Report on Image processing of Toposphere Units

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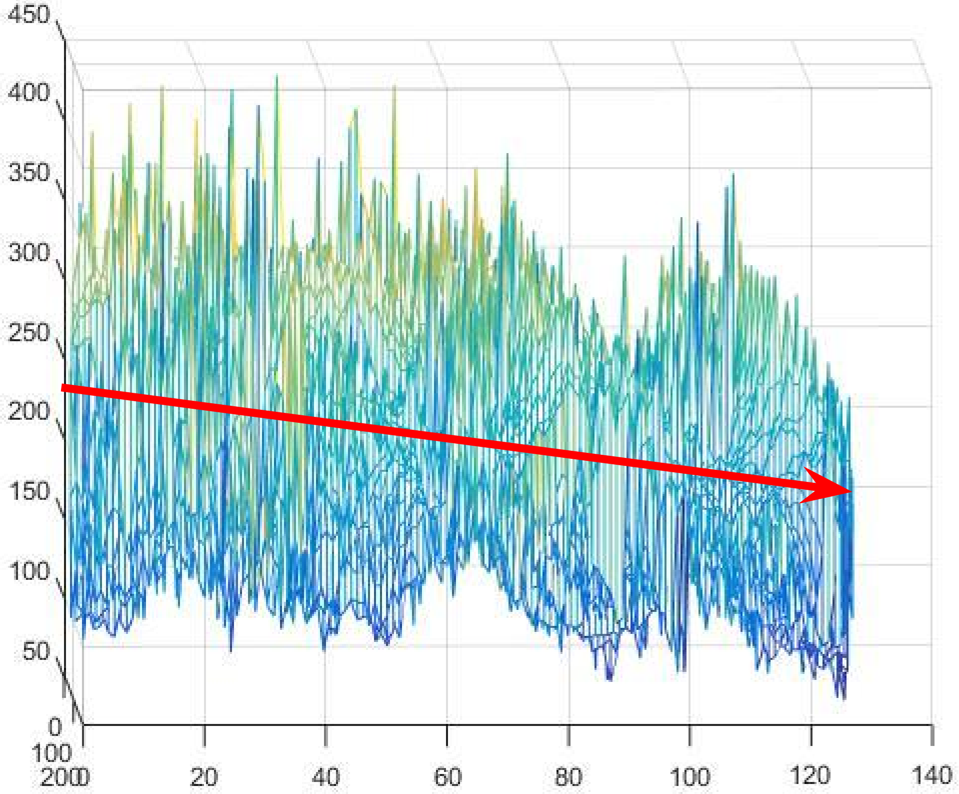
Correction of intensity variation in each tile

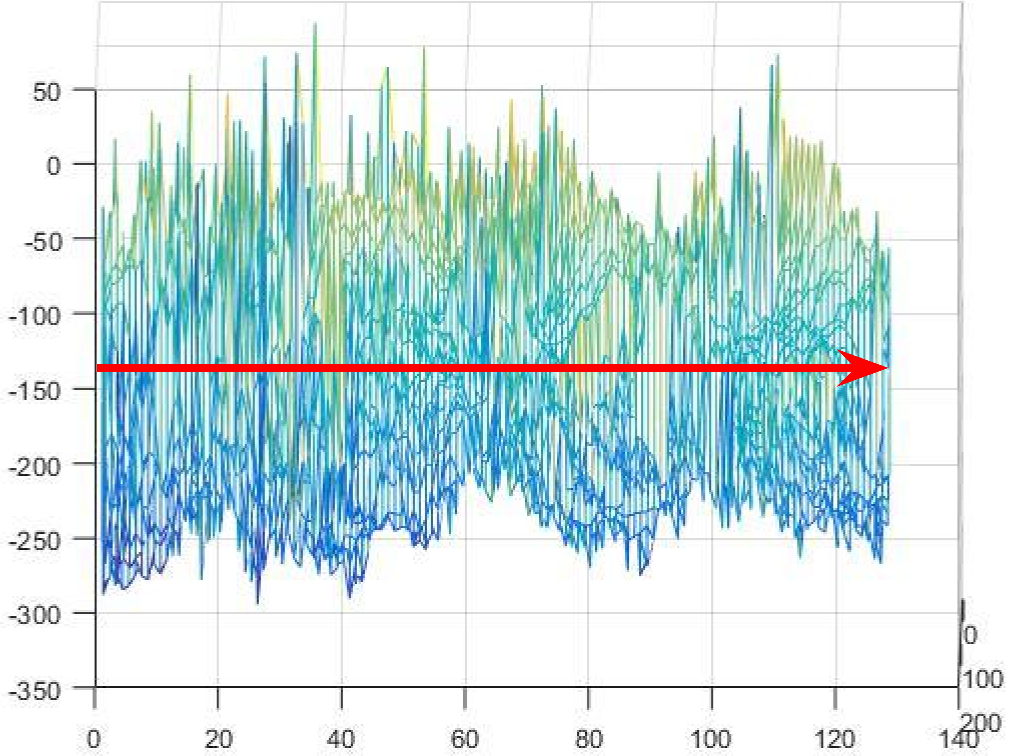
- **Problem:** Tiles appear to be lighter in one corner than the other
- **Impact:** When stitched with other tiles, this leads to a checkerboard effect
- **Idea:** Imagine a 3-d space, whose x-y dimensions are the rows and columns of image pixels, and z dimension is the grayscale intensity of the image. Each image forms a surface in this space. We want to find a tilted plane of intensity that best models the background intensity (excluding the wells), and subtract it point-wise from each pixel.
- **Challenge:** Minimizing mean square error (L2-norm) as is usually done in linear regression (line or plane fitting) can lead outliers (e.g. wells) to fit an average plane instead of the most probable plane.
- **Method:** Optimize the parameters (slope, intercept) that define a plane such that the L1-norm of the difference in pixel intensities and the plane is minimized. We do not want to use L2-norm, because L1-norm is known to produce sparse solution. Which means, most pixels, presumably from the background, will be very close (difference tending to zero) to a plane found using L1-minimization.

We find the plane by minimizing the L1-norm (as opposed to L2-norm used in MSE) of the distance of each point from the plane

(All the images are for the patches ‘tile_x004_y004_z001.tif’ and ‘tile_x005_y004_z001.tif’)



