

# Template

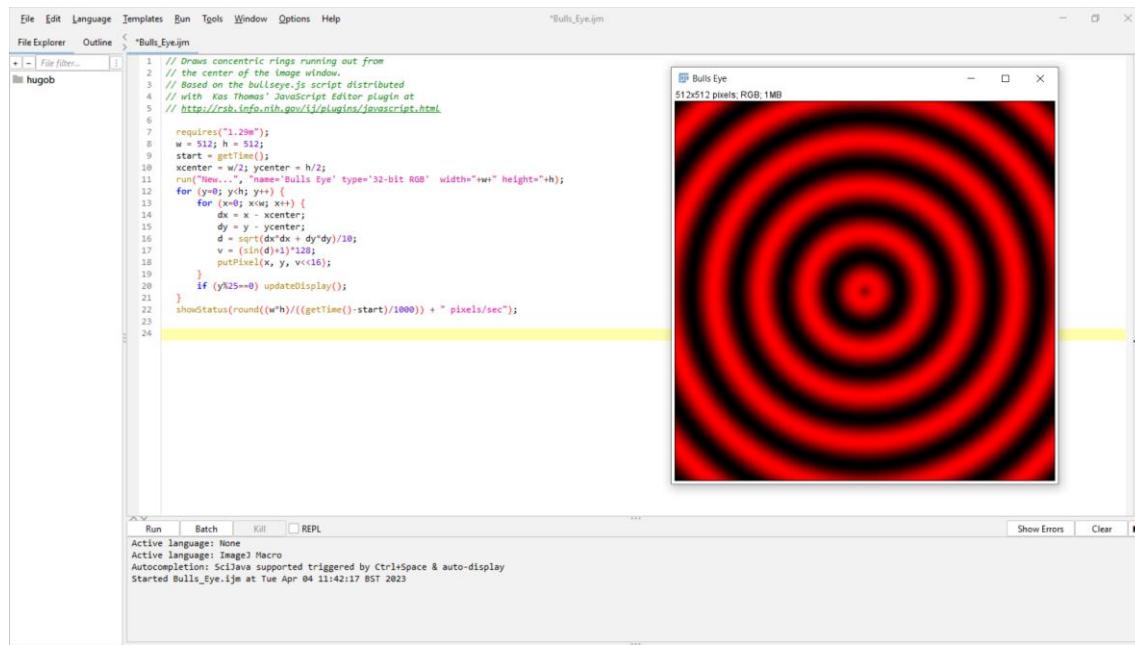
## Respostas às Tarefas 1, 2, 3, 4 e 5

### T1

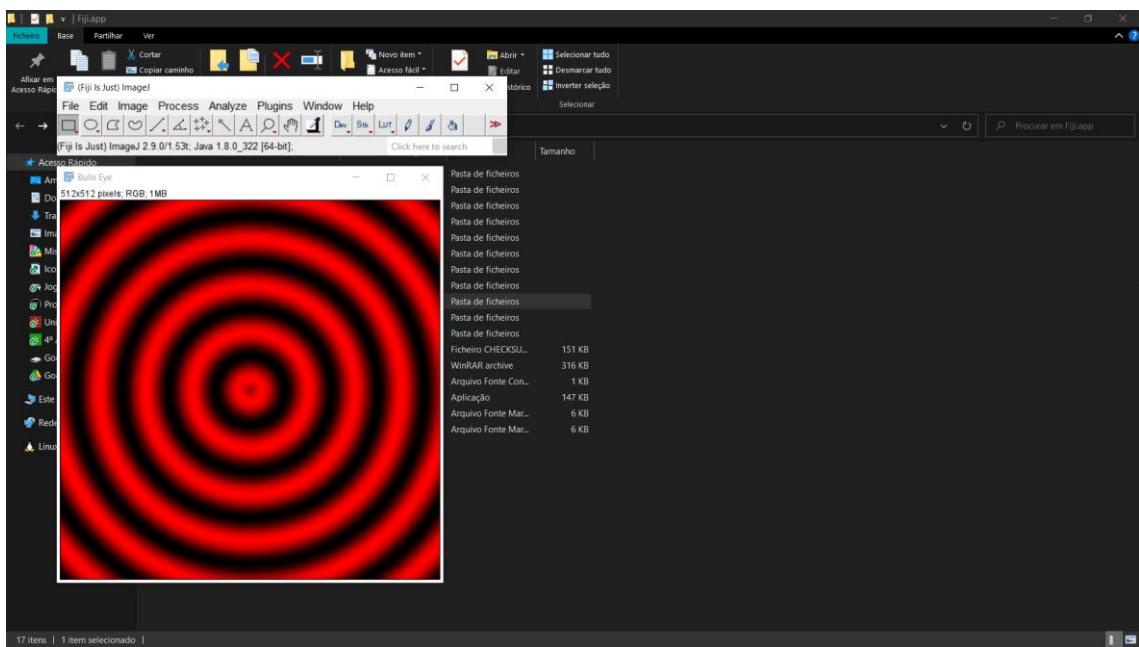
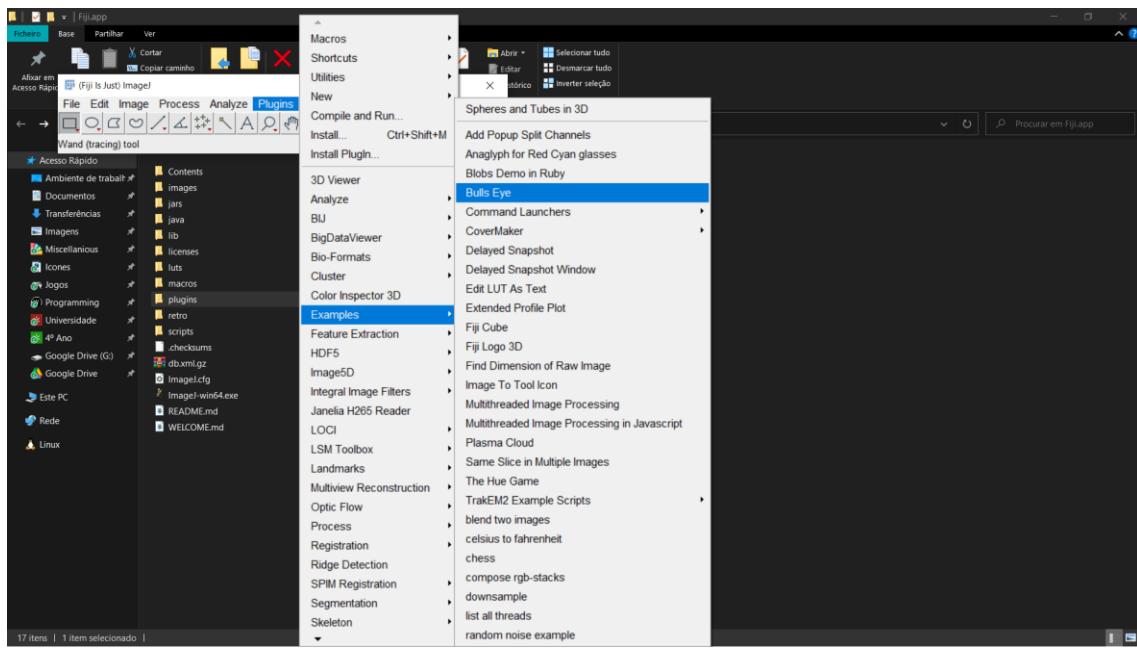
#### T1.4 ImageJ Folders

