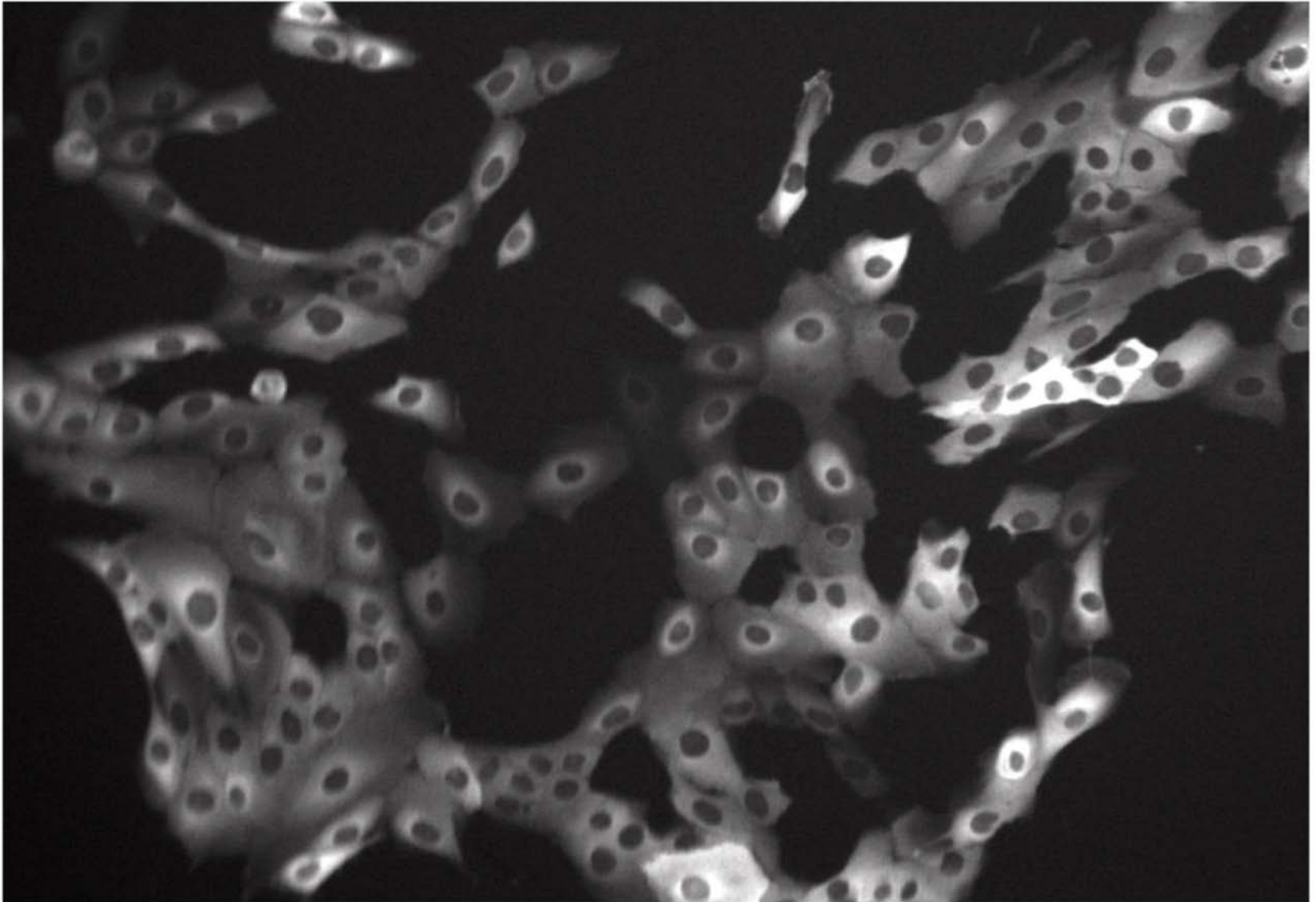


FOXO single cell tracking

Jerry

2014/09/15

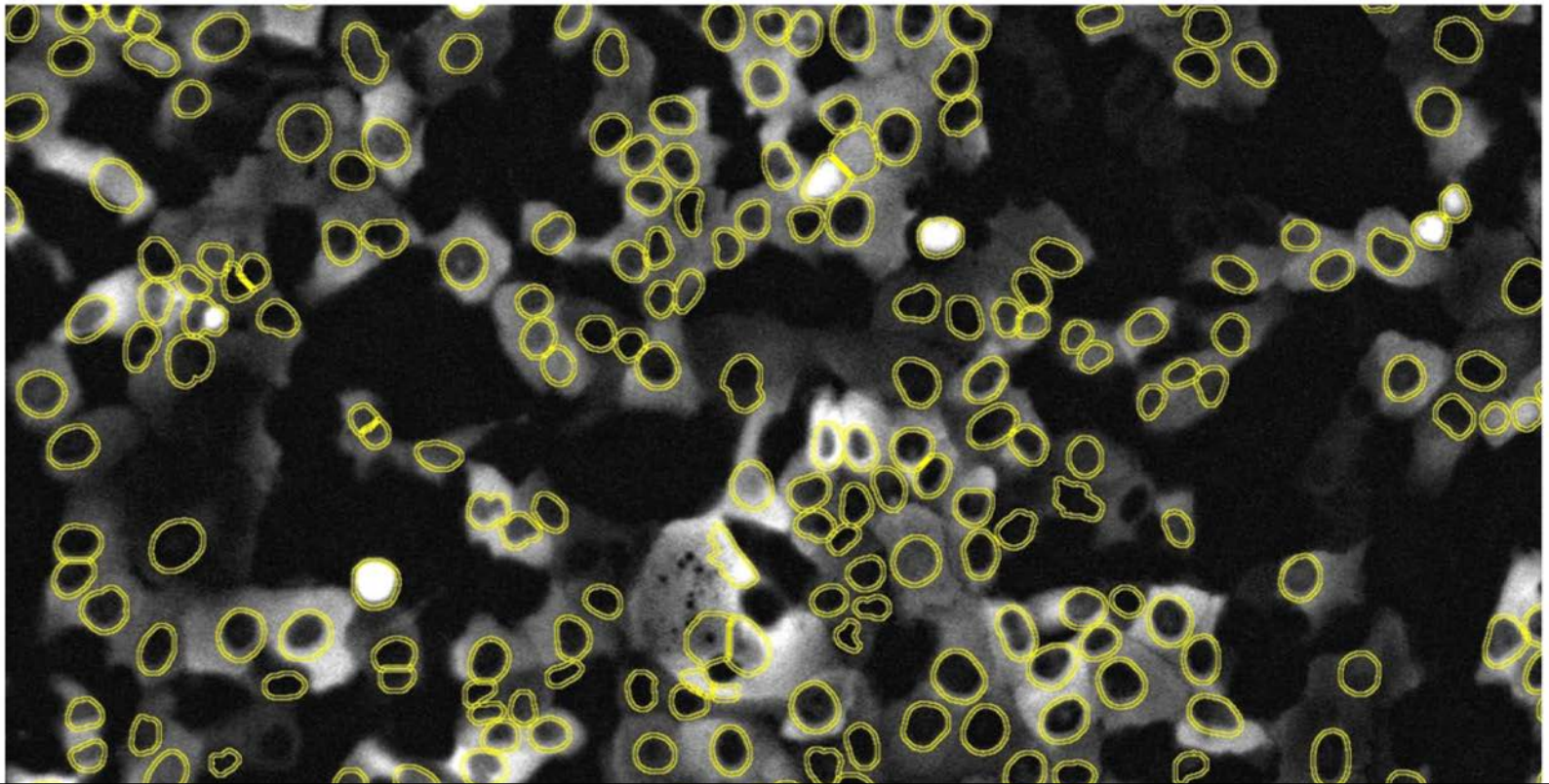
FOXO sensor with IAA treatment



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	709.238	249.531	358	1352	1248.85	6.377	47.799	0.897	633	100	1	1.028	0.973	0.939	
2	345	787.245	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	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
5	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
6	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
7	444	453	610.091	268.103	26	1233	91.092	16.425	78.184	0.931	606	100	2	1.355	0.738	0.968
8	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
9	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
10	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
11	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
12	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
13	450	633	1264.967	636.493	23	2583	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-processing: Removing incorrect tracks by mean/area checking; removing duplicate tracks