Uncorrected intensity map

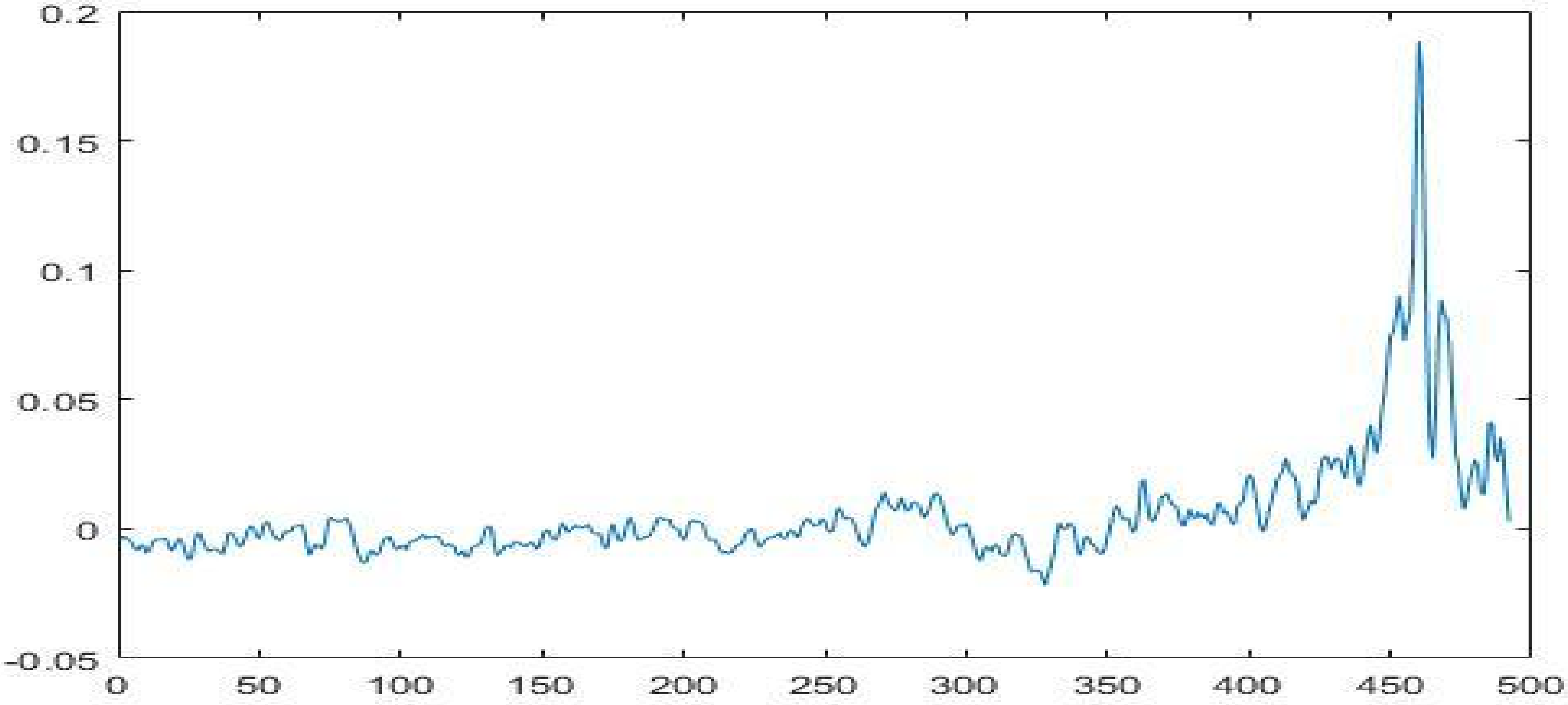


Corrected Intensity map

Finding the overlap region

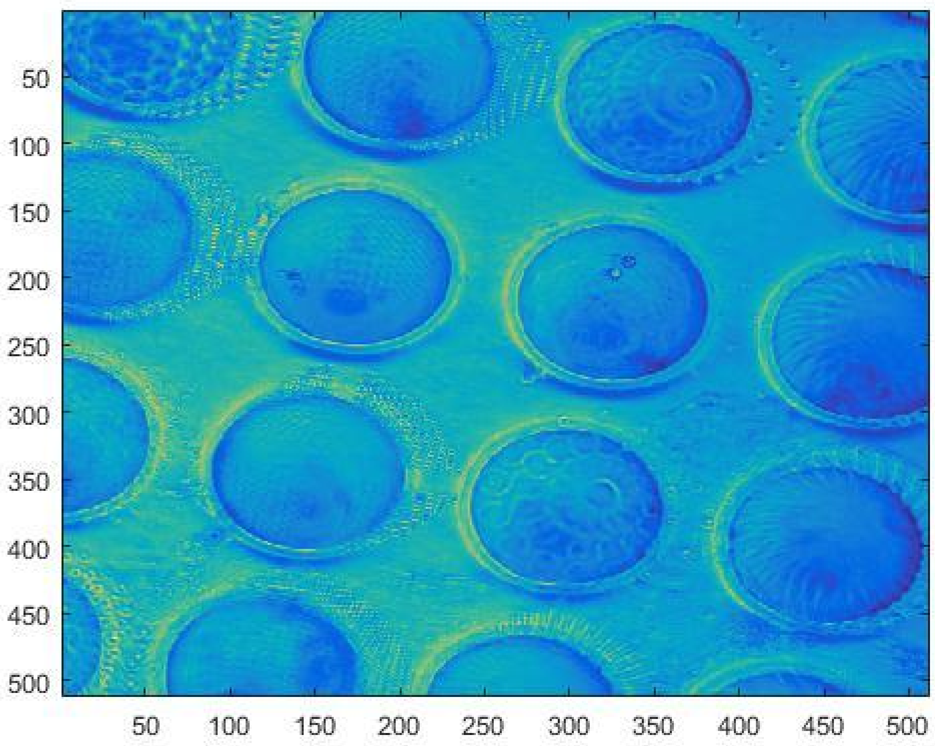
- **Problem:** Find the area of overlap between two neighbouring tiles to enable stitching
- **Idea:** Match the two image assuming one pixel (column or row) overlap, and test the quality of match. Then test for two pixel overlap, and so on, until a best quality match is found.
- **Challenge:** Due to differences in intensities of overlapping pixels of two neighbouring tiles, a simple difference in intensities may not work
- **Method:** For each n-pixel overlap, find the average cosine of image intensity gradient direction. Concordance between direction of intensity change due to local features is likely to be more robust to differences in overall intensities of two neighbouring tiles.

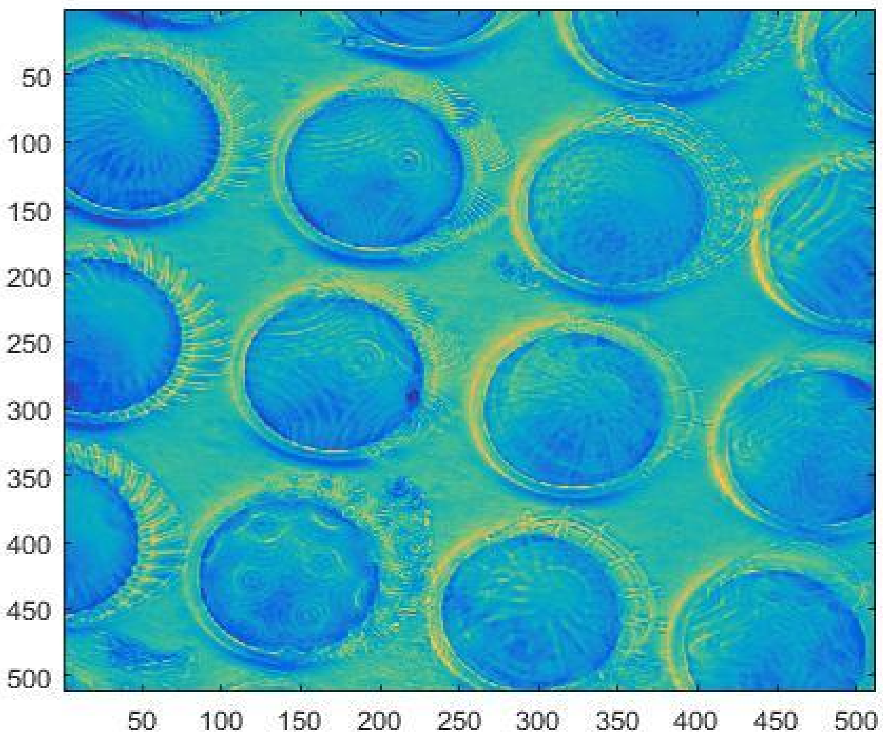
- **X-axis:** Area of overlap (number of columns)
- **Y-axis:** Average cosine between intensity gradient directions
- **Observation:** A very strong peak shows that we have found the right number of overlapping pixels

