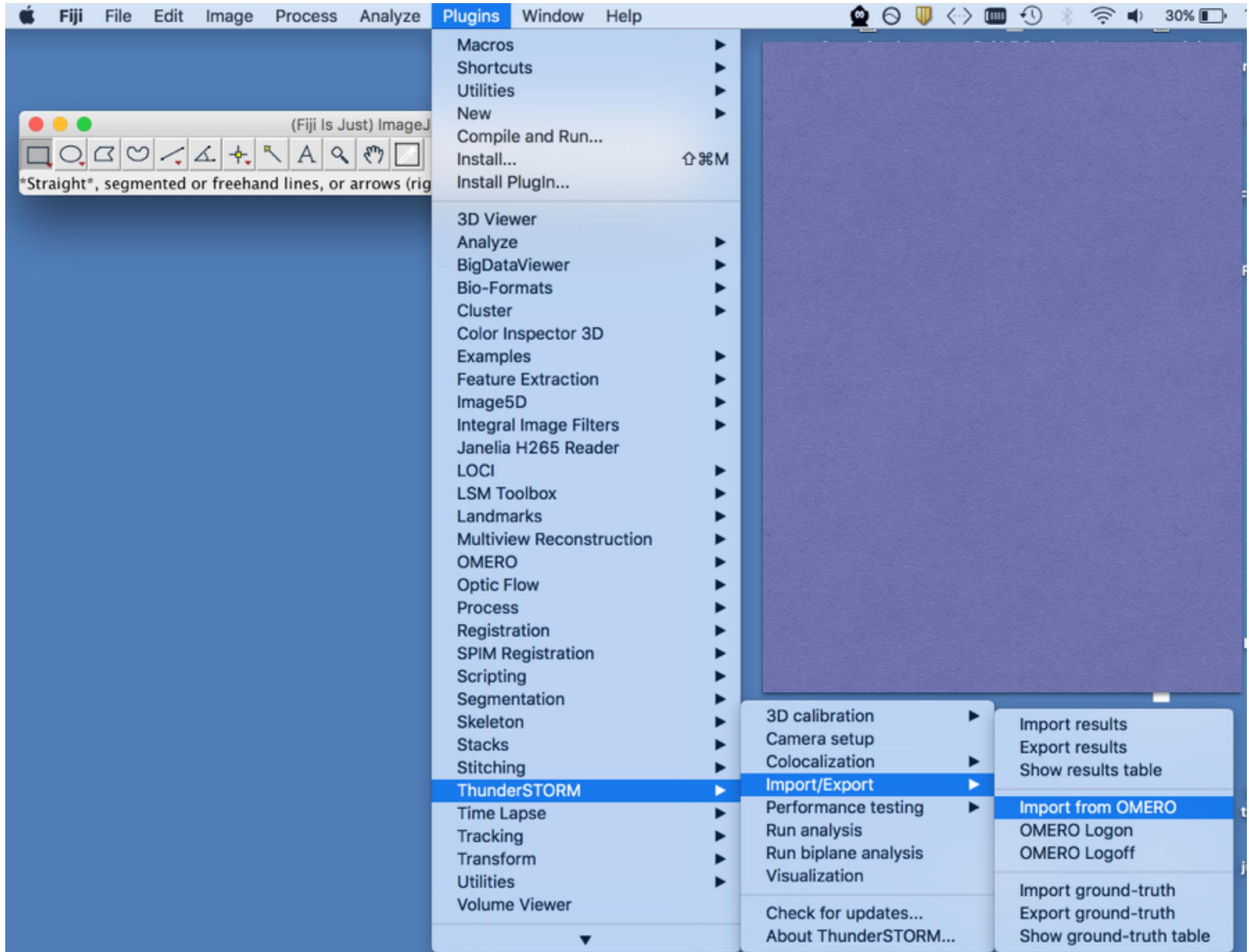
cell-555-R4_1_ts_lst-sr.h5
cell-555-R4_1_ts_lst.xls

