FOXO single cell tracking

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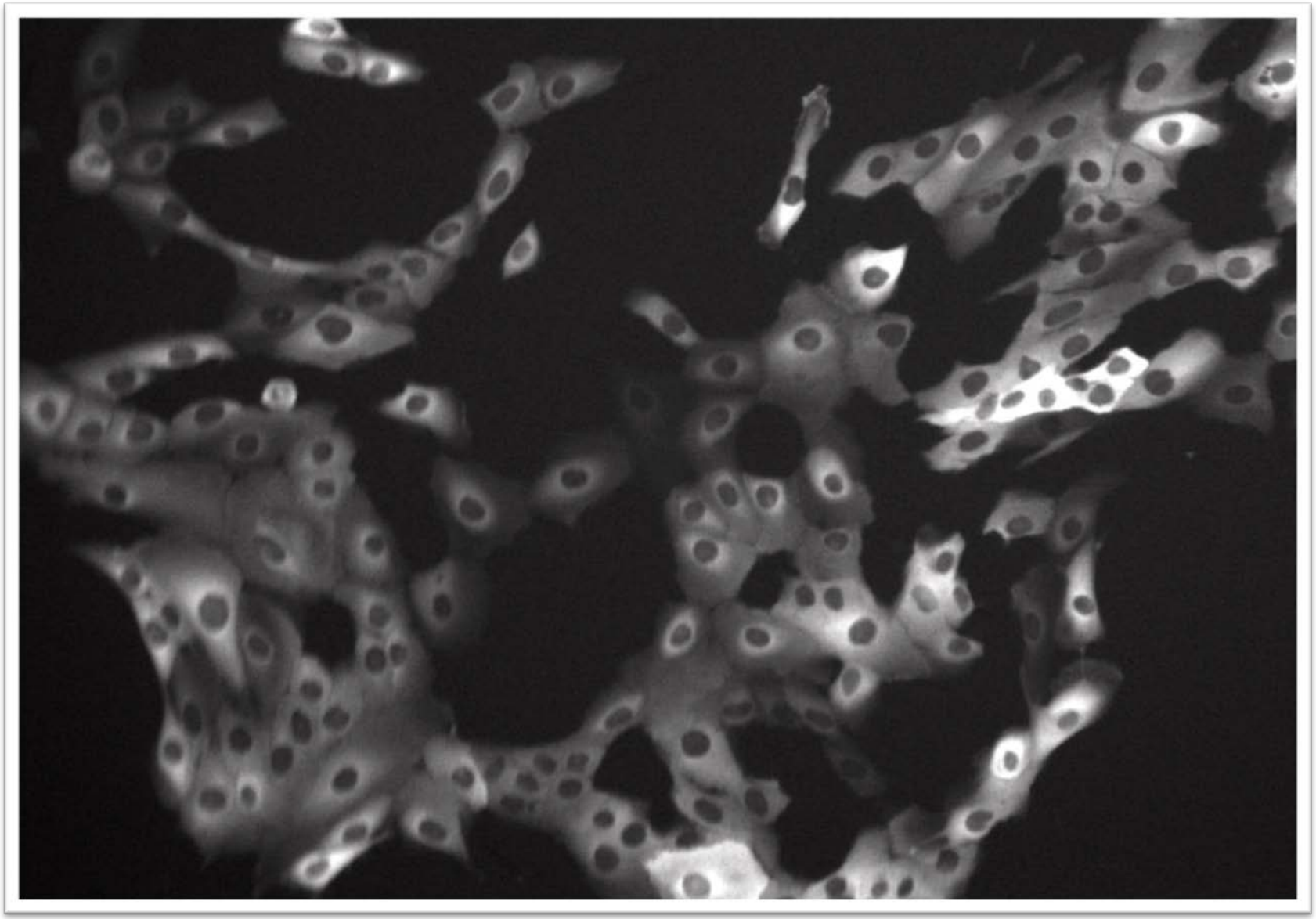
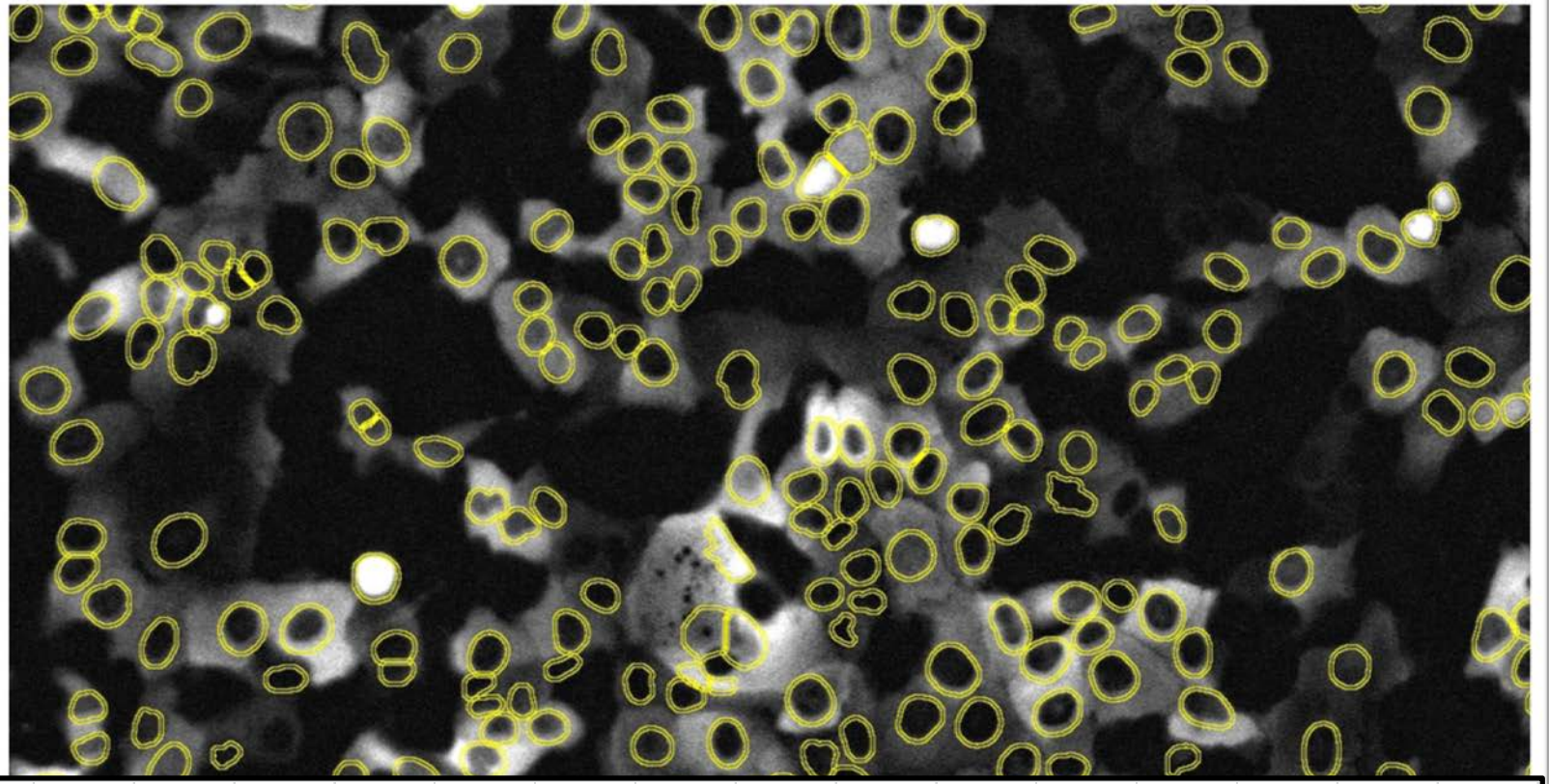
FOXO sensor with IAA treatment