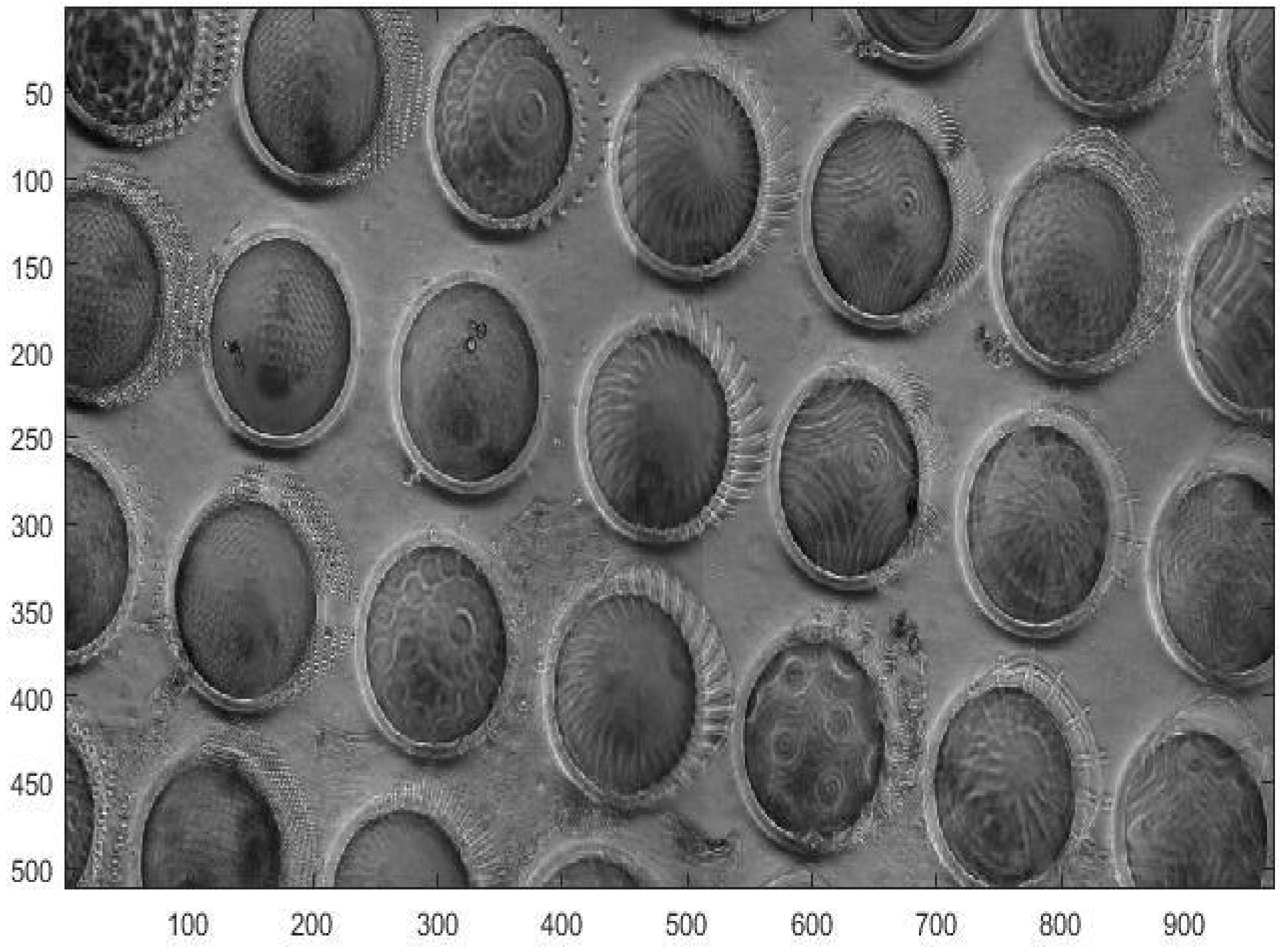


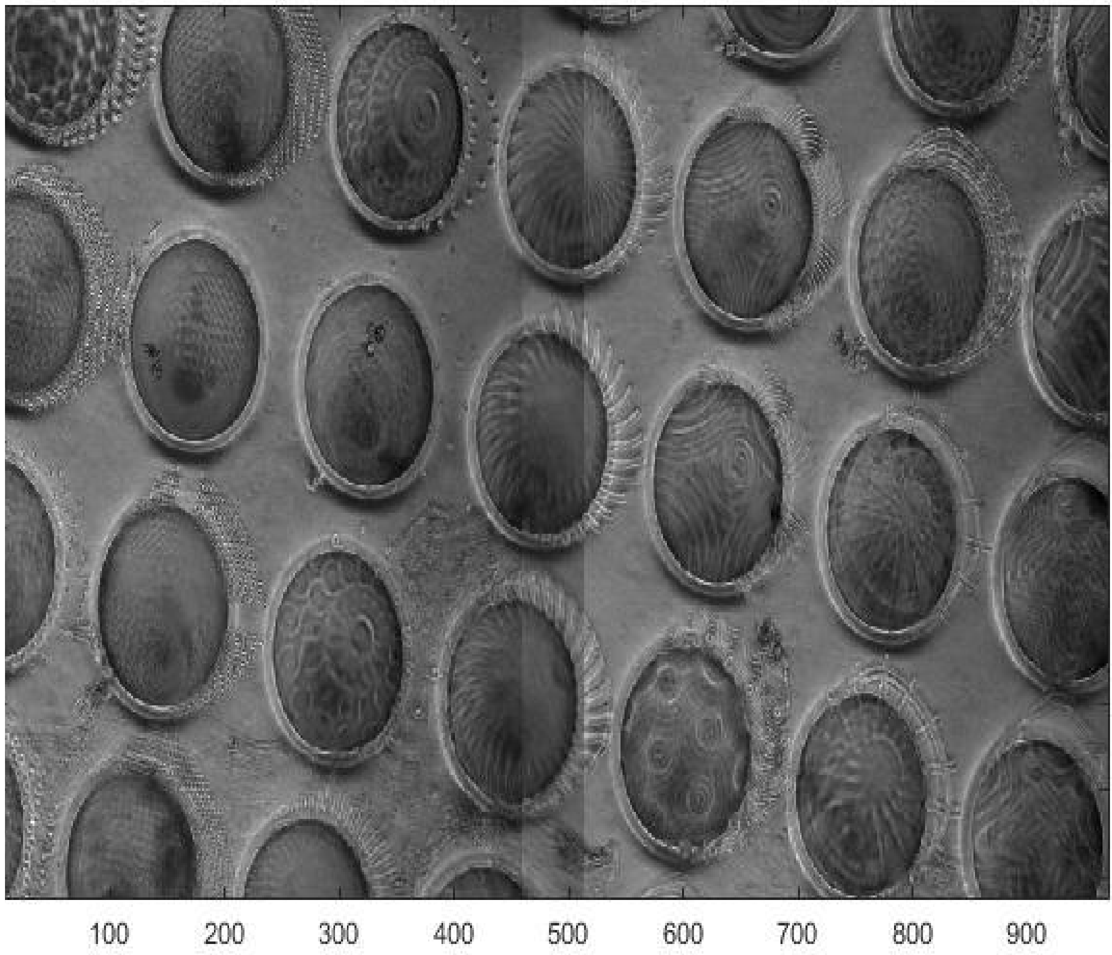
Results of stitching tiles by averaging pixels after intensity correction and finding area of overlap

- Two neighbouring tiles shown here in pseudo colour
- Results shown on next slide