- a) Enter the macros folder. Drag the macro RandomOvals.txt onto the ImageJ launcher window. The macro will be opened in a macro-editor. Run the macro from the menu Macros>Run Macro of the editor.

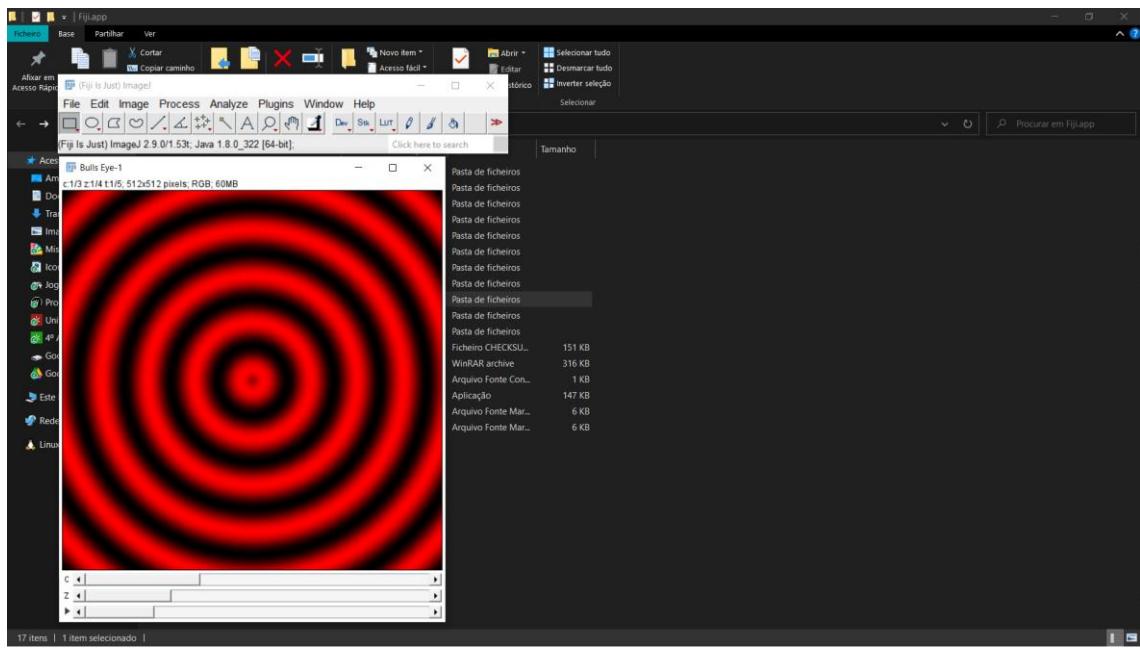
Como a diretoria macros não tinha nenhum ficheiro chamado RandomOvals.txt, eu utilizei o Bulls\_Eye.txt encontrado em plugins>Macros.



- b) Save the macro RandomOvals.txt under the name RandomOvals.ijm into the folder ImageJ/plugins/Examples. You can use the command File>Save As... from the macro-editor. Close all open windows in ImageJ. Call Help>Refresh Menus from the ImageJ launcher window. Now run the macro from the menu Plugins>Examples>RandomOvals.