Single cell tracking

- **Segmentation** (ImageJ)
  → *macro-create-mask-xx-opp/nic.ijm*
  → output: measure-mask/cytosol-xxxxxx.csv
- **Tracking** (Perl)
  → *trackXX.pl*
  → output: output.ratio/pos/mean….-xxxxxx.csv
- **Single-cell image generation** (ImageJ)
  → *macro_cropX.ijm*
  →output:: single cell movies
- **Post-analysis** (Matlab)
  → *normplotX.m*
  → output:: single-trace plots & parameters

Segmentation



	Area	Mean	StdDev	Min	Max	X	Y	Perim.	Circ.	Median	%Area	Slice	AR	Round	Solidity
1	163	709.233	249.531	358	1352	1248.85	6.377	47.799	0.897	633	100	1	1.028	0.973	0.939
2	345	787.246	371.616	0	1551	1248.888	8.141	75.113	0.768	779	99.42	1	1.222	0.818	0.796
3	236	1089.339	308.922	633	1787	461.042	10.025	55.941	0.948	1065	100	1	1.089	0.918	0.94
4	493	1096.56	360.438	80	1790	461.131	10.908	83.841	0.881	1119	100	1	1.069	0.936	0.898
5	175	619.549	143.744	358	994	85.066	15.563	50.284	0.87	587	100	1	1.72	0.582	0.933
6	445	593.542	257.697	0	1179	85.08	15.585	78.184	0.915	607	99.551	1	1.405	0.712	0.964
7	216	843.176	232.082	504	1512	358.144	15.963	57.113	0.832	794	100	1	1.682	0.595	0.929
8	512	737.16	425.286	0	1728	358.107	15.895	84.426	0.903	732	98.828	1	1.388	0.721	0.962
9	309	1289.576	493.06	397	2423	384.267	21.319	67.113	0.862	1158	100	1	1.53	0.654	0.941
10	647	1244.328	664.088	0	2423	384.23	21.44	95.012	0.901	1296	99.845	1	1.344	0.744	0.965
11	162	508.864	157.27	268	1065	494.506	25.593	46.284	0.95	471	100	1	1.177	0.85	0.931
12	416	618.966	304.248	0	1255	494.572	25.591	74.184	0.95	556	99.76	1	1.105	0.905	0.963
13	286	1164.818	395.789	330	2189	827.584	40.871	61.941	0.937	1033	100	1	1.185	0.844	0.95

Tracking: Algorithm

	Area	Mean	StdDev	Min	Max	X	Y	Perim.	Circ.	Median	%Area	Slice	AR	Round	Solidity
1	163	709.233	249.531	358	1352	1248.85	6.377	47.799	0.897	633	100	1	1.028	0.973	0.939
2	345	787.246	371.616	0	1551	1248.888	8.141	75.113	0.768	779	99.42	1	1.222	0.818	0.796
3	236	1089.339	308.922	633	1787	461.042	10.025	55.941	0.948	1065	100	1	1.089	0.918	0.94
4															
5															
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10															
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12															
13															

	Area	Mean	StdDev	Min	Max	X	Y	Perim.	Circ.	Median	%Area	Slice	AR	Round	Solidity
441	197	781.234	235.874	455	1313	1246.464	8.967	52.284	0.906	704	100	2	1.304	0.767	0.934
442	422	815.739	342.553	0	1375	1246.429	10.273	79.598	0.837	850	99.289	2	1.047	0.955	0.859
443	183	626.842	150.11	319	1007	91.085	16.451	50.87	0.889	602	100	2	1.604	0.623	0.943
444	453	610.091	268.103	26	1231	91.092	16.425	78.184	0.931	606	100	2	1.355	0.738	0.968
445	218	1085.367	332.883	517	1802	466.858	16.807	53.698	0.95	1072	100	2	1.024	0.977	0.942
446	502	1081.309	378.035	115	1858	466.835	16.777	81.598	0.947	1156	100	2	1.023	0.978	0.966
447	227	839.256	217.302	487	1516	367.196	16.104	56.87	0.882	801	100	2	1.498	0.668	0.946
448	521	780.825	321.325	80	1550	367.162	16.049	84.184	0.924	761	100	2	1.309	0.764	0.968
449	299	1303.97	517.891	510	2581	391.49	26.704	65.941	0.864	1132	100	2	1.553	0.644	0.937
450	633	1264.967	636.493	23	2581	391.538	26.762	93.841	0.903	1283	100	2	1.353	0.739	0.963