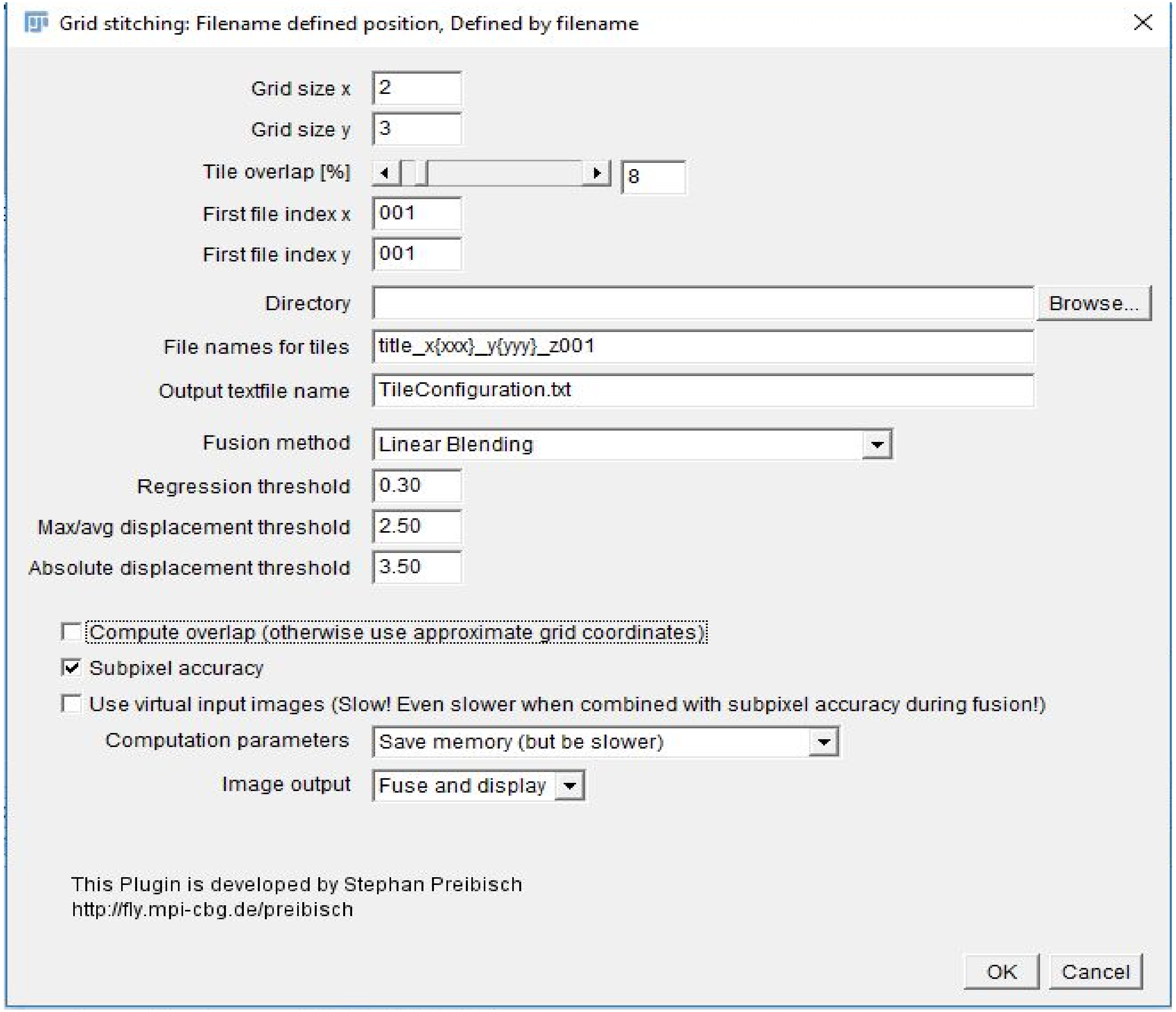
Stitched image of background intensity corrected patches

Stitched image of raw patches

We can observe that the stitching-artifact (around 470-500 pixels) is more profound in the right image than that of the left one's

Stitching Tiles Method 2: Using ImageJ

Small titles of images with varying X,Y, Z co-ordinates are stitched using ImageJ with the following parameters as specified in the figure given above.



Grid stitching: Filename defined position, Defined by filename

Grid size x: 2
Grid size y: 3
Tile overlap [%]: 8
First file index x: 001
First file index y: 001
Directory:
File names for tiles: title_x{xxx}_y{yyy}_z001
Output textfile name: TileConfiguration.txt
Fusion method: Linear Blending
Regression threshold: 0.30
Max/avg displacement threshold: 2.50
Absolute displacement threshold: 3.50

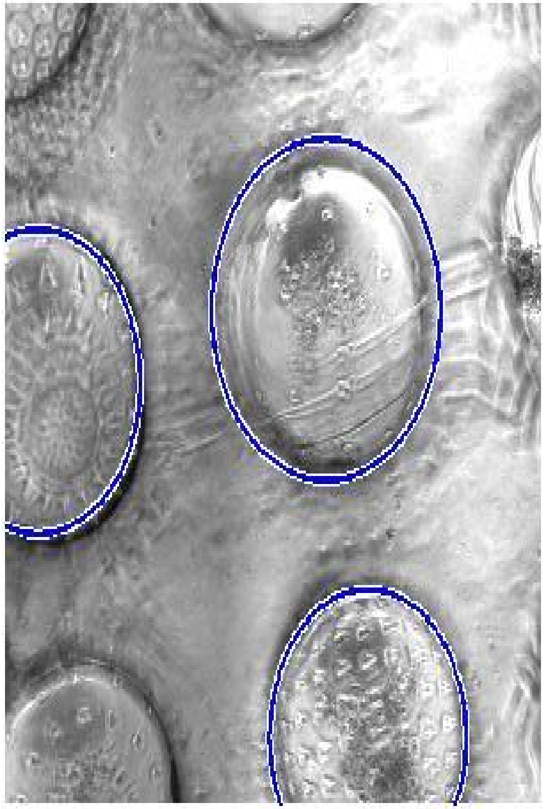
□ Compute overlap (otherwise use approximate grid coordinates)
☑ Subpixel accuracy
□ Use virtual input images (Slow! Even slower when combined with subpixel accuracy during fusion!)

Computation parameters: Save memory (but be slower)
Image output: Fuse and display

