



Solution 02 - Basic tools 1

Solution 2.1: Pixel information, zooming and scrolling

- The maximum zoom is 3200%, the minimum zoom 3.1%
- The grey-value of the pixel with the coordinates $x=549$ and $y=305$ is 199. The grey-value of the pixel with the coordinates $x=58$ and $y=246$ is 197.
- width = 550 pixel, height = 515 pixel, type = 8-bit, size = 327K.
- x-coordinates become bigger from left to right, y-coordinates from top to bottom. This is a coordinate-system of the form:

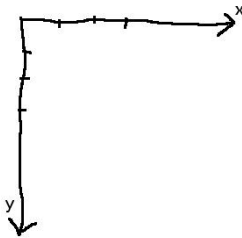


Illustration 1: The coordinate system - y grows from top to bottom.

- y-coordinates become now bigger from bottom to top. This is a coordinate-system of the form:

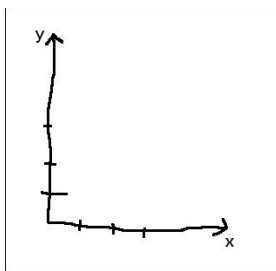


Illustration 2: The coordinate system with inverted y-coordinates.

Solution 2.2: Area selection tools

- Shift - Selection is constrained to a square
 - Alt - Width / height are changed with arrow keys one pixel at a time



- Ctrl - Selection is resized around the center
- Ctrl Alt - Current aspect ratio is maintained while resizing

- b)
- c)
- d)
- e)
- f)

g) *Image>Crop* crops the image to the selections bounding box, cutting away everything outside of the bounding box. *Image>Duplicate* creates a new image with the content of the selections bounding box.

Solution 2.3: Measuring and the results table

- a)
- b) New measurements are always added to the table names *Results*. If no table with this name is opened, a new one is created and shown.
- c) See b)
- d) Mean, std-dev, min and max can be found in the summarized results-table, the mode can be found in the distribution image.

File	Edit	Font	Results						
	Label	Area	Mean	StdDev	Mode	Min	Max	IntDen	RawIntDen
1	A4 dapi 1.tif	7329	145.482	36.712	157	15	239	1066241	1066241
2	A4 dapi 1.tif	7911	140.185	40.695	150	11	246	1109002	1109002
3	A4 dapi 1.tif	6144	130.745	28.865	132	9	211	803297	803297
Mean		7128	138.804	35.424	146.333	11.667	232	992846.667	
SD		900.485	7.465	6.019	12.897	3.055	18.520	165541.333	
Min		6144	130.745	28.865	132	9	211	803297	
Max		7911	145.482	40.695	157	15	246	1109002	

Illustration 3: Results table with summerized values.

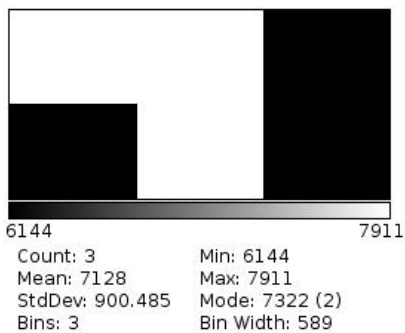
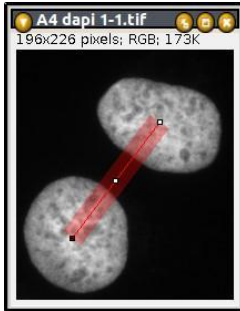


Illustration 4: The distribution of the area values.

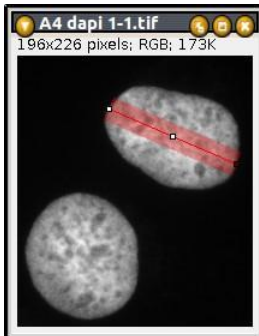
Solution 2.4: Line selection tools

a)



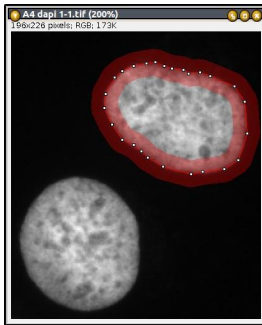
*Illustration 5:
Measuring the
distance between
two nuclei.*

b)