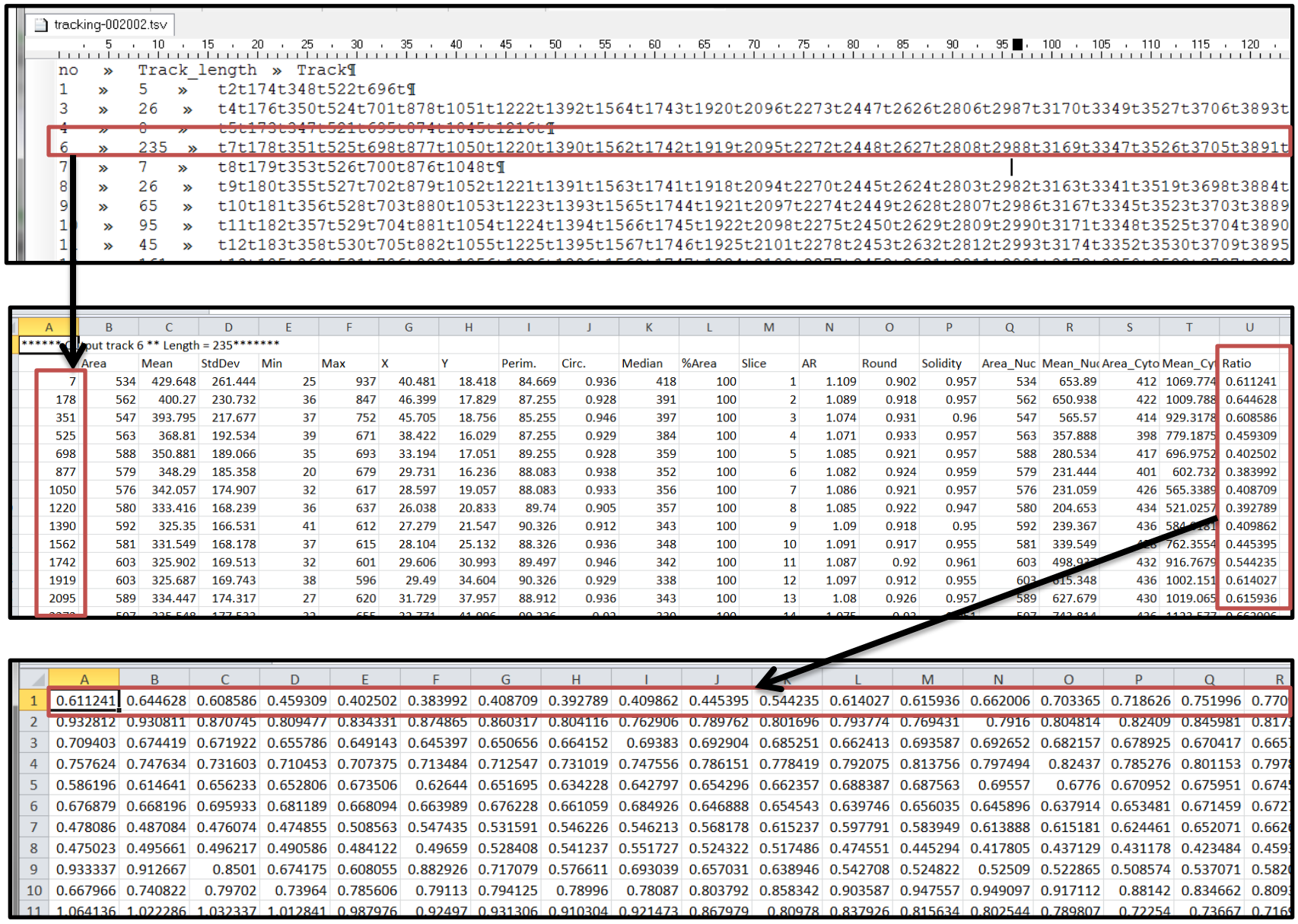
Read Input file (X,Y,Slice,Mean,Area)

Finding Shortest points in adjacent slices

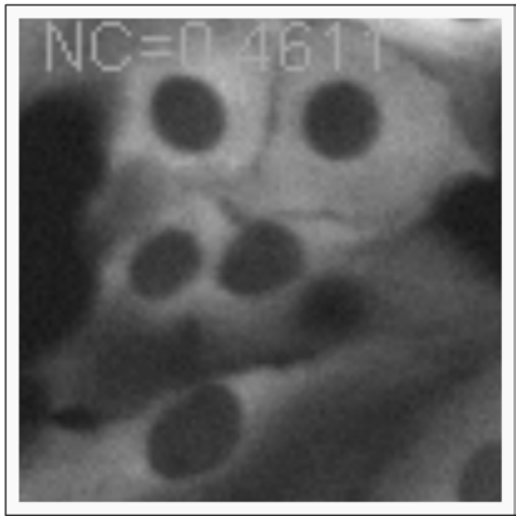
Back-Tracking: for new objects (mitosis?)

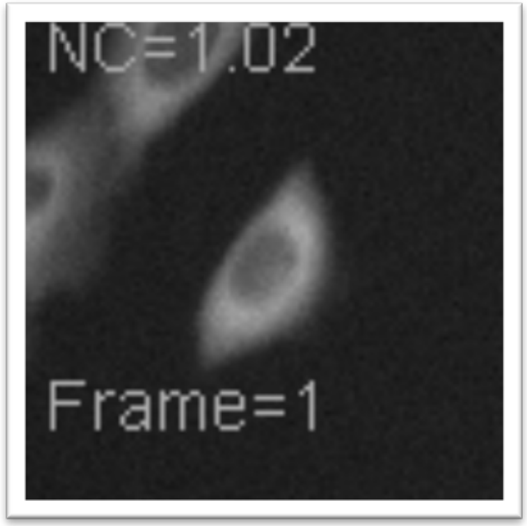
Post-possessing: Removing incorrect tracks by mean/area checking; removing duplicate tracks