Tracking: output

no		Track_length	Track
1	5		t2t174t348t522t696t
3	26		t4t176t350t524t701t878t1051t1222t1392t1564t1743t1920t2096t2273t2447t2626t2806t2987t3170t3349t3527t3706t3893t
4	0		t5t173t347t521t695t874t1045t1216t
6	235		t7t178t351t525t698t877t1050t1220t1390t1562t1742t1919t2095t2272t2448t2627t2808t2988t3169t3347t3526t3705t3891t
7	7		t8t179t353t526t700t876t1048t
8	26		t9t180t355t527t702t879t1052t1221t1391t1563t1741t1918t2094t2270t2445t2624t2803t2982t3163t3341t3519t3698t3884t
9	65		t10t181t356t528t703t880t1053t1223t1393t1565t1744t1921t2097t2274t2449t2628t2807t2986t3167t3345t3523t3703t3889
1	95		t11t182t357t529t704t881t1054t1224t1394t1566t1745t1922t2098t2275t2450t2629t2809t2990t3171t3348t3525t3704t3890
1	45		t12t183t358t530t705t882t1055t1225t1395t1567t1746t1925t2101t2278t2453t2632t2812t2993t3174t3352t3530t3709t3895

Track
file

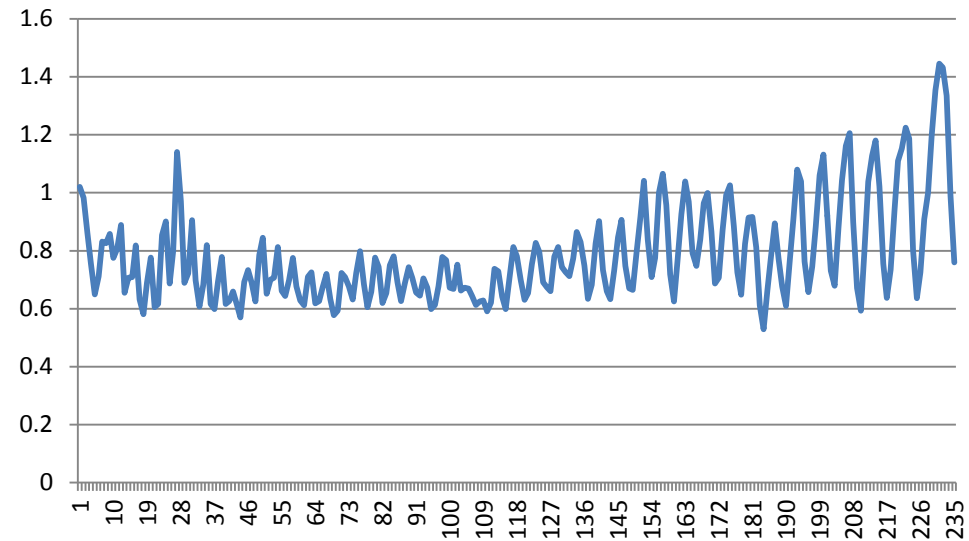
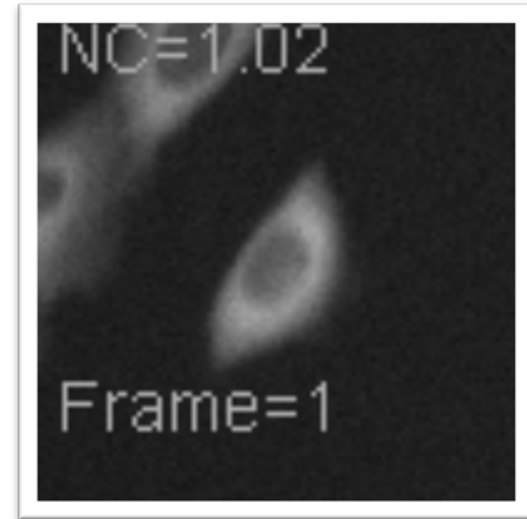
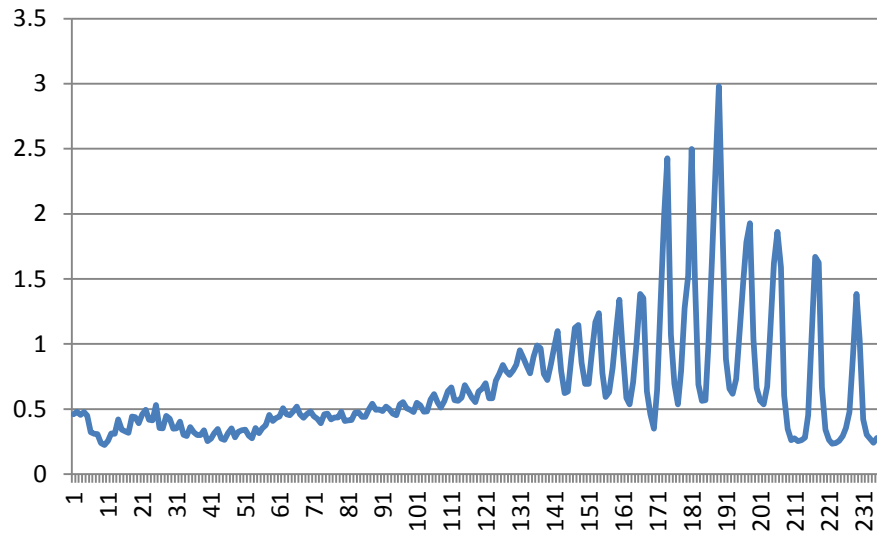
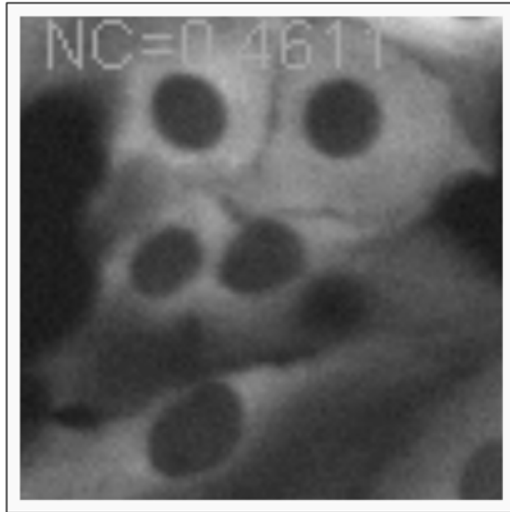
*****Input track 6 ** Length = 235*****																					
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	
Area	Mean	StdDev	Min	Max	X	Y	Perim.	Circ.	Median	%Area	Slice	AR	Round	Solidity	Area_Nuc	Mean_Nuc	Area_Cyto	Mean_Cy	Ratio		
7	534	429.648	261.444	25	937	40.481	18.418	84.669	0.936	418	100	1	1.109	0.902	0.957	534	653.89	412	1069.774	0.611241	