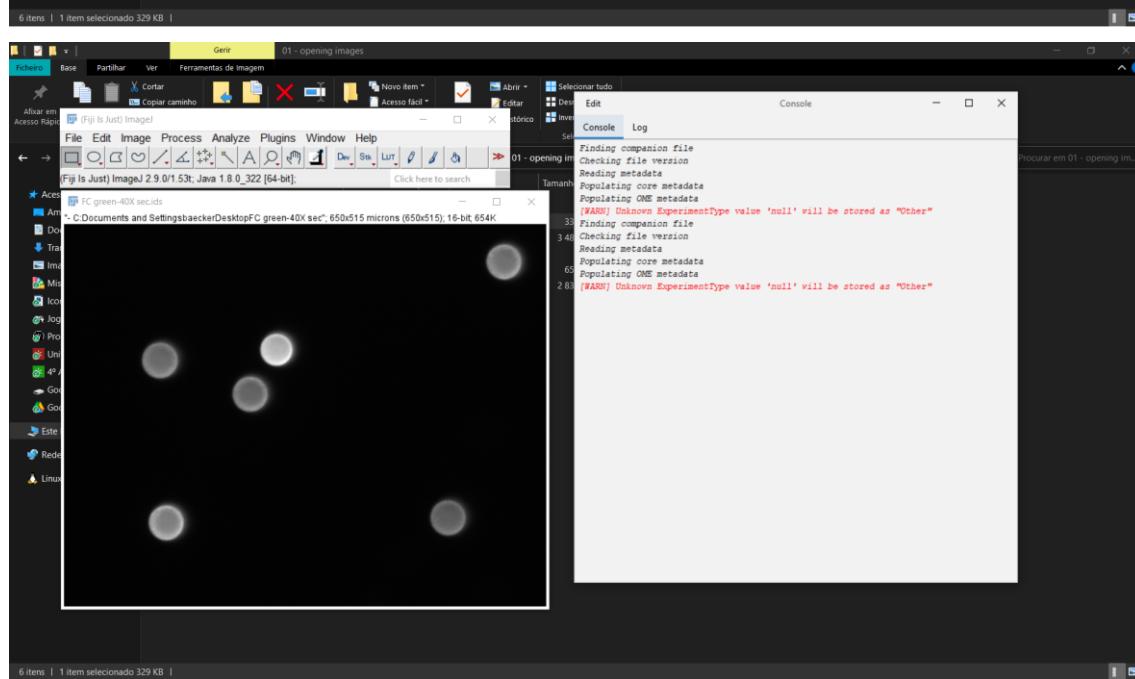
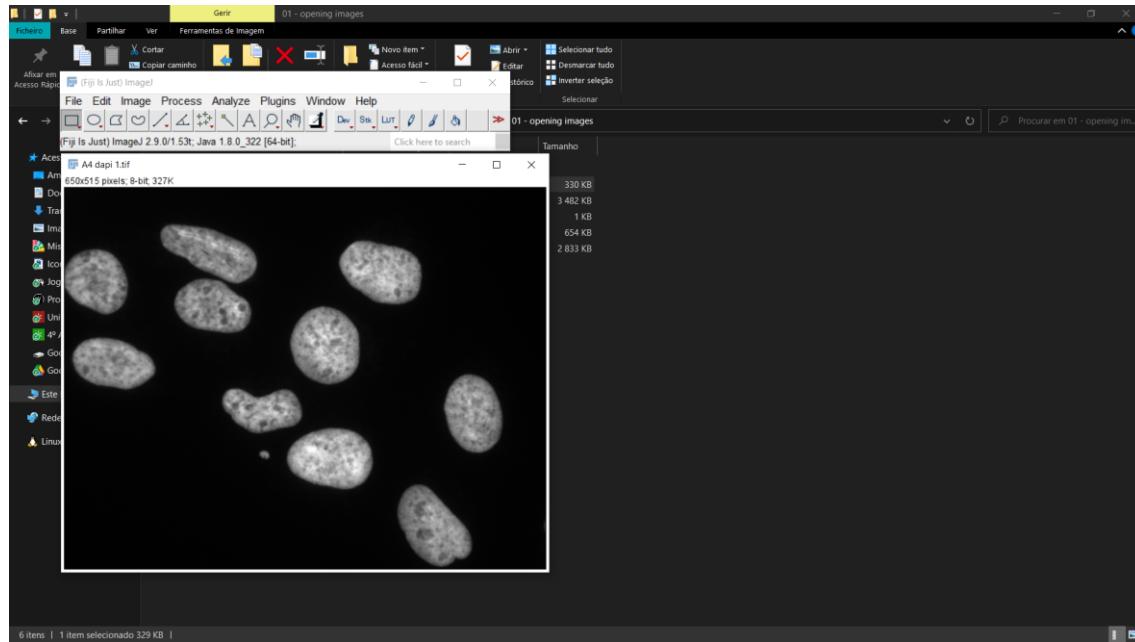
c) Hold down shift and click on Plugins>Examples>RandomOvals. What happens?



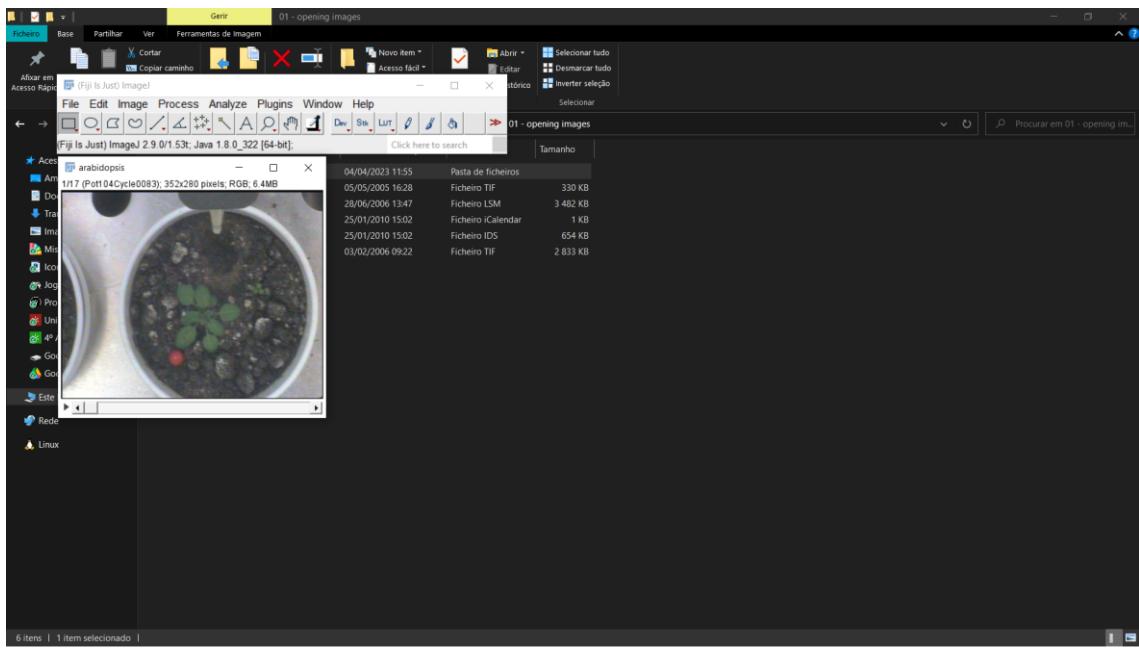
Ao correr o plugin com a tecla Shift, aparecem algumas opções de visualização, com os canais, “c”, com 3 valores, as slices, “z”, com 4, e os frames, “t”, com 5. Neste caso, a imagem original apenas aparece quando todos estes estão no valor 1.