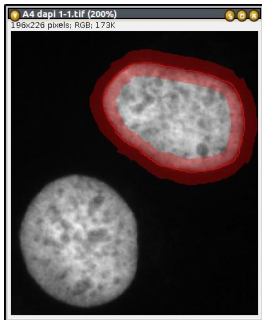
*Illustration 6:
Measuring the
diameter of a
nucleus.*

c)



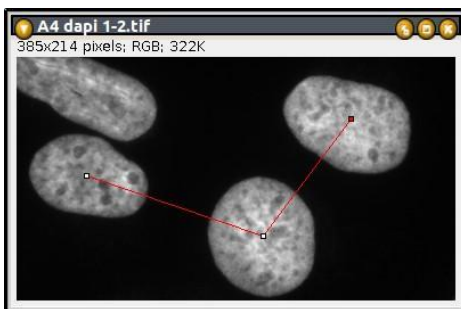
*Illustration 7:
Measuring the
perimeter with the
segmented line tool.*

d)



*Illustration 8:
Measuring the
perimeter with the
freehand line tool.*

e)



*Illustration 9: Measuring the angle
between the center points of three
nuclei.*

Solution 2.5: Profile plots

- a) Depending on the display settings the object may appear bigger or smaller. In the profile plot the background baseline is often good visible and you can measure the diameter above

of it.

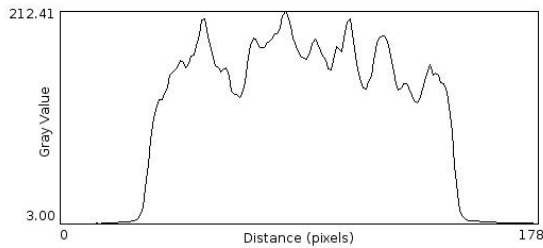


Illustration 10: An intensity profile of a line across a nucleus.

- b) The profile is smoother because it is averaged over the line-width.

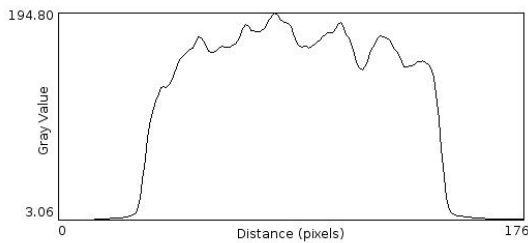


Illustration 11: The same profile plot as above but with a line-width of 21.

- c) The plots have different sizes. However they have different scales as well, so that measurements in the plots correspond to distances in the original image. So you should measure the same distance between two corresponding couples of points in the two plots. The properties dialog reveals that the pixel size is different in the two plots.



Illustration 12: Plot with a shorter line.

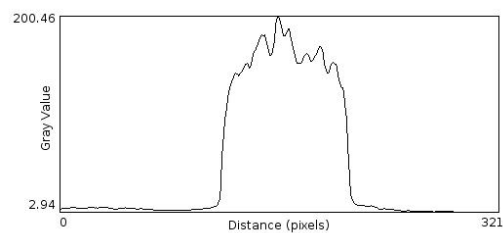


Illustration 13: Plot with a longer line.

- d)

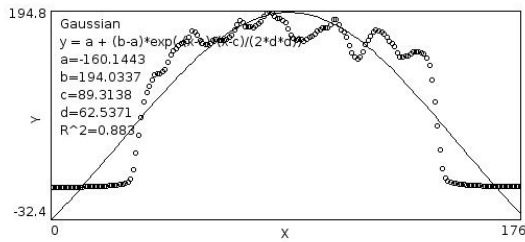
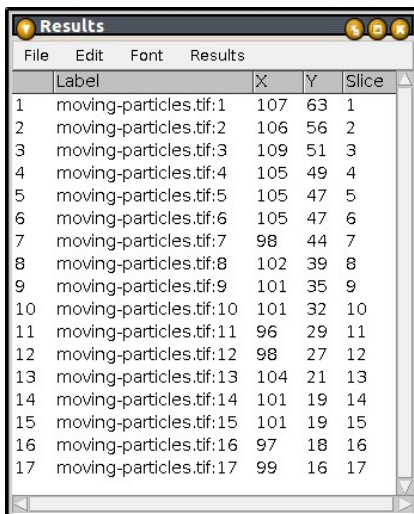


Illustration 14: A gaussian fit of the profile plot.