Ratings

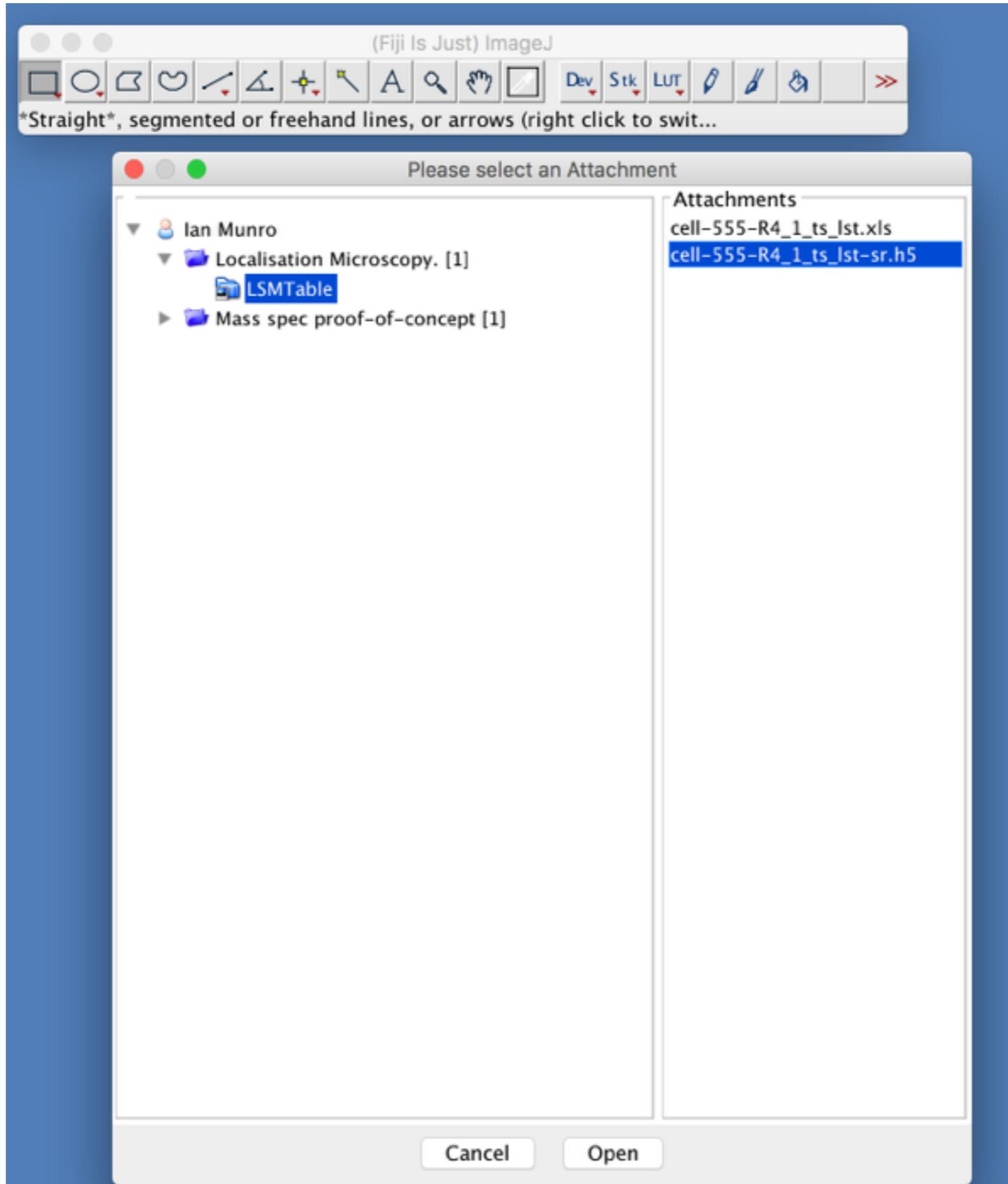
Comments

Located in

ThunderSTORM UI additions - Fiji



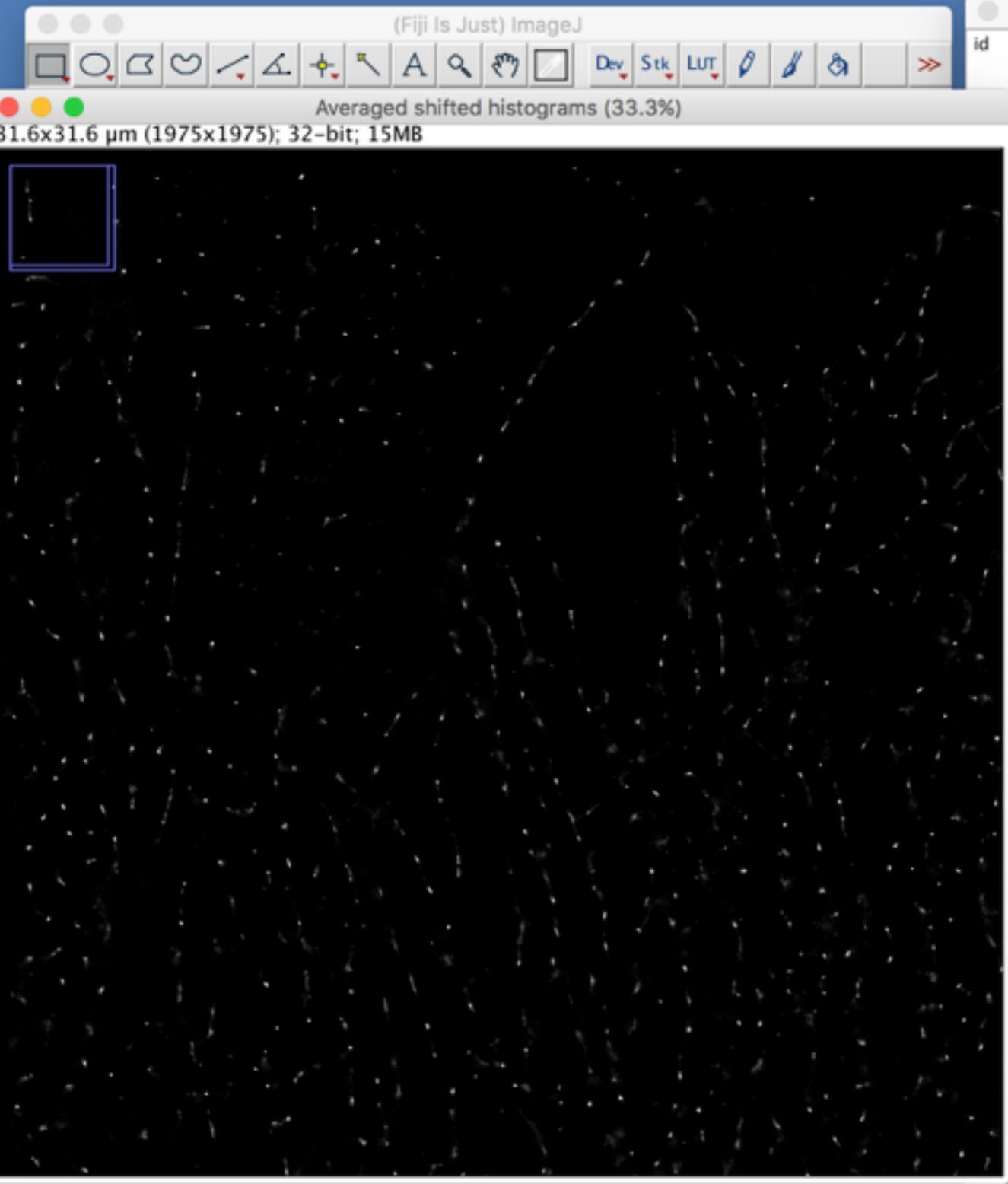
ThunderSTORM UI - select an “Attachment”



ThunderSTORM - result of Import from OMERO

(Fiji Is Just) ImageJ

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	22536	153	10618.288	2068.133	206.464	18.816