## T1.5 Opening Images

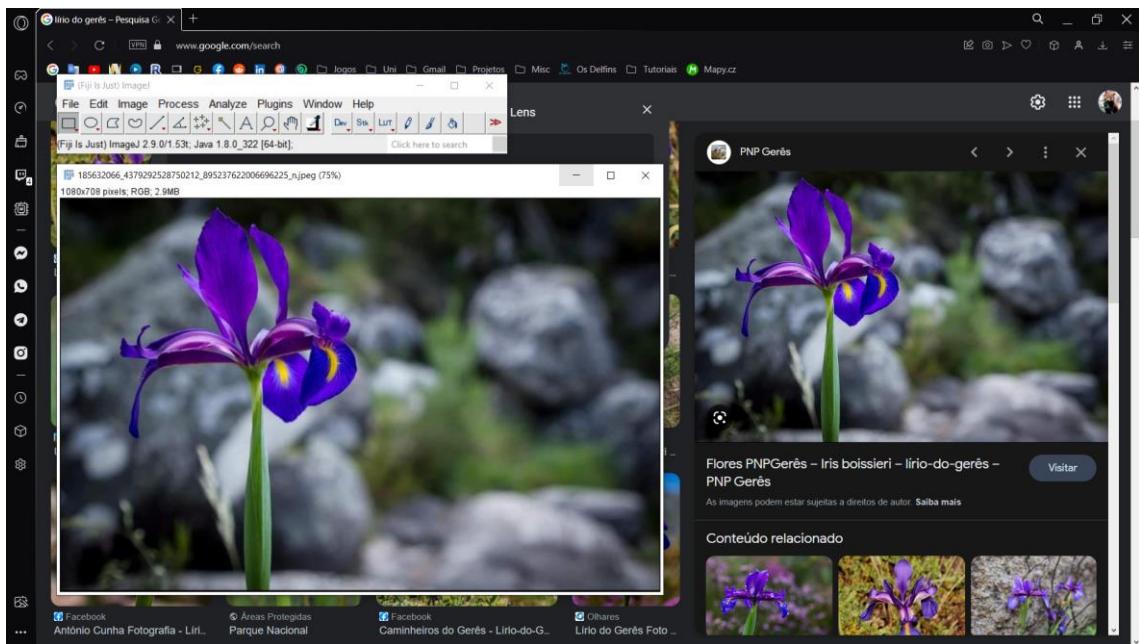
- a) Open the image A4 dapi 1.tif from folder 01 - opening images. Try different possibilities. Try **ctrl+shift+o** to switch to the next image in the folder. Try to open multiple images at the same time using drag and drop and by using the file-dialogue.



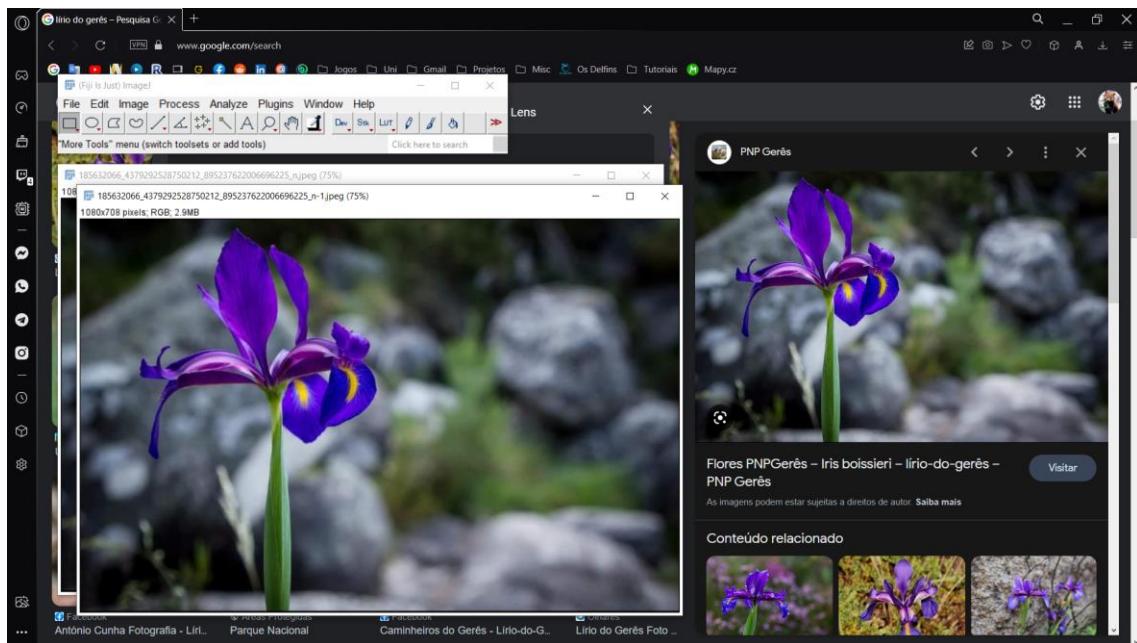
- b) Open the images in arabidopsis by dragging the folder onto the ImageJ-launcher. Open them using **File>Import>Image Sequence...**



- c) Drag and drop work with image URLs (online links) as well. Search an image with the web- browser, for example using google and drag it onto the ImageJ-launcher.