Solution 2.6: Point selections

- The number of the following points is decremented by one.
- The selection is saved with the tif-image and can still be modified after the image is loaded again.
- Each click adds a line with measurements to the results table.
- You can get a results-table with the x,y,z(slice)-coordinates as a result:



	Label	X	Y	Slice
1	moving-particles.tif:1	107	63	1
2	moving-particles.tif:2	106	56	2
3	moving-particles.tif:3	109	51	3
4	moving-particles.tif:4	105	49	4
5	moving-particles.tif:5	105	47	5
6	moving-particles.tif:6	105	47	6
7	moving-particles.tif:7	98	44	7
8	moving-particles.tif:8	102	39	8
9	moving-particles.tif:9	101	35	9
10	moving-particles.tif:10	101	32	10
11	moving-particles.tif:11	96	29	11
12	moving-particles.tif:12	98	27	12
13	moving-particles.tif:13	104	21	13
14	moving-particles.tif:14	101	19	14
15	moving-particles.tif:15	101	19	15
16	moving-particles.tif:16	97	18	16
17	moving-particles.tif:17	99	16	17

Illustration 15: Results of a manual particle-tracking.

Solution 2.7: Thresholding and the magic wand tool

- shortest perimeter: 288.8 pixel
 - longest perimeter: 608.0 pixel
 - shortest diameter: 95.8 pixel
 - longest diameter: 188.4 pixel

Results			
File	Edit	Font	Results
	Label	Perim.	Feret
1	A4 dapi 1.tif	340.475	123.988
2	A4 dapi 1.tif	310.149	109.041
3	A4 dapi 1.tif	339.463	117.720
4	A4 dapi 1.tif	301.664	111.571
5	A4 dapi 1.tif	332.534	107.824
6	A4 dapi 1.tif	333.806	118.119
7	A4 dapi 1.tif	297.321	107.201
8	A4 dapi 1.tif	607.955	188.404
9	A4 dapi 1.tif	307.078	115.521
10	A4 dapi 1.tif	288.836	95.776
Mean		345.928	119.516
SD		93.943	25.411
Min		288.836	95.776
Max		607.955	188.404

Illustration 16: Results of the diameter (Feret) and perimeter measurements.

- b) The complete image has an area of 334750 pixel. For the nuclei I measured an area of 64885 pixel. The percentage of the area covered by nuclei is therefore: $64885p * 100\% / 334750p = 19.4\%$

Solution 2.8: The roi-manager

- a)
b)
c)
d) It creates an RGB-snapshot of the image with the particle boundaries and labels.

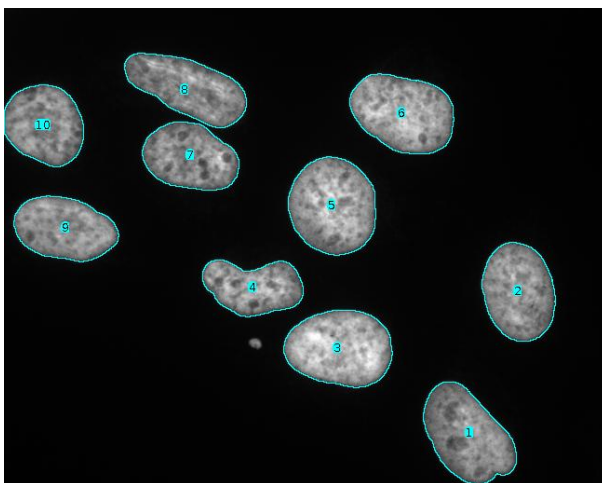


Illustration 17: Flattened image with particle boundaries and labels from the particles in the roi-manager.

- e) *Create selection* creates one single selection containing all nuclei. The *split* command on the roi-manager splits the selection into selections of connected particles and adds them to the roi-manager.
- f) The selections in the roi-manager are applied to the active window. This means you have transferred the selections of the nuclei from the dapi-image to the rhod-image. You can now measure the intensities within the nuclei in the rhod-image.