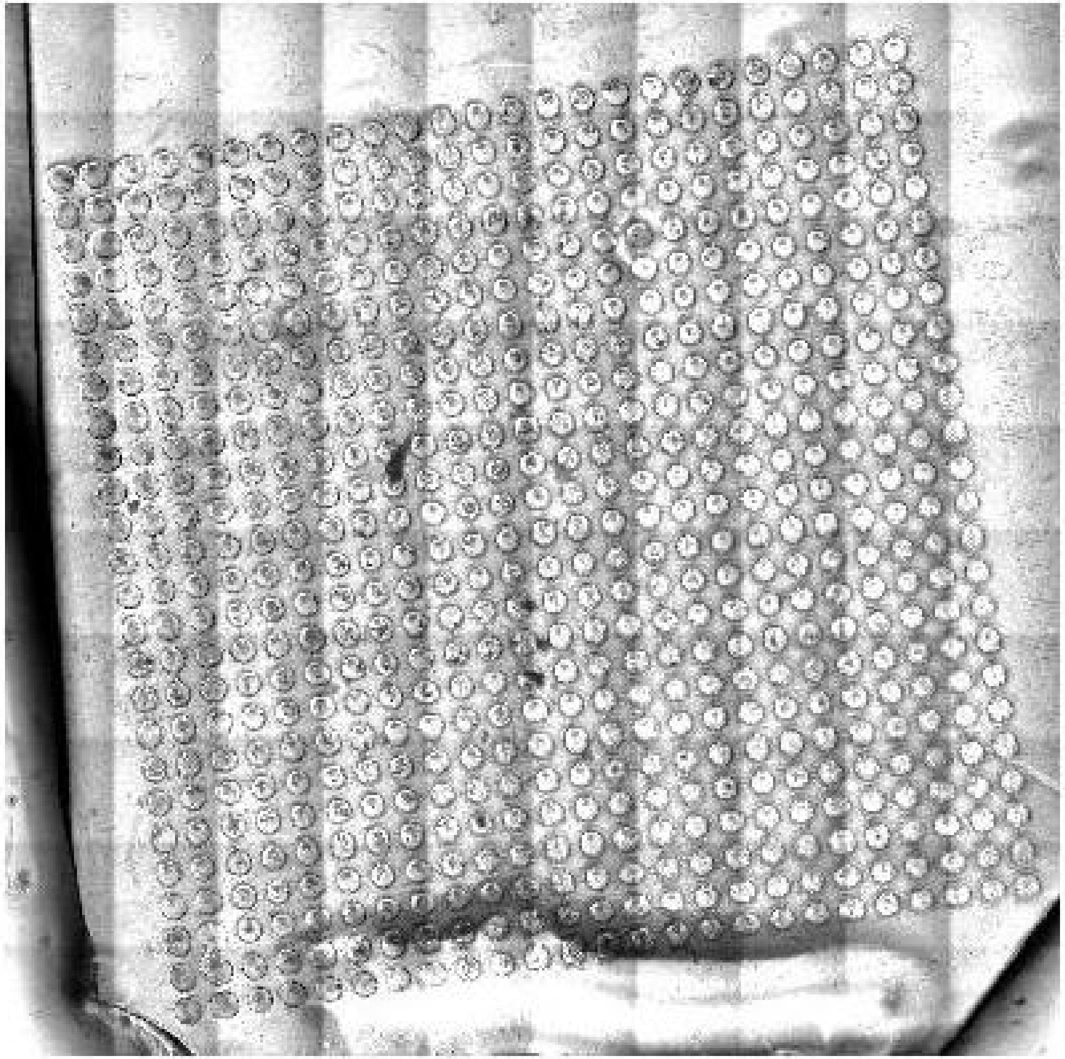
This Plugin is developed by Stephan Preibisch
http://fly.mpi-cbg.de/preibisch

Image rotation to align with a Cartesian grid

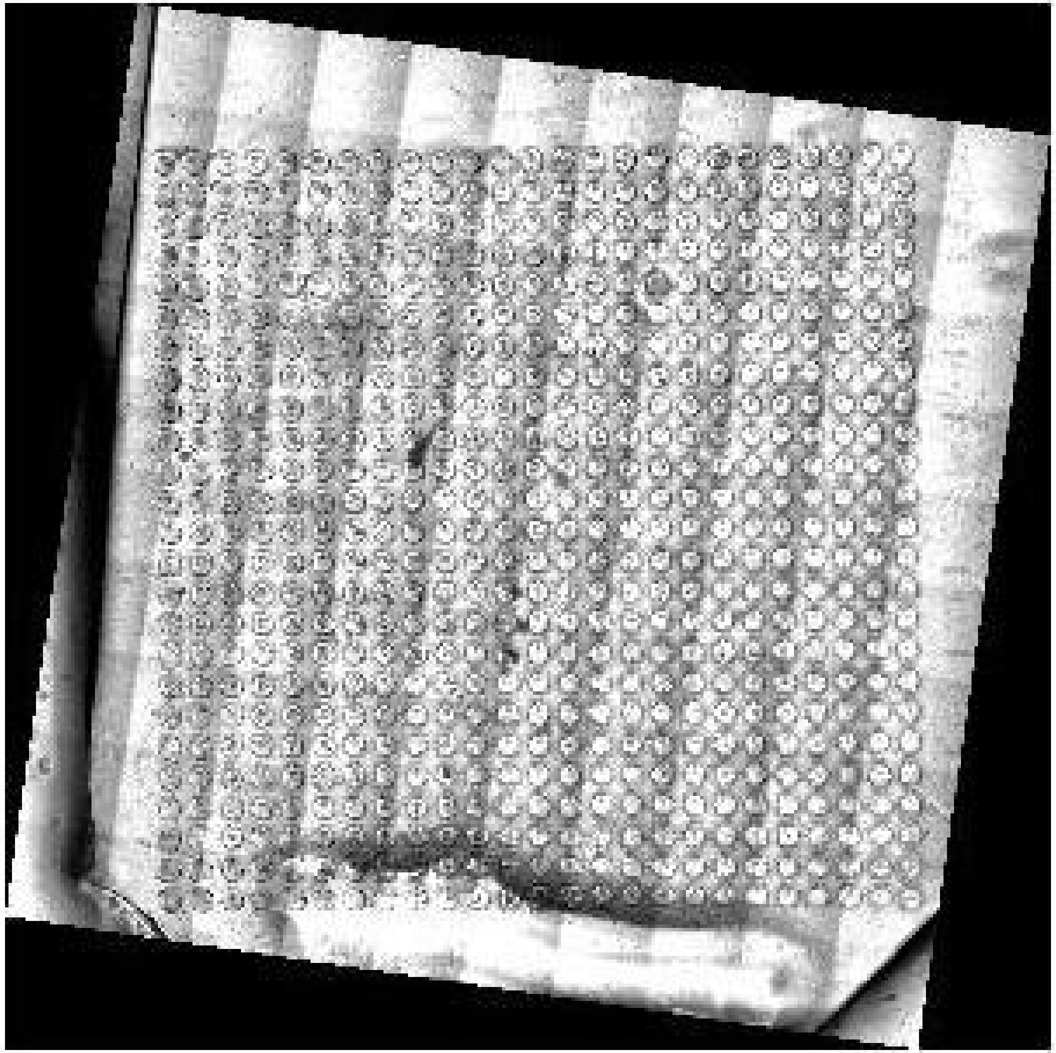
- Small portion of the image is cropped and Hough transform along with Canny edge detection is applied, to find the circles in this this small stack.
- Slope of the line joining any two neighboring centers is determined, and image is rotated by this angle so that all circles can be aligned along X-Y axis.