178	562	400.27	230.732	36	847	46.399	17.829	87.255	0.928	391	100	2	1.089	0.918	0.957	562	650.938	422	1009.788	0.644628	
351	547	393.795	217.677	37	752	45.705	18.756	85.255	0.946	397	100	3	1.074	0.931	0.96	547	565.57	414	929.3178	0.608586	
525	563	368.81	192.534	39	671	38.422	16.029	87.255	0.929	384	100	4	1.071	0.933	0.957	563	357.888	398	779.1875	0.459309	
698	588	350.881	189.066	35	693	33.194	17.051	89.255	0.928	359	100	5	1.085	0.921	0.957	588	280.534	417	696.9752	0.402502	
877	579	348.29	185.358	20	679	29.731	16.236	88.083	0.938	352	100	6	1.082	0.924	0.959	579	231.444	401	602.732	0.383992	
1050	576	342.057	174.907	32	617	28.597	19.057	88.083	0.933	356	100	7	1.086	0.921	0.957	576	231.059	426	565.3389	0.408709	
1220	580	333.416	168.239	36	637	26.038	20.833	89.74	0.905	357	100	8	1.085	0.922	0.947	580	204.653	434	521.0257	0.392789	
1390	592	325.35	166.531	41	612	27.279	21.547	90.326	0.912	343	100	9	1.09	0.918	0.95	592	239.367	436	584.2181	0.409862	
1562	581	331.549	168.178	37	615	28.104	25.132	88.326	0.936	348	100	10	1.091	0.917	0.955	581	339.549	428	762.3554	0.445395	
1742	603	325.902	169.513	32	601	29.606	30.993	89.497	0.946	342	100	11	1.087	0.92	0.961	603	498.923	432	916.7679	0.544235	
1919	603	325.687	169.743	38	596	29.49	34.604	90.326	0.929	338	100	12	1.097	0.912	0.955	603	615.348	436	1002.151	0.614027	
2095	589	334.447	174.317	27	620	31.729	37.957	88.912	0.936	343	100	13	1.08	0.926	0.957	589	627.679	430	1019.065	0.615936	