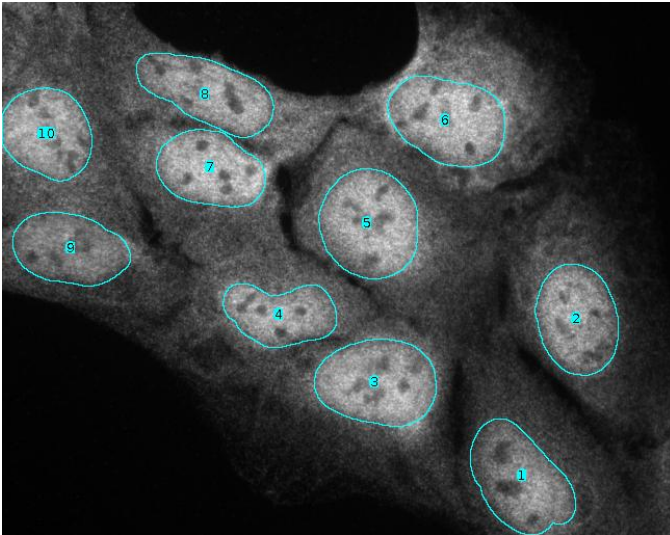


Illustration 18: The selections of the nuclei on the rhod-image.

Solution 2.9: Measuring objects with the Particle Analyzer

- a)
- b) Objects that are not entirely in the image do not have the correct size and will therefore falsify the measurements of mean or total values that are based on the size (area, diameter, perimeter, etc.). The option *exclude on edges* allows to ignore these objects.
- c)
- d) 0.7 as a minimum circularity excludes the two nuclei with the lowest circularity values.

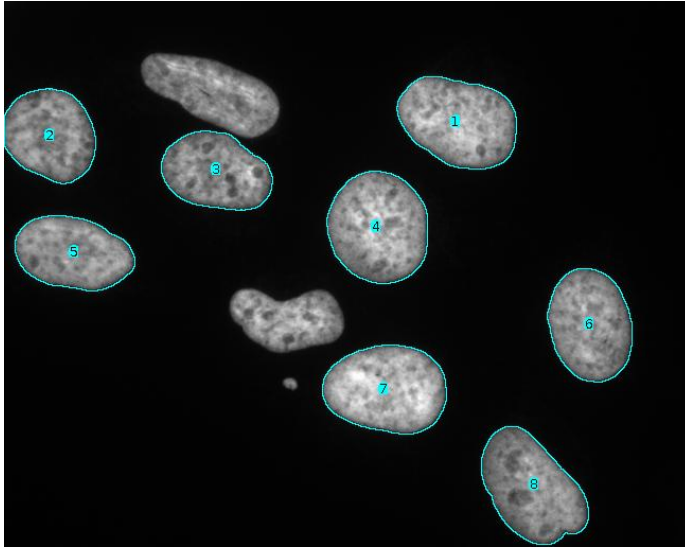


Illustration 19: The two nuclei with the lowest circularity have been excluded by the particle analyzer.

- e) With the include holes option only one big particle is found, containing the hole and the inner particle. Without the include hole option the inner and the outer particle are found. Be careful although measuring with the particle analyzer yields the right results in the second case, the roi added to the roi manager for the outer particle, contains the hole and the inner particle.
- f) Excluding the blobs touching the edges, I count 46 blobs of all sizes and forms. I measure an average area of 414.5 pixel.

Solution 2.10: Arrows, annotations and overlays

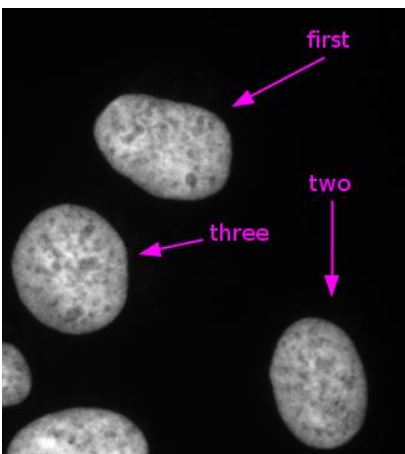


Illustration 20: Annotations added to the image, using the arrow-tool and the text-tool.