Original image

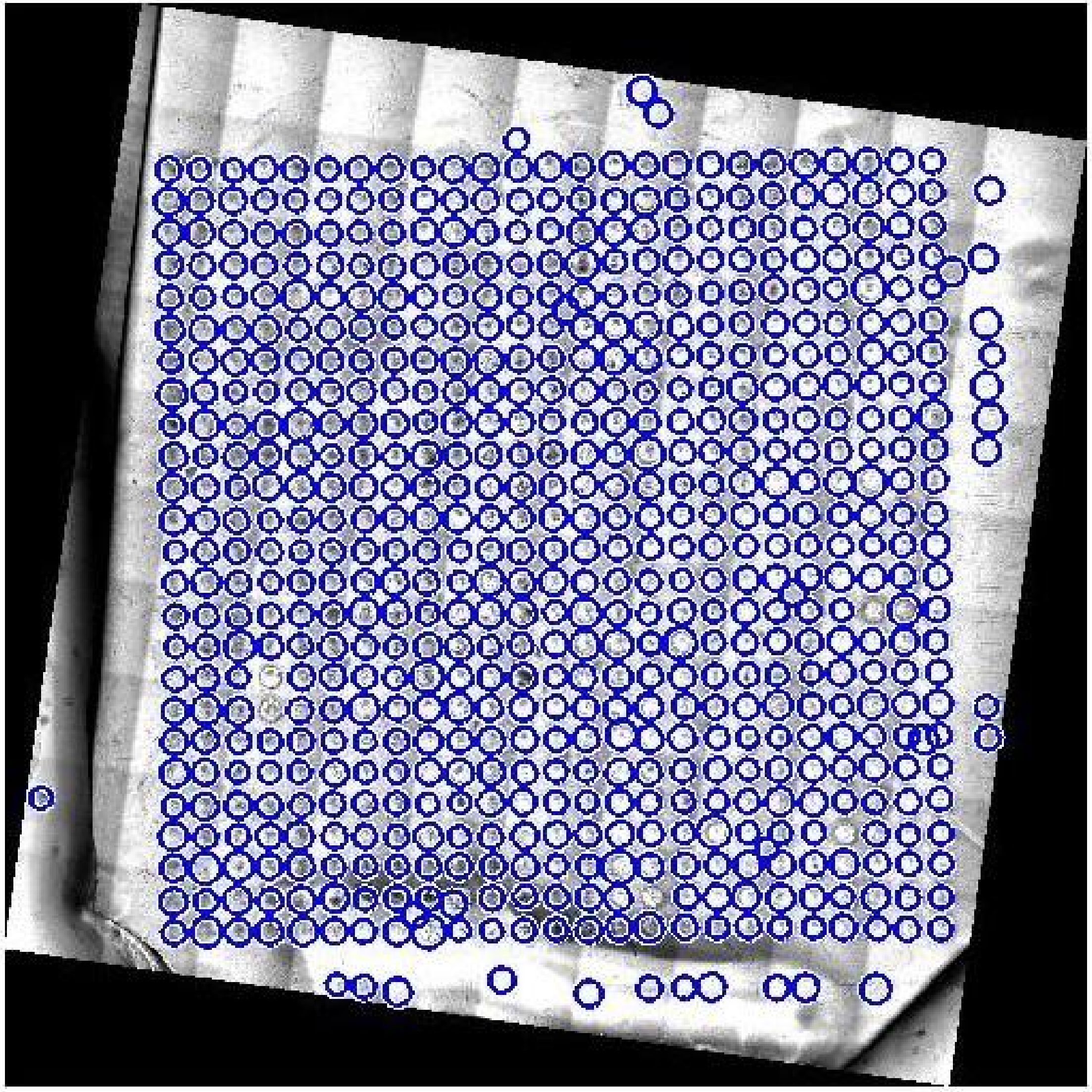


Rotated Image

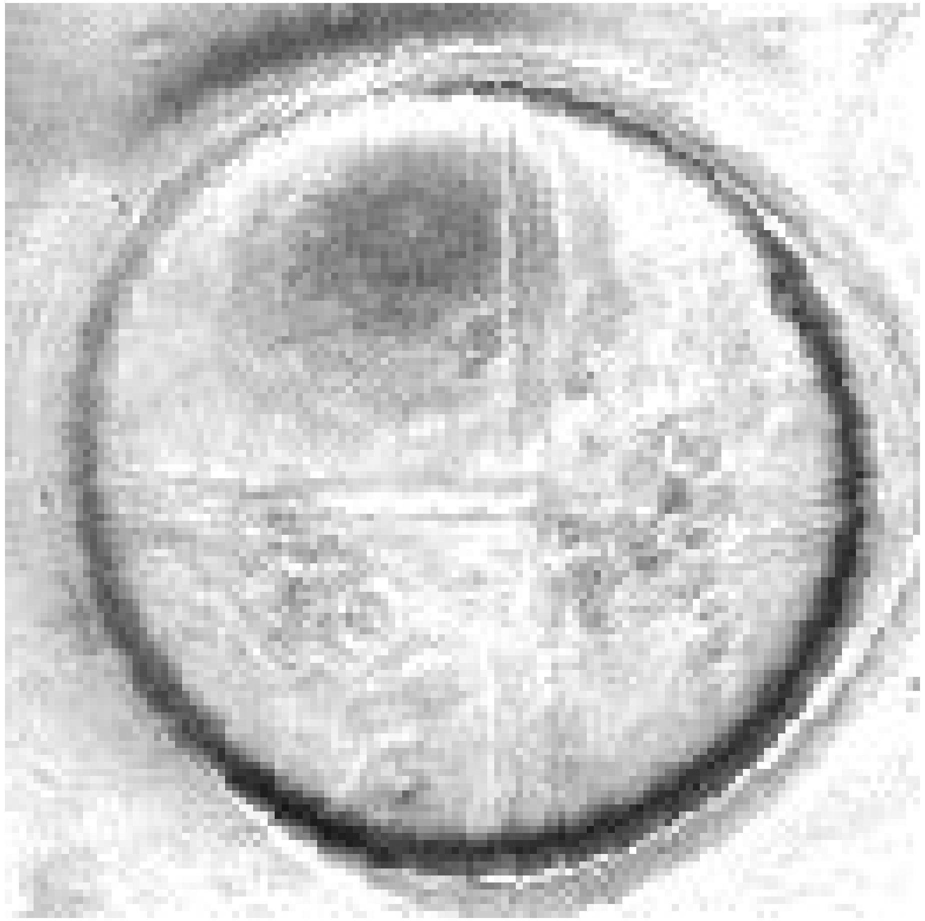


Circle Detection

- We apply Hough transform along with canny edge detection is on a particular stack of image to extract the circles from the rotated images.



Video of circles cropped from original image.



Move the mouse pointer on the image and click on play button to see the video.