Data
file

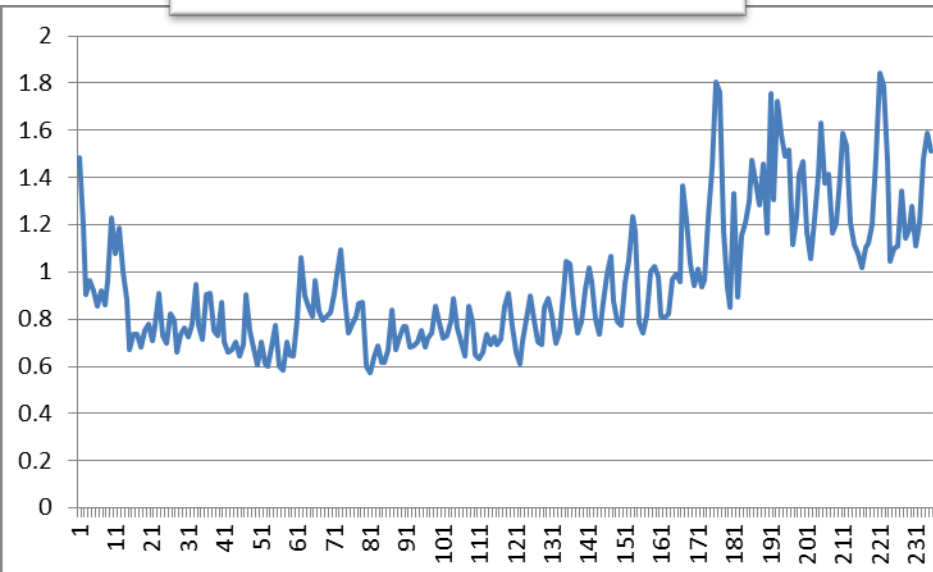
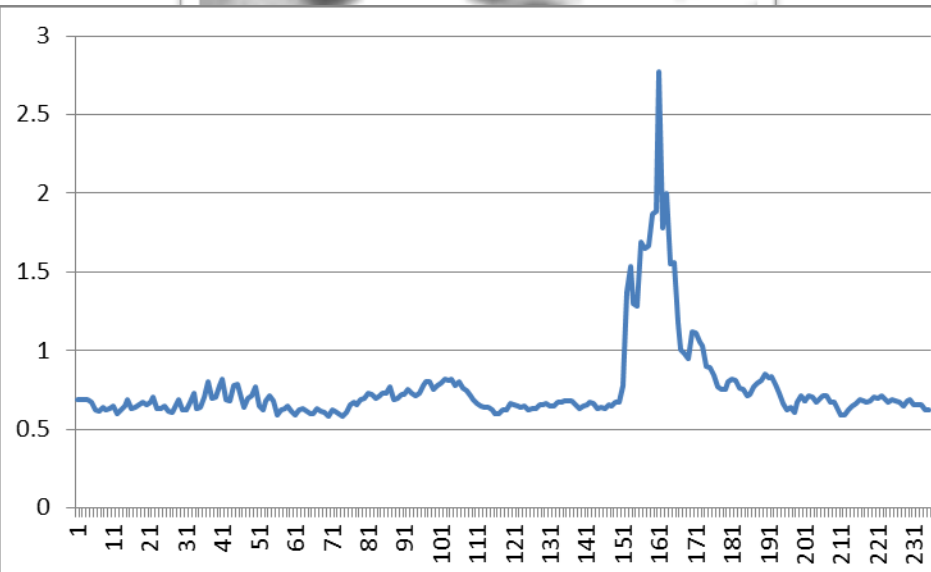
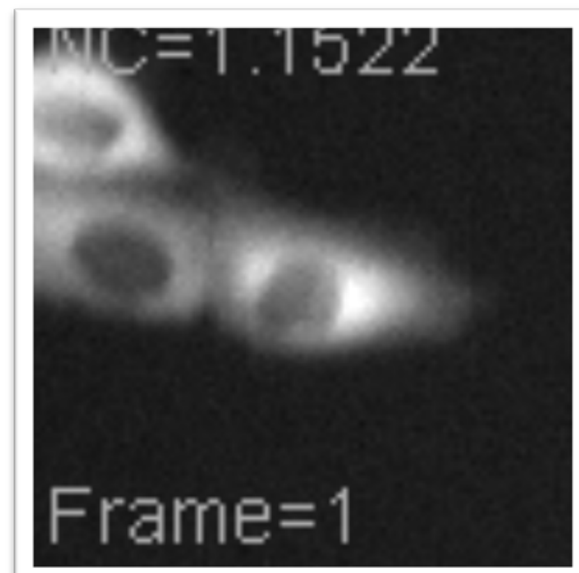
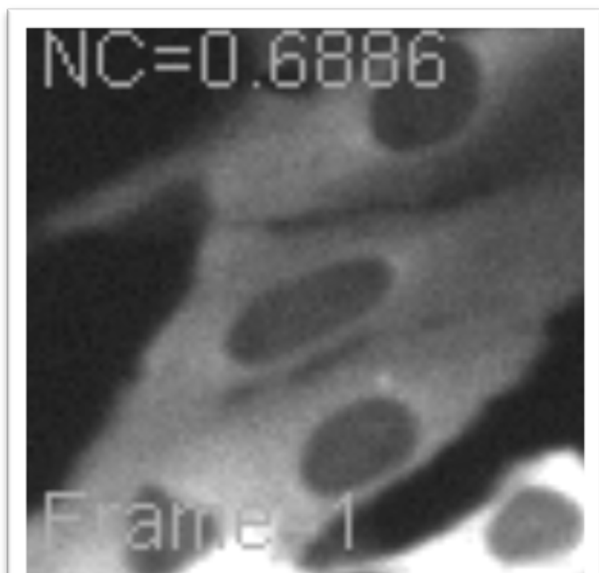
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1		0.611241	0.644628	0.608586	0.459309	0.402502	0.383992	0.408709	0.392789	0.409862	0.445395	0.544235	0.614027	0.615936	0.662006	0.703365	0.718626	0.751996	0.770
2		0.932812	0.930811	0.870745	0.809477	0.834331	0.874865	0.860317	0.804116	0.762906	0.789762	0.801696	0.793774	0.769431	0.7916	0.804814	0.82409	0.845981	0.817
3		0.709403	0.674419	0.671922	0.655786	0.649143	0.645397	0.650656	0.664152	0.69383	0.692904	0.685251	0.662413	0.693587	0.692652	0.682157	0.678925	0.670417	0.665
4		0.757624	0.747634	0.731603	0.710453	0.707375	0.713484	0.712547	0.731019	0.747556	0.786151	0.778419	0.792075	0.813756	0.797494	0.82437	0.785276	0.801153	0.797
5		0.586196	0.614641	0.656233	0.652806	0.673506	0.62644	0.651695	0.634228	0.642797	0.654296	0.662357	0.688387	0.687563	0.69557	0.6776	0.670952	0.675951	0.674