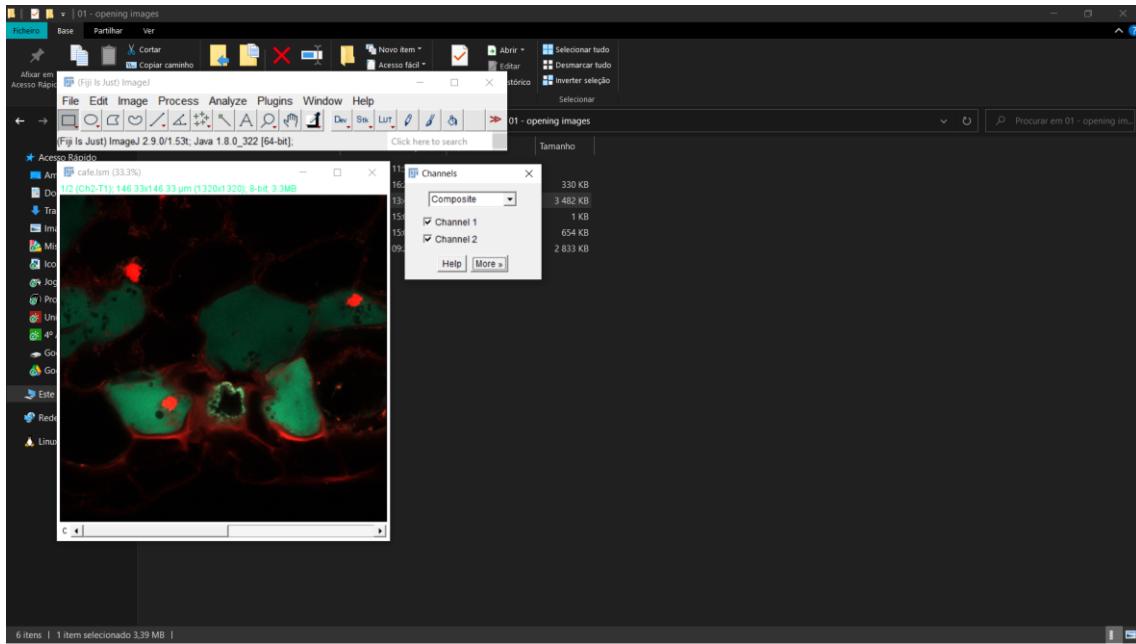


- d) Open the same image twice. What happens to the title of the image when the same image is opened multiple times?



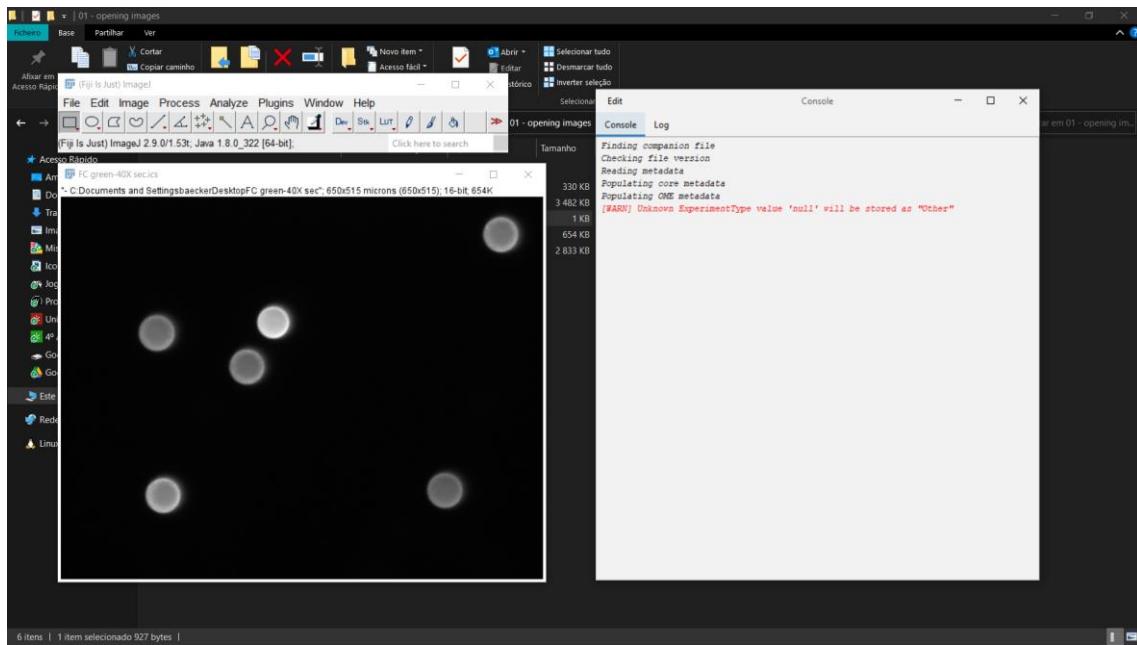
Quando se abre a mesma imagem duas vezes, a segunda imagem fica com um “-1” no final do nome.

- e) Open the image `cafe.lsm`. The image is opened as a so-called hyperstack. The slider on the bottom of the window changes the channel that is displayed. Run `Image>Color>Channels Tool...` and select `Composite` instead of `Color` as display mode to see an overlay of both channels.