X-Coordinates of 625 circles

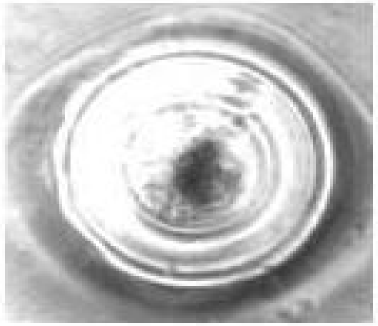
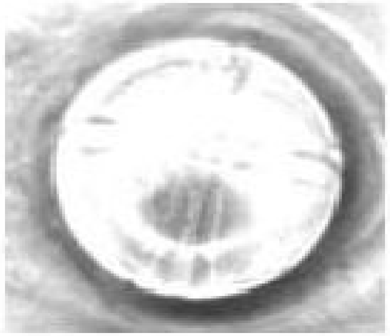
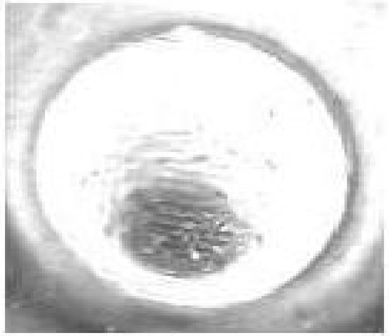
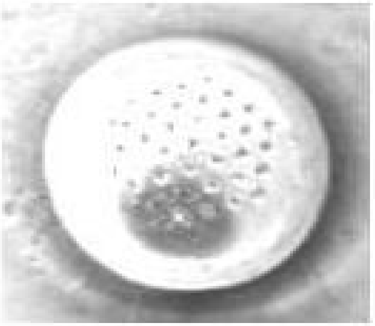
821.7298	974.8285	1144.791	1295.977	1451.683	1616.267	1769.339	1924.318	2088.556	2241.661	2396.052	2563.946	2716.803	2872.197	3038.323	3195.945	3351.399	3518.933	3671.815	3825.9	3988.407	4033.317	4144.945	4299.644	4461.35
822.0092	976.07	1142.465	1299.375	1455.103	1619.684	1775.472	1929.85	2095.991	2245.288	2401.332	2570.542	2720.031	2876.82	3042.46	3198.18	3354	3515.496	3668.125	3828.624	3989.266	4033.317	4139.415	4301.23	4471.463
822.0512	976.2335	1145.603	1301.175	1457.363	1621.222	1774.958	1935.353	2096.015	2241.493	2397.333	2559.524	2718.763	2883.467	3040.266	3189.611	3355.997	3519.083	3678.619	3827.174	3985.761	4033.317	4145.695	4311.096	4463.8
825.8331	988.0657	1147.353	1298.162	1462.394	1626.402	1779.661	1933.082	2096.277	2249.961	2408.327	2572.004	2723.168	2883.031	3044.07	3200.935	3357.887	3520.276	3676.139	3832.95	3992.472	4033.317	4147.243	4309.262	4467.218
827.3014	991.889	1150.513	1303.127	1463.623	1625.97	1779.875	1941.33	2094.396	2248.015	2411.527	2570.672	2724.208	2885.225	3040.461	3200.953	3360.336	3521.638	3674.25	3833.245	3996.673	4033.317	4143.45	4310.596	4465.898
828.1143	995.8571	1153.706	1304.949	1469.059	1627.282	1783.69	1942.541	2096.061	2250.325	2413.549	2572.434	2724.563	2890.147	3043.467	3198.108	3370.159	3517.738	3673.866	3842.592	3998.073	4033.317	4148.119	4321.34	4470.667
833.7198	997.1087	1156.484	1304.23	1472.794	1626.987	1782.99	1946.531	2096.89	2251.595	2413.753	2570.268	2726.02	2892.924	3040.518	3201.146	3367.822	3518.984	3678.242	3842.5	4001.506	4033.317	4153.4	4323.996	4469.095
839.0943	999.9358	1155.864	1309.006	1475.628	1624.183	1784.078	1947.493	2098.924	2255.269	2413.721	2570.696	2727.108	2893.158	3044.257	3197.92	3368.148	3524.822	3678.695	3844.953	4002.982	4033.317	4153.614	4316.251	4471.602
839.8926	1001.886	1159.416	1313.655	1475.409	1631.293	1788.965	1949.022	2099.761	2253.699	2416.347	2572.405	2727.992	2893.995	3050.665	3202.138	3370.257	3525.263	3680.867	3843.859	3997.017	4033.317	4150.713	4317.097	4472.901
846.8645	1004.86	1162.588	1315.414	1480.058	1629.691	1793.774	1952.073	2104.155	2260.394	2412.552	2573.368	2731.702	2896.317	3043.962	3205.096	3380.47	3525.43	3682.949	3850.855	3999.091	4033.317	4157.925	4318.008	4474.986
841.738	1004.839	1163.483	1318.103	1478.09	1628.214	1792.414	1950.733	2101.287	2257.374	2417.617	2573.579	2733.604	2894.873	3045.539	3212.351	3377.537	3526.808	3686.61	3849.961	3998.273	4033.317	4159.841	4311.24	4469.422
849.8684	1005.068	1161.856	1325.877	1483.225	1634.135	1800.751	1948.51	2104.05	2262.83	2420.99	2573.727	2736.935	2895.614	3054.342	3217.122	3374.246	3530.717	3691.812	3847.747	3994.114	4033.317	4165.886	4317.66	4475.43
854.0496	1006.373	1162.529	1325.87	1481.399	1634.84	1800.736	1954.107	2107.329	2271.332	2423.953	2574.962	2736.866	2895.54	3058.29	3222.238	3377.713	3532.219	3697.492	3853.7	4005.05	4033.317	4166.923	4324.949	4476.975
854.7005	1007.97	1167.517	1329.439	1481.198	1636.45	1801.278	1946.041	2105.358	2274.811	2415.71	2577.262	2736.915	2893.156	3056.03	3215.229	3378.124	3532.984	3701.529	3849.15	4007.694	4033.317	4162.815	4326.791	4476.477
856.6009	1009.842	1168.538	1327.721	1481.832	1637.935	1795.77	1954.666	2110.79	2269.554	2418.61	2580.811	2745.535	2893.214	3057.352	3211.299	3378.304	3535.331	3695.578	3850.772	4007.826	4033.317	4171.128	4321.836	4486.679
858.4566	1008.575	1175.393	1328.894	1479.494	1639.616	1802.348	1950.88	2112.299	2268.646	2421.298	2576.127	2746.618	2892.973	3054.213	3220.581	3368.958	3536.304	3692.515	3853.747	4011.243	4033.317	4172.28	4321.836	4484.195
855.8333	1009.034	1168.566	1328.894	1484.395	1642.056	1798.577	1948.507	2107.739	2274.364	2426.622	2585.445	2747.95	2882.061	3060.754	3220.341	3380.093	3540.716	3701.765	3851.742	4020.396	4033.317	4167.663	4330.124	4486.95
851.8305	1006.676	1170.882	1328.894	1476.968	1642.558	1794.313	1959.391	2115.473	2266.644	2420.028	2585.765	2751.608	2897.249	3054.626	3211.944	3394.105	3543.298	3699.718	3855.212	4020.554	4033.317	4173.296	4326.949	4493.443
851.9011	1004.845	1172.123	1326.222	1480.625	1644.148	1800.669	1958.394	2115.818	2272.445	2425.14	2594.51	2754.166	2899.953	3054.626	3216.097	3380.944	3554.782	3705.407	3859.478	4023.782	4033.317	4175.489	4331.097	4497.75
852.3684	1001.147	1168.305	1325.966	1473.891	1650.217	1802.129	1956.737	2119.769	2269.082	2428.35	2595.778	2750.87	2900.394	3066.434	3211.005	3383.198	3553.573	3708.108	3859.765	4028.888	4033.317	4182.26	4334.834	4496.898
849.1039	1001.171	1168.996	1323.949	1483.413	1639.4	1799.127	1956.216	2111.604	2272.806	2426.008	2584.843	2751.252	2904.961	3066.696	3211.728	3385.996	3552.652	3705.369	3865.197	4031.509	4033.317	4186.523	4336.494	4504.56
846.4449	1005.554	1167.756	1323.477	1470.505	1648.003	1799.913	1958.657	2115.859	2268.516	2427.907	2595.61	2755.148	2910.376	3070.498	3224.407	3389.152	3538.373	3707.401	3867.774	4031.509	4033.317	4185.672	4345.083	4503.812
847.1724	1005.072	1169.586	1322.558	1477.339	1645.921	1801.274	1962.661	2115.297	2266.475	2438.744	2595.657	2753.692	2911.778	3061.237	3216.927	3394.774	3555.506	3707.74	3870.657	4031.509	4033.553	4185.672	4351.177	4508.2
845.3257	1009.191	1166.173	1325.626	1485.402	1648.528	1801.292	1960.737	2114.576	2266.475	2431.055	2597.867	2753.55	2916.895	3059.616	3216.927	3402.88	3553.788	3694.407	3869.25	4031.509	4032.636	4177.745	4341.307	4502.482
848.2259	1011.5	1169.893	1324.591	1492.883	1650.342	1792.111	1962.07	2114.895	2269.039	2431.112	2591.562	2750.704	2913.955	3063.226	3213.609	3394.131	3555.419	3708.508	3866.052	4031.509	4033.76	4179.785	4352.716	4499.624

Y-Coordinates of 625 circles.