6		0.676879	0.668196	0.695933	0.681189	0.668094	0.663989	0.676228	0.661059	0.684926	0.646888	0.654543	0.639746	0.656035	0.645896	0.637914	0.653481	0.671459	0.672
7		0.478086	0.487084	0.476074	0.474855	0.508563	0.547435	0.531591	0.546226	0.546213	0.568178	0.615237	0.597791	0.583949	0.613888	0.615181	0.624461	0.652071	0.662
8		0.475023	0.495661	0.496217	0.490586	0.484122	0.49659	0.528408	0.541237	0.551727	0.524322	0.517486	0.474551	0.445294	0.417805	0.437129	0.431178	0.423484	0.459
9		0.933337	0.912667	0.8501	0.674175	0.608055	0.882926	0.717079	0.576611	0.693039	0.657031	0.638946	0.542708	0.524822	0.52509	0.522865	0.508574	0.537071	0.582
10		0.667966	0.740822	0.79702	0.73964	0.785606	0.79113	0.794125	0.78996	0.78087	0.803792	0.858342	0.903587	0.947557	0.949097	0.917112	0.88142	0.834662	0.809
11		1.064136	1.022286	1.032337	1.012841	0.987976	0.92497	0.931306	0.910304	0.921473	0.867979	0.80978	0.837926	0.815634	0.802544	0.789807	0.72254	0.73667	0.716