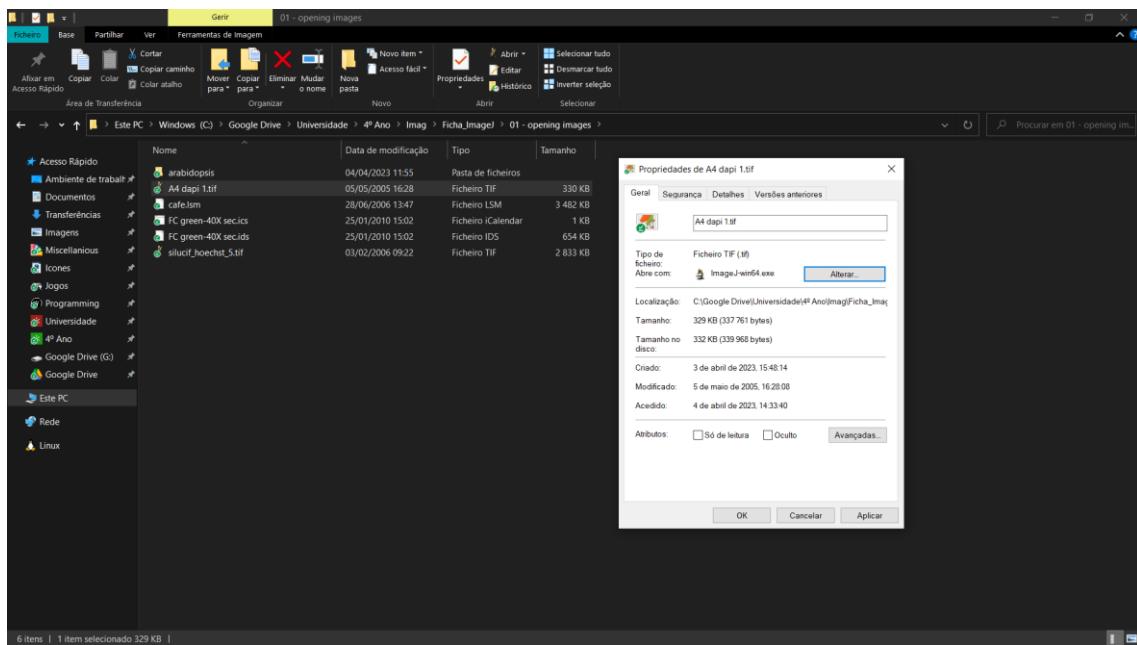


- f) Try to open the image `FC green-40X sec.ics`. You will get an error. ImageJ doesn't know how to open the ics-format. Use `Help>Plugins` to open the ImageJ plugins website in your web browser. Search for loci and click on the link. On this page click on Bio-Formats Downloads and drag the link `loci_tools.jar` onto the ImageJ launcher. This will open a file-dialogue. Click ok to save the plugin into the plugins folder. Try

to open the image again. Hint: Select Autoscale in the colour options of the Bio-Formats Import Options to have the image displayed correctly.



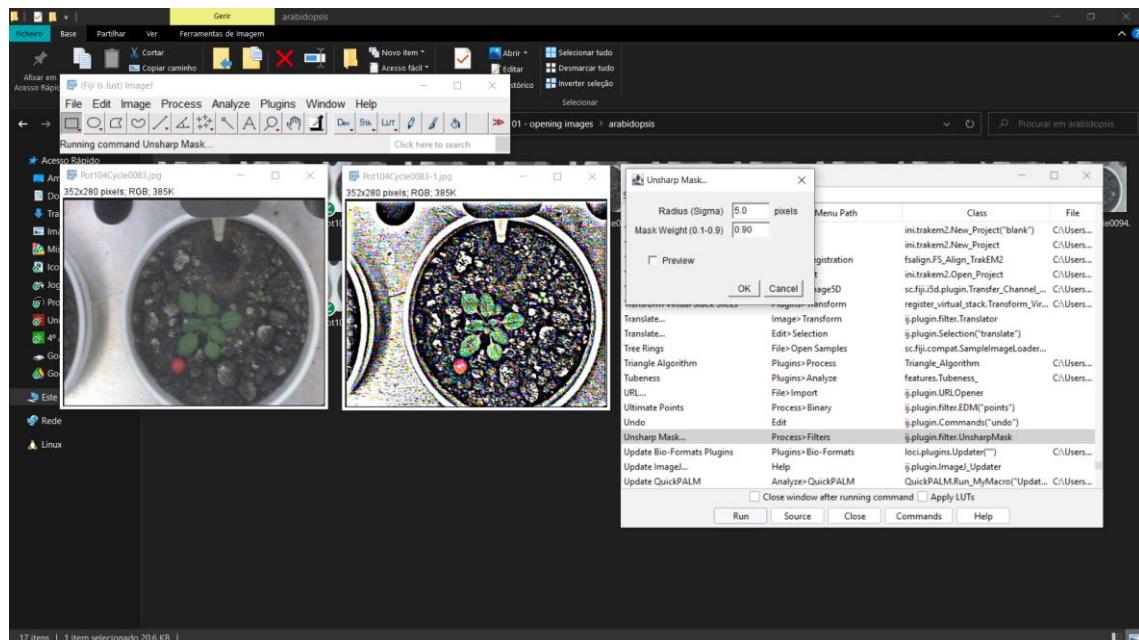
- g) Activate the single instance listener Edit>Options>Misc... in ImageJ and configure ImageJ as the default application for tif-images in your operating system.



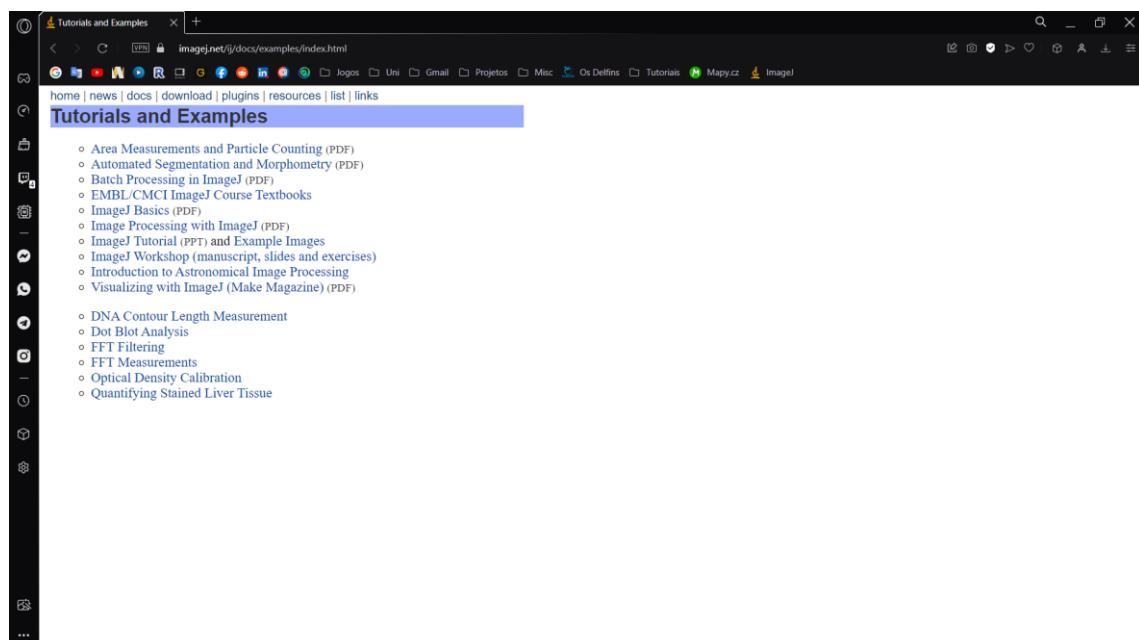
## T1.6 The ImageJ Website

- a) Go to the Documentation part of the website and have a look at the “ImageJ User Guide”. Read the explication of the Unsharp mask command. How does it work? Try the command on one of the images in the 01 - opening images/arabidopsis folder.