Filter Density filter Remove duplicates Merging Drift correction

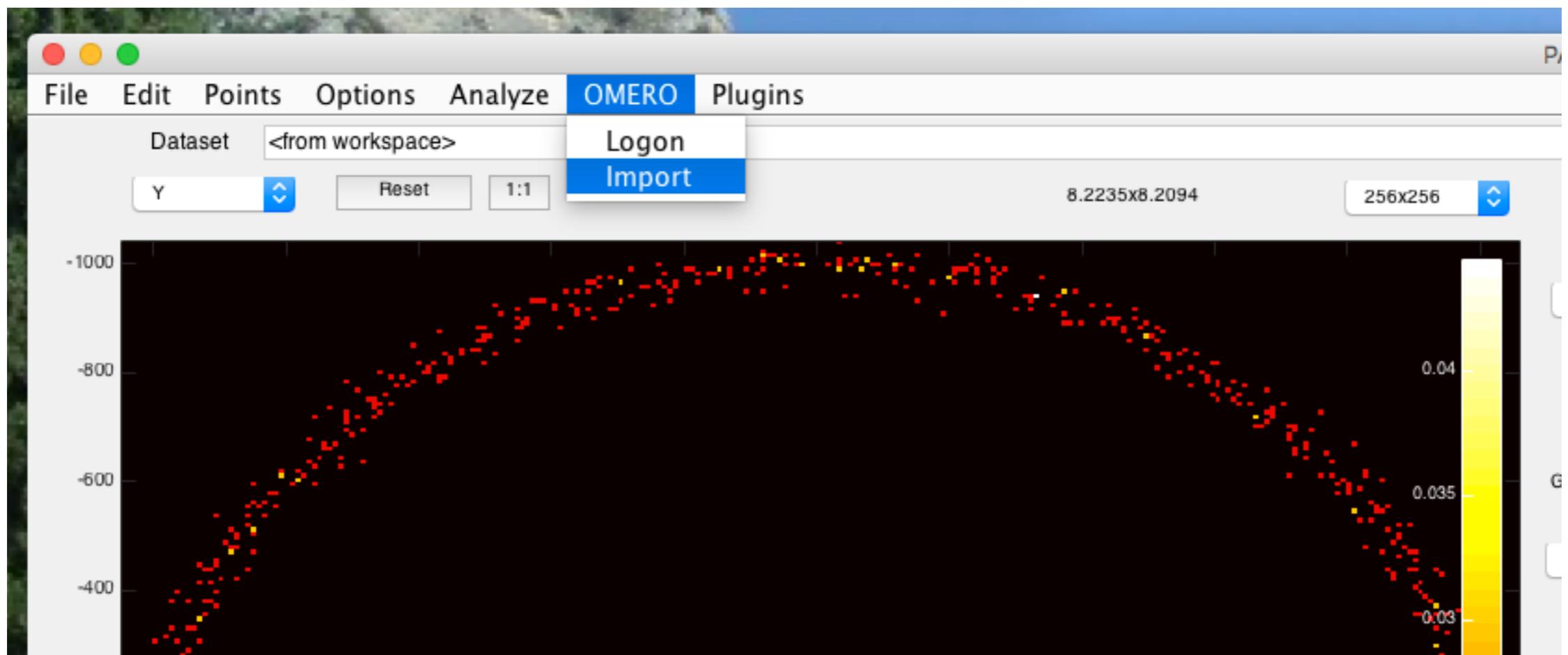
Filter: Apply Restrict to ROI

-processing history: -

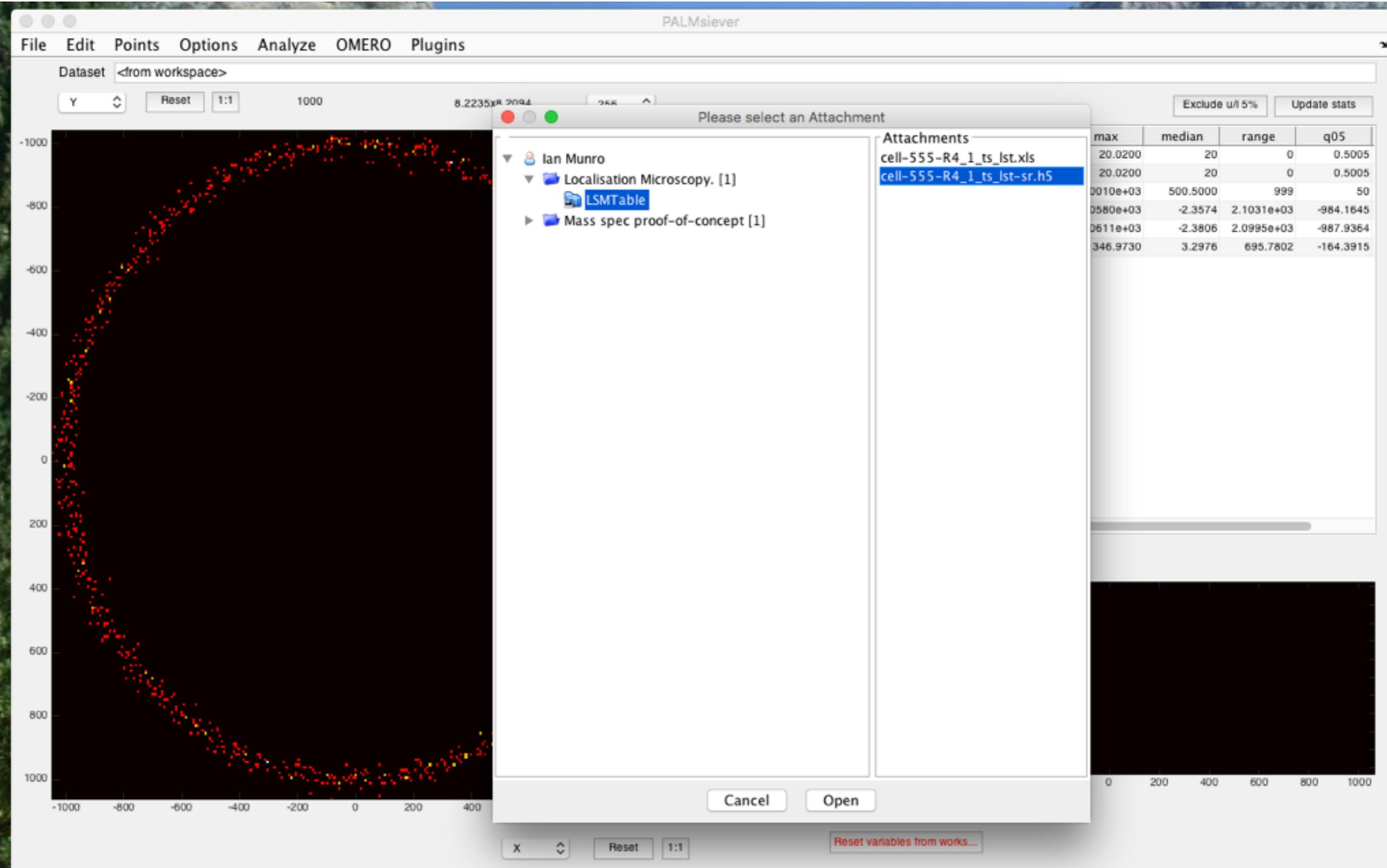
Reset

Preview Defaults Plot histogram Visualization Import Export

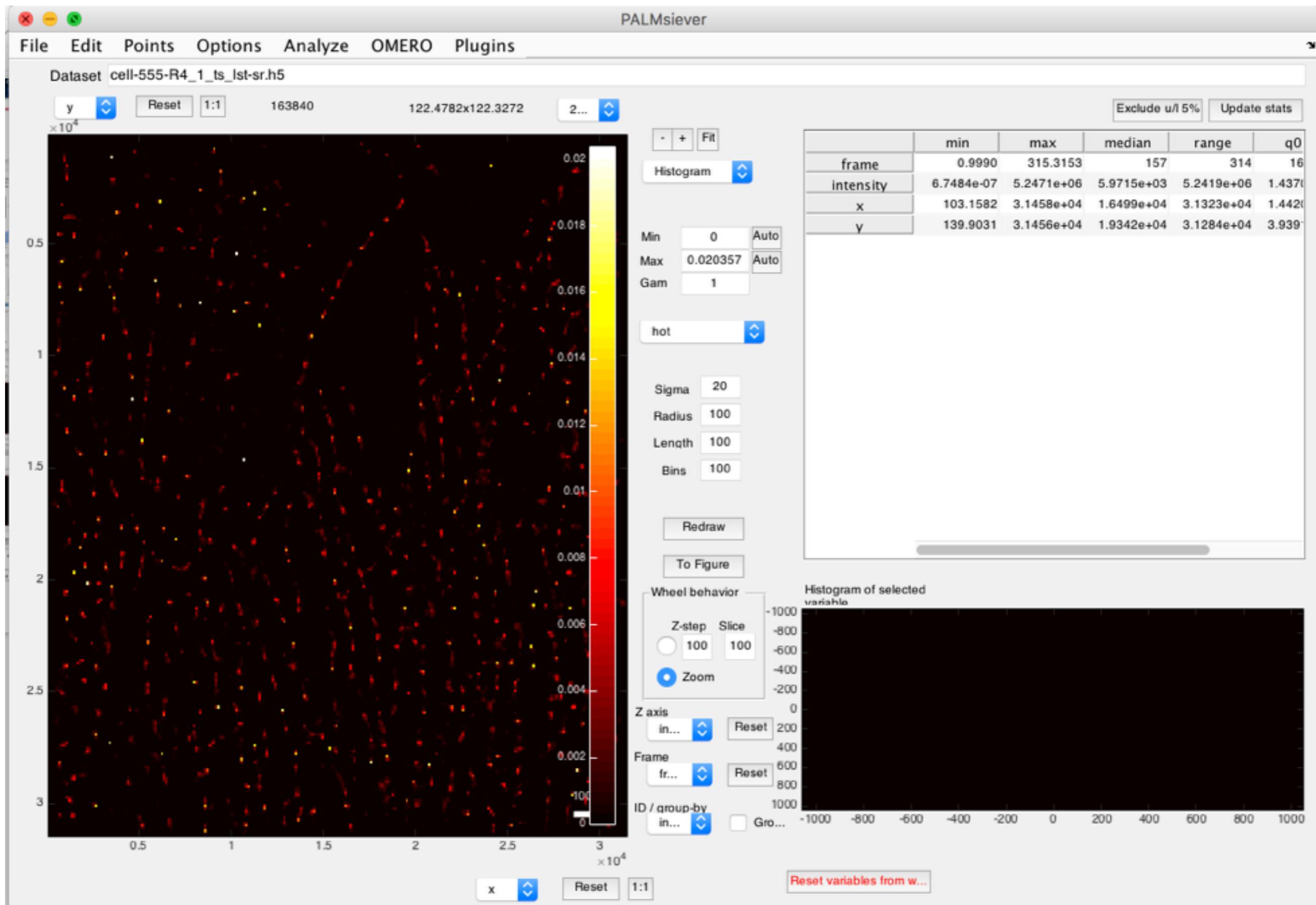
PALMsiever UI additions



PALMsiever UI - select an “Attachment”



PALMsiever - result of Import from OMERO



Progress so far

<https://github.com/imunro/palm-siever>

Feedback, ideas etc wanted

<https://github.com/imunro/thunderstorm>

The next step ?

1. CreateOMERO.tables from Localisation data

Which formats?

What form? Initially a stand-alone Java tool?

2. Add more OMERO functionality to PALMsiever.

Read Image, Save Image - other?

3. Look at ThunderSTORM- Imagej-OMERO interface.

How useable is it?

Share Login - important?

4. Optimise Table Read for speed

Dundee - Jason Swedlow, Colin Blackburn, Jean-Marie Burel, Mark Carroll,
Gus Ferguson, Helen Flynn, Kenny Gillen, Roger Leigh, Simon Li, Dominik
Lindner, Josh Moore, Will Moore, Balaji Ramalingam, Aleksandra Tarkowska,
Petr Walczysko, Eleanor Williams,

Glencoe Software - Chris Allan, Joshua Balance, Andreas Knab, Melissa
Linkert, Emil Rozbicki, Liza Unson, Rebecca Walker, Wilma Woudenberg.

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