Time
Series
file

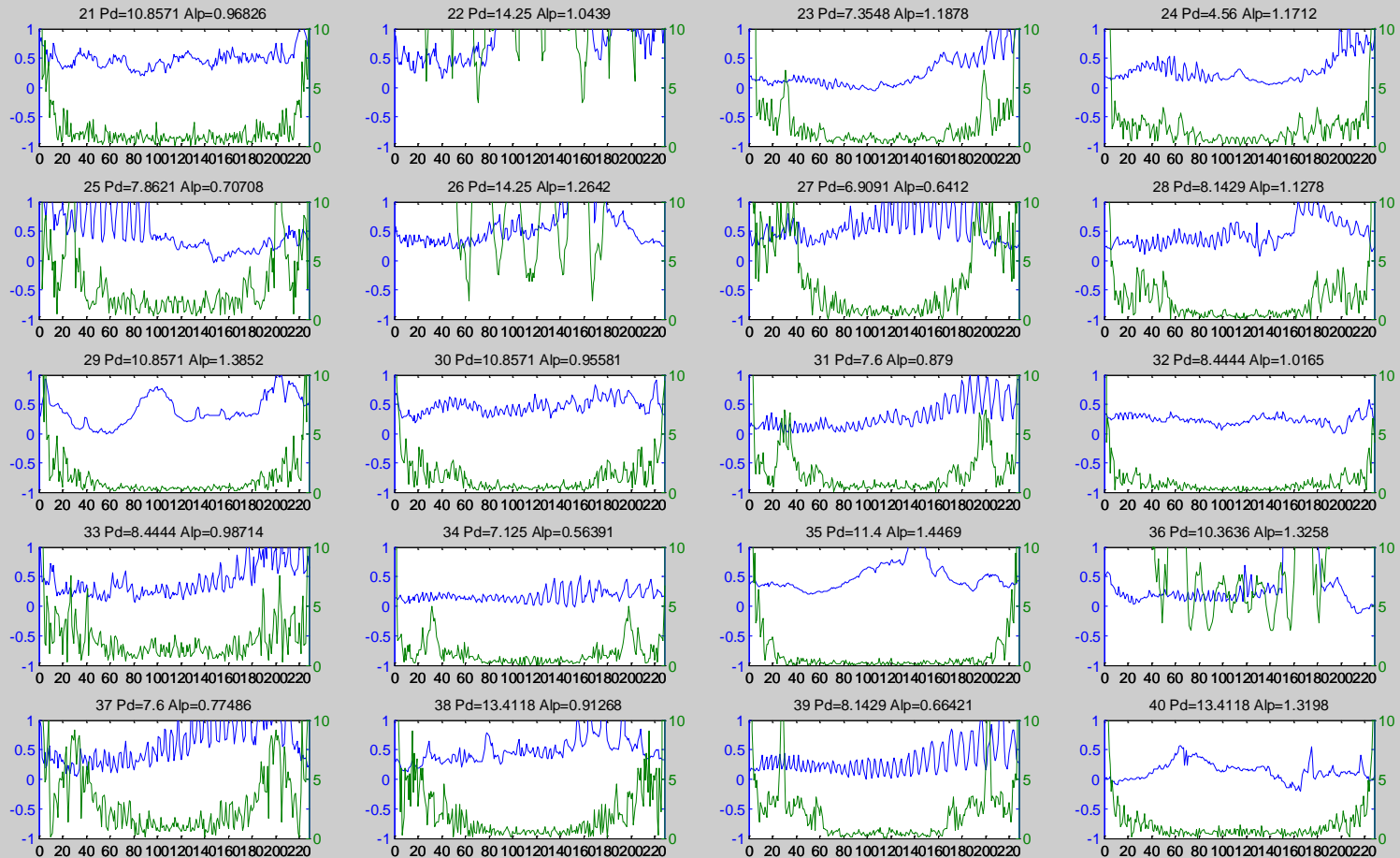
Single cell traces



Single cell traces