O comando “unsharp mask” torna as bordas da imagem mais nítidas, subtraindo uma versão desfocada (a “unsharp mask”) à imagem original. Tem 2 parâmetros, o radius (sigma), que corresponde ao desvio-padrão do desfoque Gaussiano, e o mask weight, que define a força da filtragem. Quanto maior o radius e o mask weight, mais nítida e contrastante fica a imagem.



- b) In the Documentation part have a look at the Tutorials and Examples.



The screenshot shows a web browser window with the following details:

- Title Bar:** Tutorials and Examples
- Address Bar:** imagej.net/ij/docs/examples/index.html
- Page Content:**
  - Tutorials and Examples** (highlighted in blue)
  - Links to:
    - Area Measurements and Particle Counting (PDF)
    - Automated Segmentation and Morphometry (PDF)
    - Batch Processing in ImageJ (PDF)
    - EMBL-CMCI ImageJ Course Textbooks
    - ImageJ Basics (PDF)
    - Image Processing with ImageJ (PDF)
    - ImageJ Tutorial (PPT) and Example Images
    - ImageJ Workshop (manuscript, slides and exercises)
    - Introduction to Astronomical Image Processing
    - Visualizing with ImageJ (Make Magazine) (PDF)
  - Links to:
    - DNA Contour Length Measurement
    - Dot Blot Analysis
    - FFT Filtering
    - FFT Measurements
    - Optical Density Calibration
    - Quantifying Stained Liver Tissue

- c) ImageJ users can ask questions on the ImageJ mailing list. Go to the list-archive. In the search text-field type colocalization. Find the name of at least one colocalization plugin from the answer of the search.

The ImageJ mailing list is a discussion group for ImageJ users and developers. ImageJ support is also available on the ImageJ Forum.

**Subscribing**  
To subscribe, go to the [Subscribe](#) or [Unsubscribe](#) page, enter your name and email address, and click "Subscribe (IMAGEJ)".

**Sending Messages**  
To send a message to all the people subscribed to the list, send a message to [imagej@list.nih.gov](mailto:imagej@list.nih.gov).

**List Archives**  
Messages sent to this list are archived and made available on the Web at [list.nih.gov/archives/imagej.html](http://list.nih.gov/archives/imagej.html).

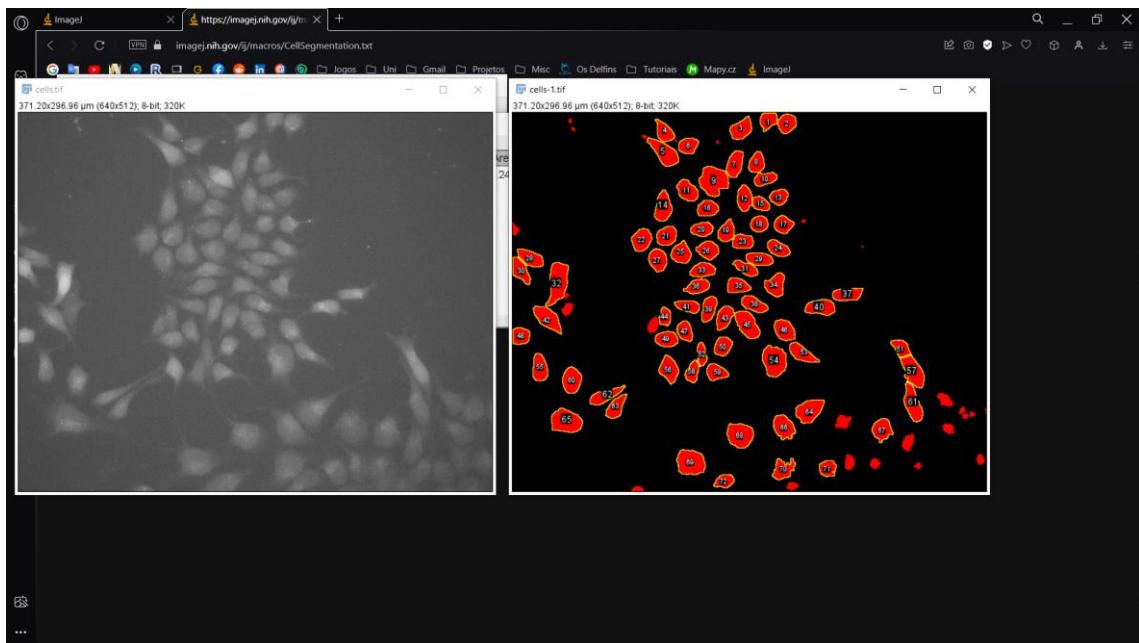
**Leaving the List**  
To unsubscribe, go to the [Subscribe](#) or [Unsubscribe](#) page, enter your email address and click "Unsubscribe (IMAGEJ)".

**More Information**  
When you subscribe to the list, you will receive a message with more information.

- d) Go to the plugins part of the website. How many colocalization plugins can you find on this page?