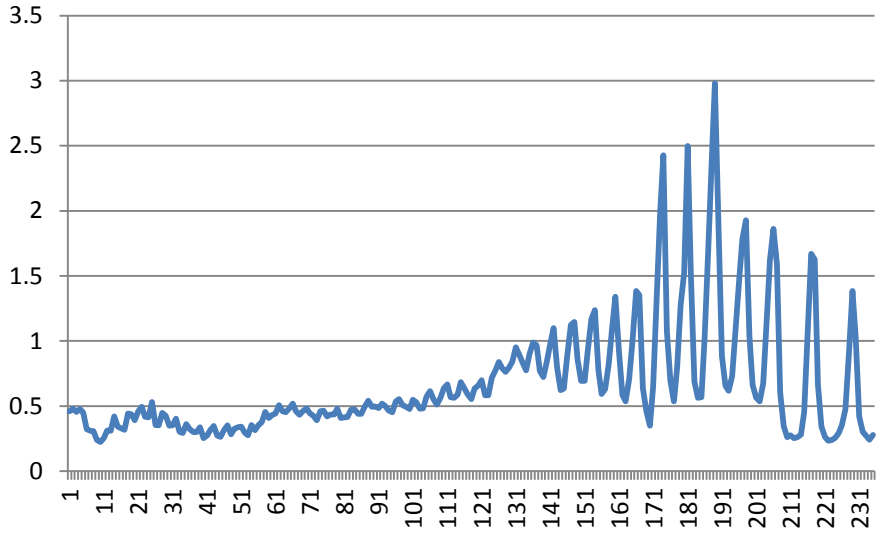
Tracking: output

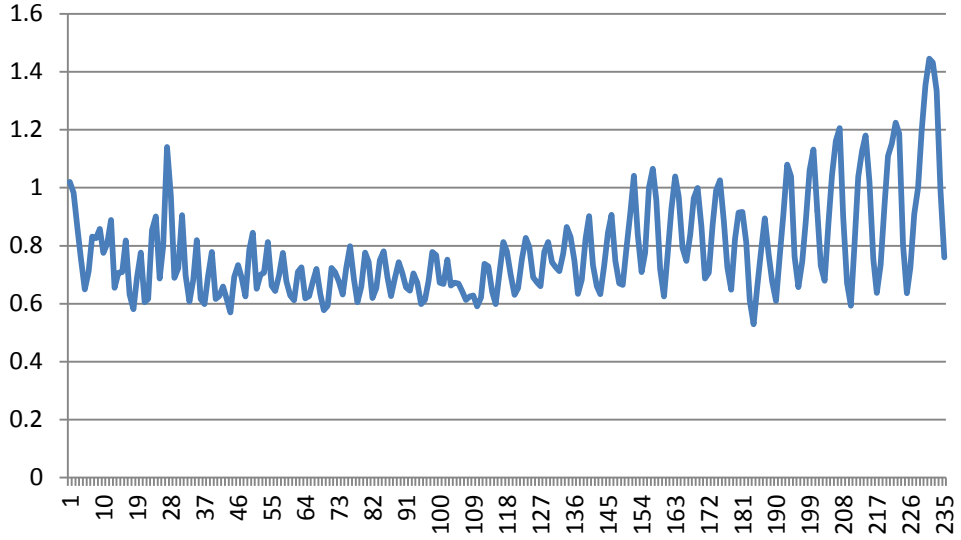


Single cell traces

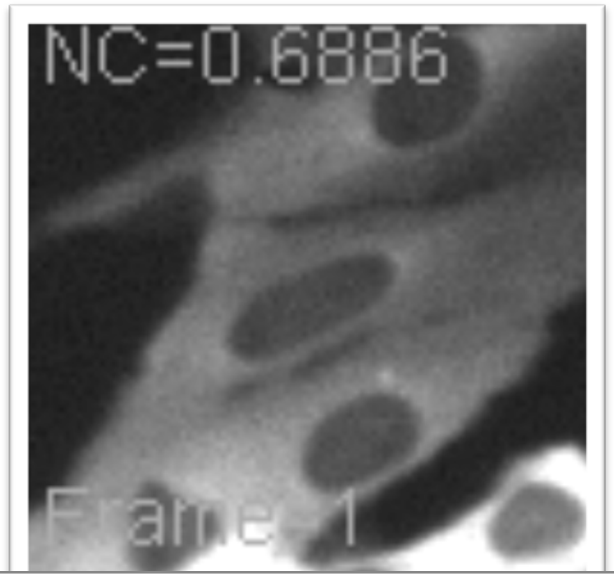


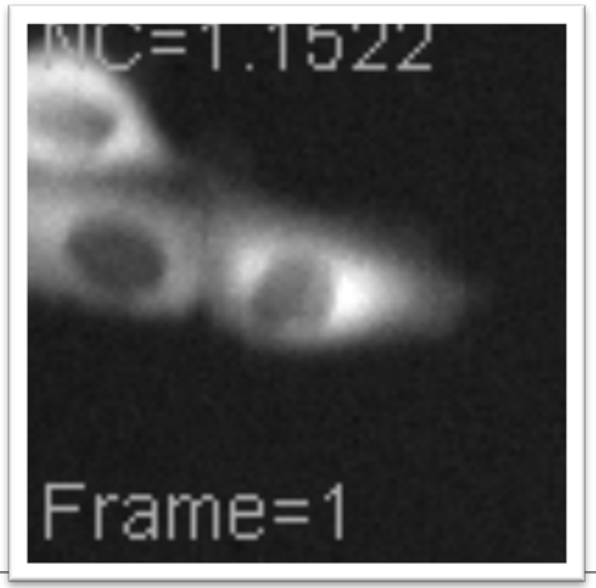


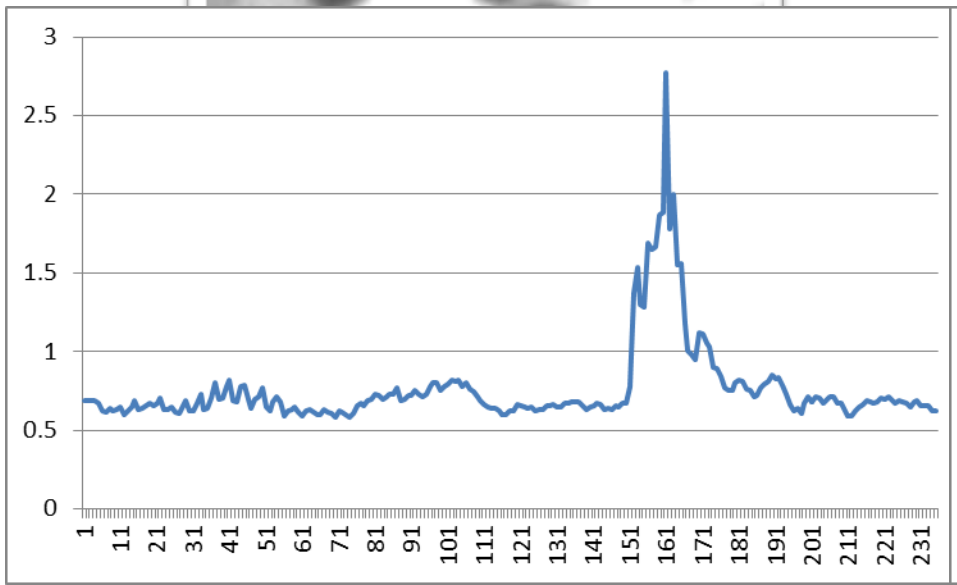


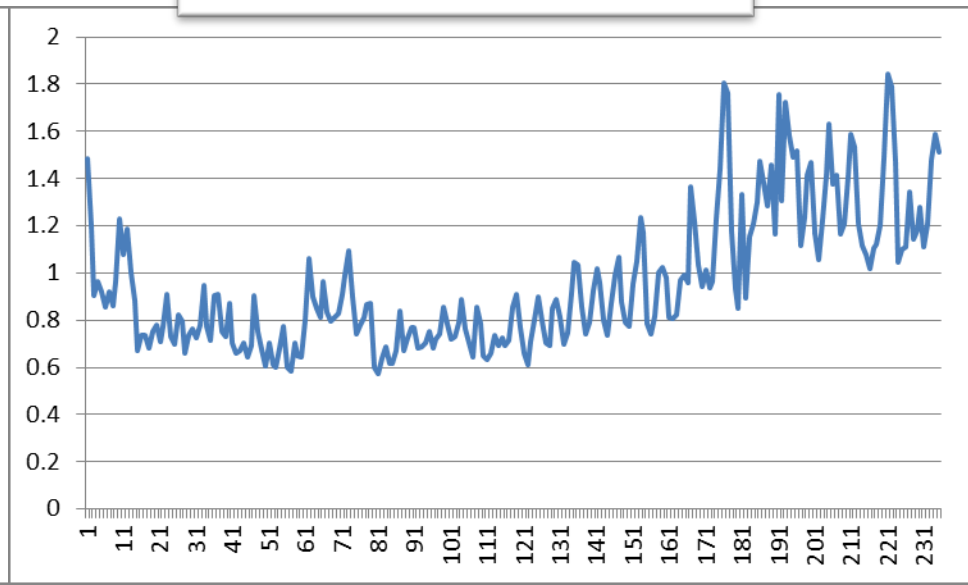


Single cell traces

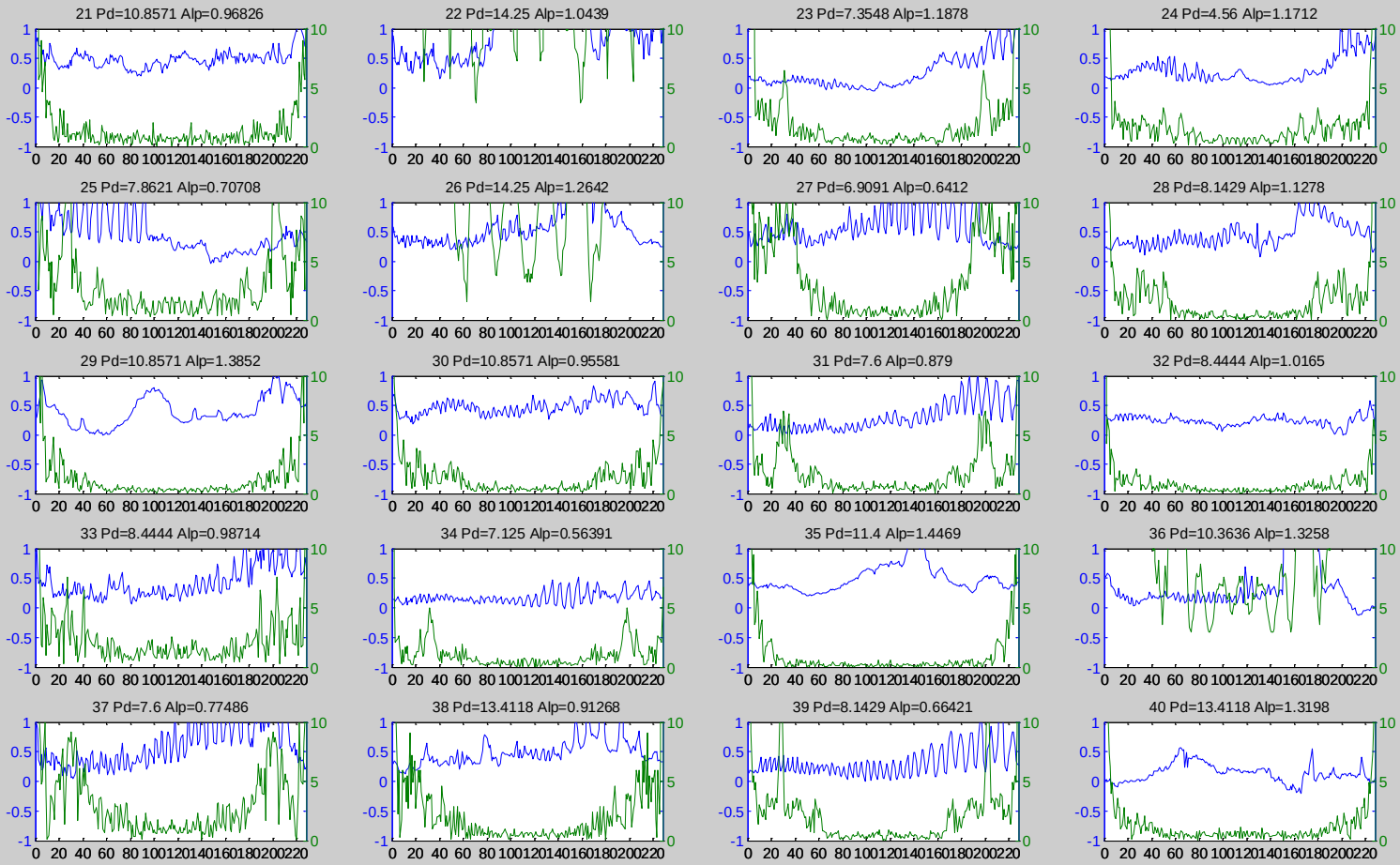






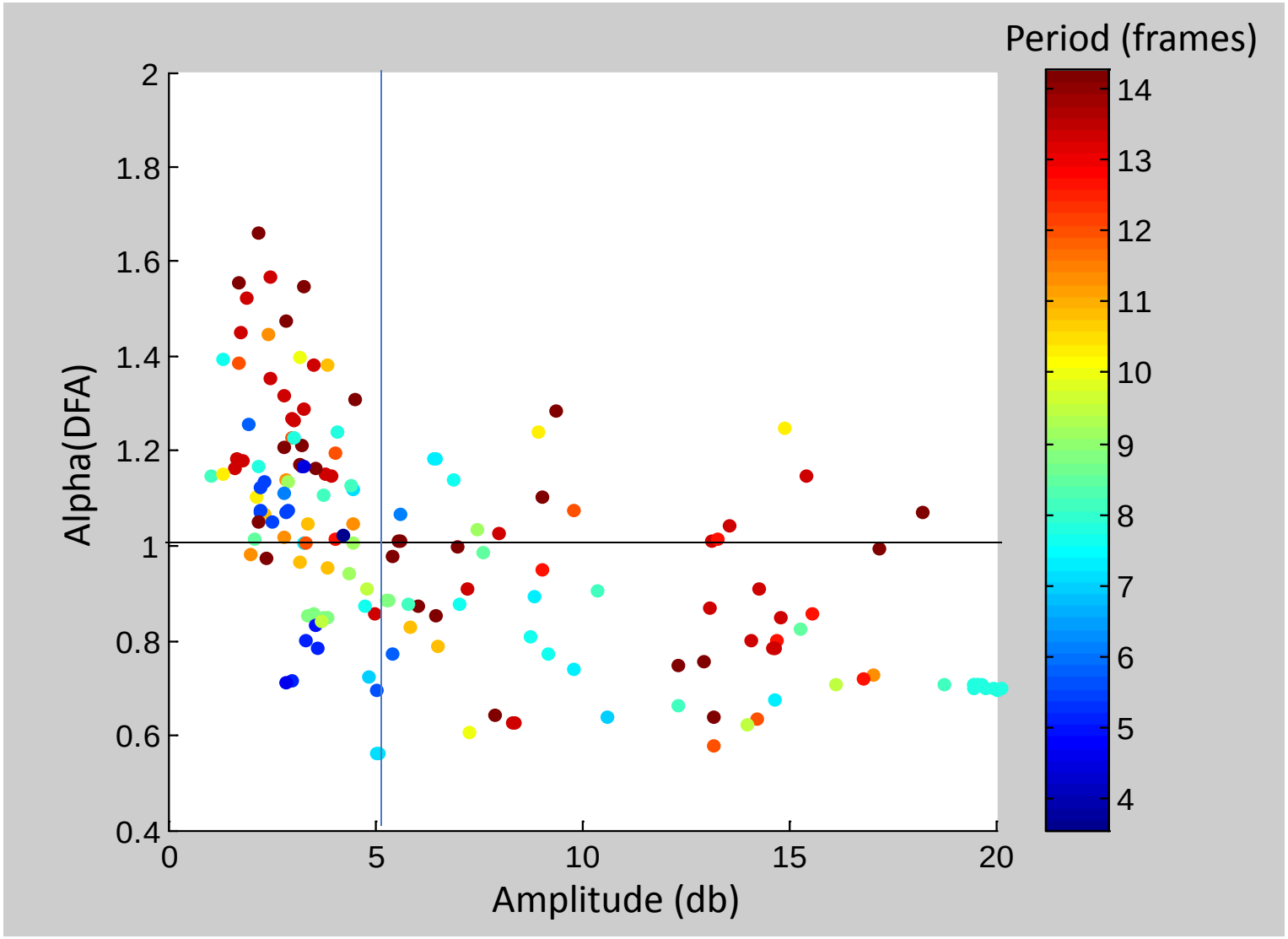


Multiple Traces analysis (Matlab)



Power Spectrum & Detrended Fluctuation Analysis (DFA)

Amplitude, Frequency & alpha(DFA)



