

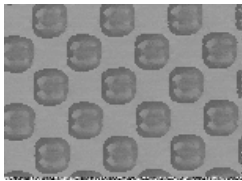
Measuring pit volume from topographical projections in ImageJ

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Example:

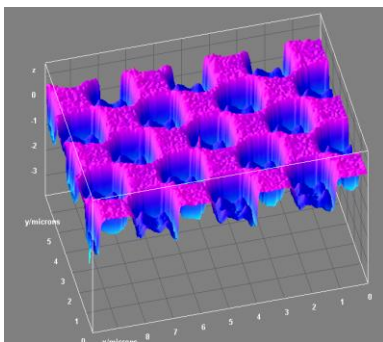
Reflected light confocal z-stack of an AFM test target. Silicon wafer with 1 micrometer-deep square wells. Image stack is loaded (and spatially calibrated if the voxel size was not read from the file)

1. generate TOPO projection with TopoJ plugin. Use the Quadratic Refinement option for more precise result. The result is a 32-bit file where pixel value corresponds to surface height (in the calibrated units, e.g, micrometers)



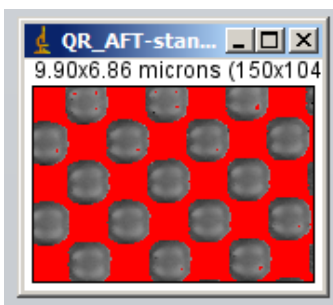
2. Crop the image as needed to remove processing artifacts on the edges of the image

3. display the interactive 3D surface plot to check if there are any outliers (hot or black pixels). If necessary, remove the outliers (process-Noise-Remove Outliers)

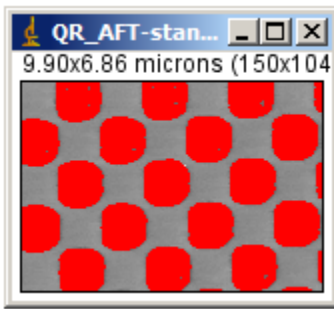


4. Level the TOPO image (TopoJ-Level)

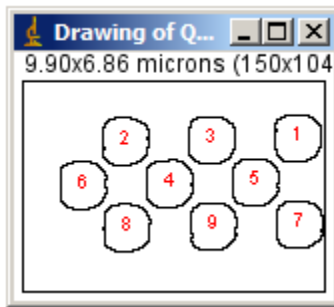
5. Threshold the image to highlight the flat surface. Measure the mean pixel value of the surface (make sure the Analyze- Set Measurements-Limit to Threshold is set and that you have sufficient number of decimal places for the result) , subtract this value from the image (Process-Math-Subtract). The flat surface now have pixel values close to zero.



6. Threshold the resulting image to highlight the pits.



7. Analyze-Set Measurements – make sure to check the Integrated Density. Then Analyze-Analyze Particles (EXCLUDE ON EDGES, INCLUDE HOLES, SHOW OUTLINE). The IntDen corresponds to the well volume. In this case, about two cubic micrometers each well.



Results:

#	label	Area	mean	IntDen	RawIntDen
1	QR_AFT-standard0005.oif - Series 1 - C=0-2	2.0430	-0.9844	-2.0110	-461.6724
2	QR_AFT-standard0005.oif - Series 1 - C=0-2	2.0691	-1.0024	-2.0740	-476.1324
3	QR_AFT-standard0005.oif - Series 1 - C=0-2	2.0604	-0.9930	-2.0460	-469.7013
4	QR_AFT-standard0005.oif - Series 1 - C=0-2	2.0647	-0.9901	-2.0443	-469.3108
5	QR_AFT-standard0005.oif - Series 1 - C=0-2	2.0430	-0.9811	-2.0043	-460.1183
6	QR_AFT-standard0005.oif - Series 1 - C=0-2	2.0647	-0.9914	-2.0469	-469.9020
7	QR_AFT-standard0005.oif - Series 1 - C=0-2	2.0560	-0.9771	-2.0090	-461.1930
8	QR_AFT-standard0005.oif - Series 1 - C=0-2	2.0430	-0.9807	-2.0036	-459.9545
9	QR_AFT-standard0005.oif - Series 1 - C=0-2	2.0299	-0.9745	-1.9781	-454.1175