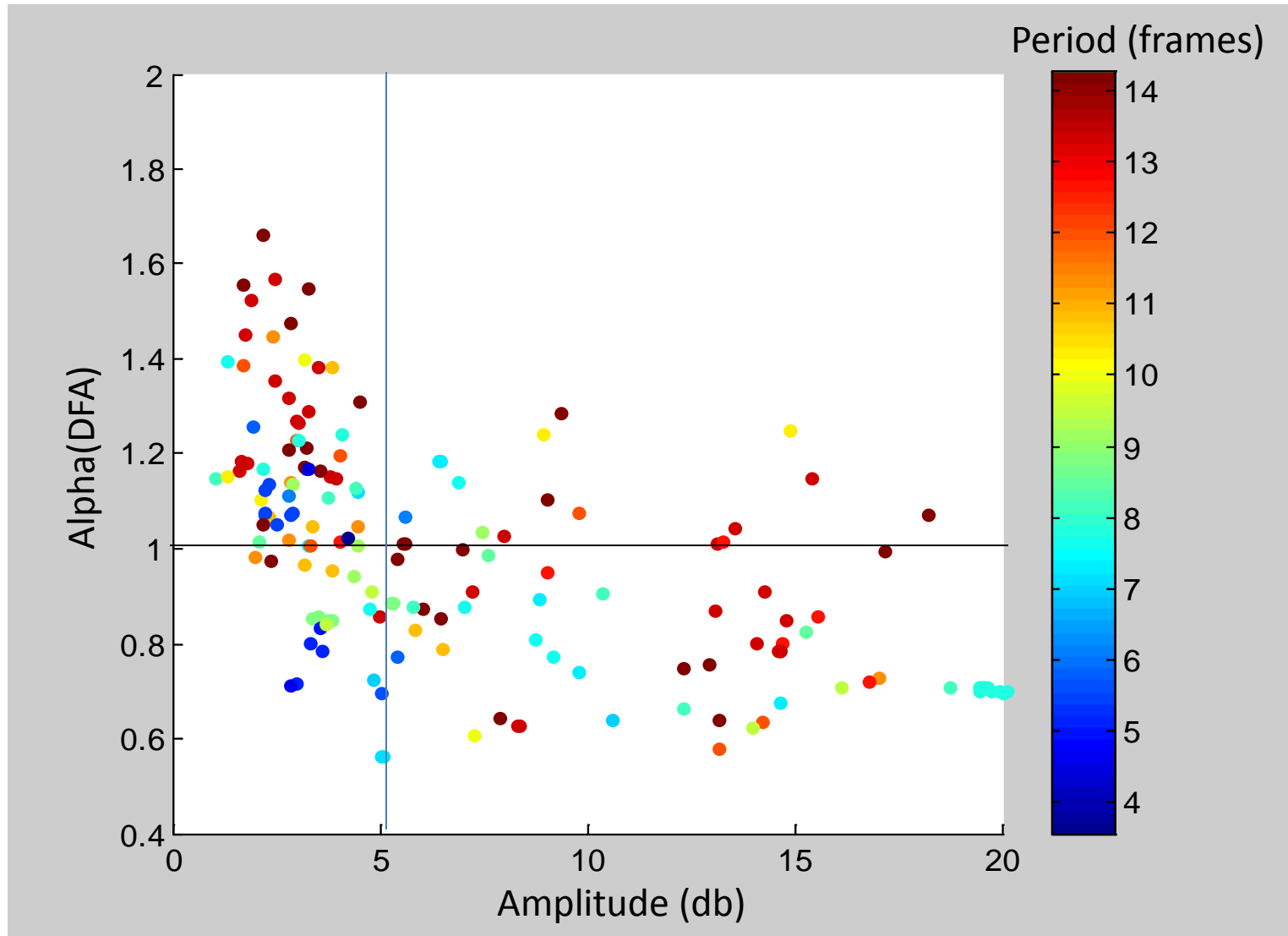


Multiple Traces analysis (Matlab)



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