Segmentation Script (ImageJ)

- ***Macro-Create-Mask-10-nic.ijm***
  → For NIC images only
  → Usage :: assign **site** number (batch model)
  → Usage :: change input & output **directories**

- ***Macro-Create-Mask-10-opp.ijm***
  → For Operetta images
  → Usage :: assign **row & col**
  → Usage :: change input & output **directories**

Tracking Script (Perl)

- ***Track12.pl***
- Move/copy this script into the output directory of segmentation outputs
- Usage (NIC)::
  >perl track12.pl ***start_site end_site 99 99***
- Usage (Opp)::
  >perl track12.pl ***start_row end_row start_col end_col***

Trace files generation (Perl)

- ***Crop01.pl***
- Copy this script to the output directory
- Usage::
  >perl crop01.pl **site/well**
- Examples::
  >perl crop01.pl s1 (NIC images)
  >perl crop01.pl 002002 (Operetta images)
- This script will create a new directory using the well/site name for trace files

Single-cell movie generation (ImageJ)

- ***Macro_crop2.ijm***
- Pre-request→ Import image sequence and rename the stack into "**Image1**"
- Usage::
  run this macro with ImageJ and then pick the trace file of interest
- Output→ single cell stack movie

Ratio CSV file importing (Matlab)

- ***Ratio_xls_import.m***
- Usage
  → change **filename**
  → assign output parameter (**WellSxx**)
- Output
  → ratio matrix (WellSxx)

Analysis & trace plot (Matlab)

- ***Normplot5.m***
- Input → the ratio matrix generated/imported by ratio_xls_import.m
- Usage::

*[**period,Db,DFA**]=normplot5(**WellSxx,number**)*

- Output:: plot single-cell traces & power-spectrum and store oscillation parameters