829.6653	830.8623	833.0667	827.0041	827.6785	826.6193	827.3008	823.9343	822.7149	828.281	822.6096	821.6156	815.0416	815.772	807.7904	806.7449	805.0452	803.1107	805.5454	801.0269	802.2479	802.2479	793.737	794.0331	789.8103
988.2842	985.2976	982.9174	981.1005	981.4881	981.3042	979.7326	980.2475	978.3798	984.1739	981.0664	986.9097	978.5387	980.1545	984.1706	988.023	974.0737	966.9436	967.4526	957.3081	955.6635	955.6635	947.7168	947.1935	947.8517
1144.438	1142.55	1150.264	1150.959	1148.955	1146.813	1141.757	1143.504	1136.154	1139.989	1140.751	1139.79	1130.871	1139.634	1135.054	1126.343	1122.176	1121.471	1122.804	1119.878	1122.846	1122.846	1114.456	1114.35	1108.524
1302.08	1306.373	1305.181	1304.581	1304.165	1304.297	1307.13	1314.641	1307.506	1305.237	1306.294	1302.281	1296.763	1300.141	1284.755	1284.494	1281.528	1276.806	1275.685	1278.377	1273.367	1273.367	1272.872	1269.421	1262.947
1468.166	1466.262	1459.546	1455.691	1457.599	1458.451	1464.354	1463.818	1461.841	1457.458	1462.767	1463.241	1454.185	1461.844	1456.161	1446.874	1452.895	1442.643	1438.98	1425.539	1428.774	1428.774	1423.181	1420.422	1417.926
1626.352	1627.985	1624.916	1629.83	1624.097	1621.928	1616.959	1613.587	1610.088	1614.134	1609.299	1602.219	1608.083	1613.197	1615.609	1610.041	1607.062	1601.704	1596.18	1596.815	1593.535	1593.535	1595.64	1594.74	1590.532
1780.289	1778.063	1785.624	1779.688	1779.132	1779.232	1780.945	1776.18	1776.766	1786.806	1779.875	1770.176	1767.966	1768.149	1772.014	1769.11	1753.408	1754.066	1753.313	1750.049	1749.437	1749.437	1744.387	1743.453	1743.034
1946.787	1935.476	1934.546	1934.273	1931.54	1937.025	1933.775	1927.47	1935.04	1934.208	1933.051	1933.322	1926.429	1932.878	1928.991	1936.15	1921.577	1919.79	1915.812	1912.331	1905.593	1905.593	1898.559	1897.182	1896.005
2100.966	2098.325	2104.351	2102.276	2101.64	2093.167	2093.592	2091.784	2084.996	2086.041	2088.253	2084.819	2081.571	2087.698	2084.798	2083.769	2081.37	2077.869	2077.986	2076.258	2071.779	2071.779	2069.665	2068.5	2064.131
2255.7	2253.337	2255.759	2251.476	2250.746	2253.824	2251.141	2255.451	2255.075	2254.605	2249.627	2249.449	2244.08	2244.329	2244.449	2242.214	2233.292	2232.767	2232.085	2227.699	2227.838	2227.838	2227.582	2222.592	2220.195
2410.703	2411.85	2410.667	2401.784	2406.555	2409.113	2408.663	2409.839	2405.581	2407.545	2409.508	2408.819	2405.885	2396.526	2407.982	2404.745	2397.71	2395.354	2385.539	2386.314	2379.814	2379.814	2383.005	2385.152	2374.782
2574.77	2576.75	2576.376	2574.709	2571.887	2566.571	2568.486	2563.441	2562.725	2563.417	2563.536	2562.976	2558.574	2564.958	2557.925	2556.353	2547.99	2545.639	2550.325	2547.966	2545.077	2545.077	2547.032	2550.241	2545.292
2730.263	2729.095	2726.476	2729.05	2728.895	2729.962	2726.342	2725.718	2725.358	2725.466	2726.15	2727.412	2723.672	2716.5	2711.88	2707.704	2703.88	2703.977	2700.696	2699.66	2696.763	2696.763	2699.365	2696.777	2695.578
2886.183	2882.262	2887.101	2881.149	2881.879	2882.121	2878.77	2878.878	2880.289	2883.206	2875.481	2883.656	2877.703	2884.207	2875.037	2880.362	2871.182	2869.956	2866.348	2861.454	2856.687	2856.687	2856.554	2858.397	2850.435
3051.62	3049.366	3051.323	3048.012	3041.269	3042.175	3036.044	3035.243	3036.493	3039.775	3037.417	3034.629	3029.509	3035.01	3036.677	3038.156	3028.161	3026.077	3028.181	3023.493	3024.057	3024.057	3020.478	3020.478	3018.717
3202.877	3203.913	3200.719	3199.537	3197.297	3201.478	3201.793	3205.044	3201.327	3203.6	3203.212	3192.328	3188.75	3192.642	3191.665	3186.229	3184.728	3178.798	3179.892	3175.551	3179.498	3179.498	3174.638	3175.032	3175.746
3363.363	3358.173	3350.587	3353.549	3353.549	3352.665	3353.778	3357.409	3351.671	3351.015	3354.097	3354.831	3348.517	3354.582	3353.824	3349.566	3350.396	3347.462	3341.472	3343.813	3333.639	3333.639	3329.721	3328.509	3328.538
3523.062	3516.27	3524.717	3518.04	3518.04	3516.342	3511.202	3508.763	3507.224	3505.529	3507.367	3504.603	3500.096	3507.263	3513.402	3503.039	3496.397	3503.124	3501.241	3492.232	3498.162	3498.162	3495.362	3489.944	3491.102
3674.096	3666.656	3673.928	3674.468	3672.782	3672.536	3673.271	3672.776	3671.135	3669.931	3669.914	3663.275	3664.094	3659.204	3660.619	3660.619	3655.668	3654.461	3653.544	3652.433	3651.03	3651.03	3654.199	3647.293	3648.812
3830.355	3826.015	3826.371	3825.956	3824.389	3829.293	3825.267	3824.874	3825.025	3830.951	3826.026	3829.125	3820.314	3820.455	3828.792	3827.724	3819.144	3813.354	3812.859	3812.902	3810.211	3810.211	3806.411	3799.945	3801.407
3994.129	3996.492	3991.018	3994.048	3991.748	3983.79	3982.311	3979.478	3984.325	3977.851	3976.499	3973.265	3975.725	3973.814	3977.208	3979.085	3971.021	3971.761	3970.378	3970.833	3975.226	3975.226	3972.17	3977.671	3972.246
4143.312	4149.565	4144.132	4144.85	4143.676	4144.428	4145.728	4146.998	4147.682	4147.736	4146.085	4143.412	4135.104	4134.379	4134.727	4130.63	4126.441	4122.635	4125.003	4127.872	4126.209	4126.209	4126.209	4132.236	4138.744
4303.416	4303.464	4294.868	4295.692	4291.943	4296.944	4293.232	4292.951	4295.08	4293.732	4292.17	4296.596	4290.676	4295.507	4299.6	4298.278	4290.142	4286.734	4289.648	4284.646	4282.22	4282.22	4281.469	4279.033	4282.288
4469.684	4469.459	4461.589	4465.518	4463.249	4451.737	4459.013	4454.056	4457.781	4451.64	4451.64	4443.522	4446.166	4450.068	4452.764	4445.47	4445.47	4448.782	4444.049	4451.791	4449.441	4449.441	4446.544	4451.05	4451.54
4624.964	4620.75	4620.4	4617.165	4616.916	4617.971	4616.047	4619.164	4619.777	4612.38	4614.191	4612.781	4606.619	4609.984	4600.282	4598.133	4596.951	4597.455	4592.662	4596.322	4598.348	4598.348	4597.953	4601.496	4606.739

Finding focused Z stack

- Using the centers of circles we crop the images of individual circles and find the top five z-stacks that are in focus.
- To do so we compute Fourier transform of images and find the power of high frequency components. The higher the power of high frequency components the better focused is the z-stack. We sort the z-stack and display the top five z-stack values.

			
Z=[4 5 6 7 8]	Z=[6 7 8 9 10]	Z=[2 3 4 5 6]	Z= [5 6 7 8 9]