Specify ROI  
Specify Line Selection  
Comment Writer  
16-bit Histogram  
Results and Text  
Draw line or point grids  
Moment Calculator  
Batch Statistics  
Cell Counter  
Oval Profile Plot  
Color Comparison  
Radial Profile Plot  
Microtubule  
MRI Analysis Calculator  
Sync Measure 3D  
Hough Circles  
Convex Hull, Circularity, Roundness  
Fractal Dimension and Lacunarity  
Measure And Label  
**Colocalization**  
Granulometry  
Texture Analysis  
Named Measurements  
Cell Outliner  
Grid Cycloid Arc  
RGB Profiler  
**Colocalization** Finder  
Spectrum Extractor  
Contact Angle  
**RG2B Colocalization**  
Color Profiler  
Hull and Circle  
MR Urography  
Template Matching  
Extract IMT from ultrasound images  
ITCN (Image-based Tool for Counting Nuclei)  
Multi Cell Outliner  
FRETcalc - FRET by acceptor photobleaching  
JACoP (Just Another **Colocalization** Plugin)

- e) Go to the macros part of the website. Drag the link to the CellSegmentation.txt macro onto the ImageJ-launcher and run the macro from the macro-editor. Do you understand what it does?



A macro CellSegmentation.txt separa e conta o número de células presentes numa imagem.

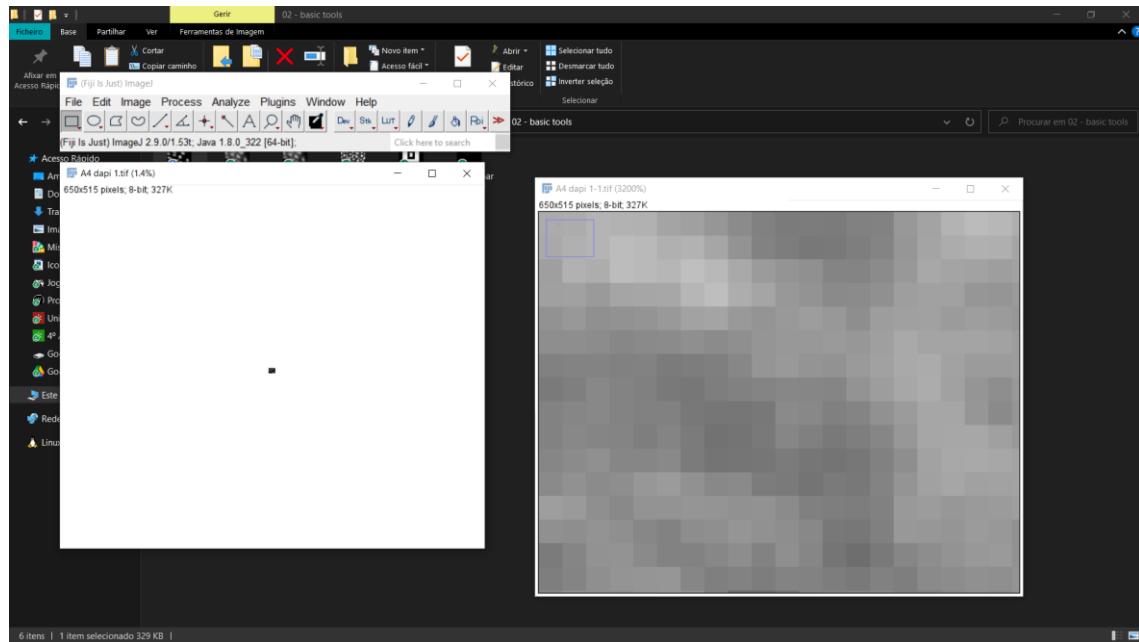
- f) Go to [https://www.medphys.it/down\\_scan\\_header.htm](https://www.medphys.it/down_scan_header.htm) where a custom plugin is available. What does it do?

O plugin presente nesse website demonstra as tags das imagens DICOM presentes numa diretoria e suas subdiretorias.

# T2

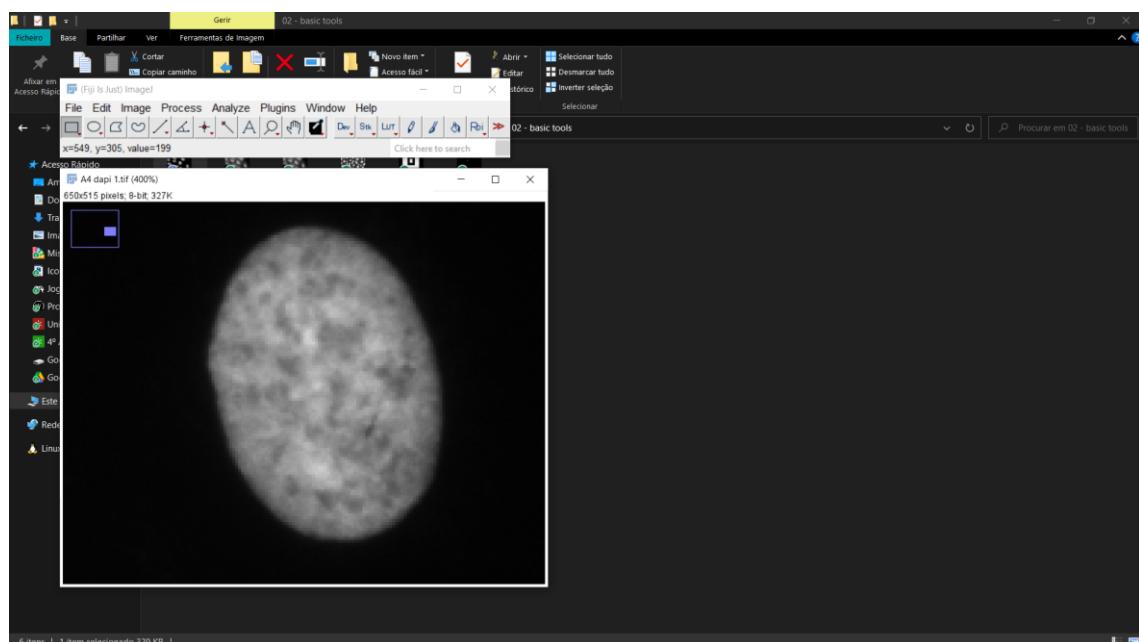
## T2.1 Pixel Information, Zooming and Scrolling

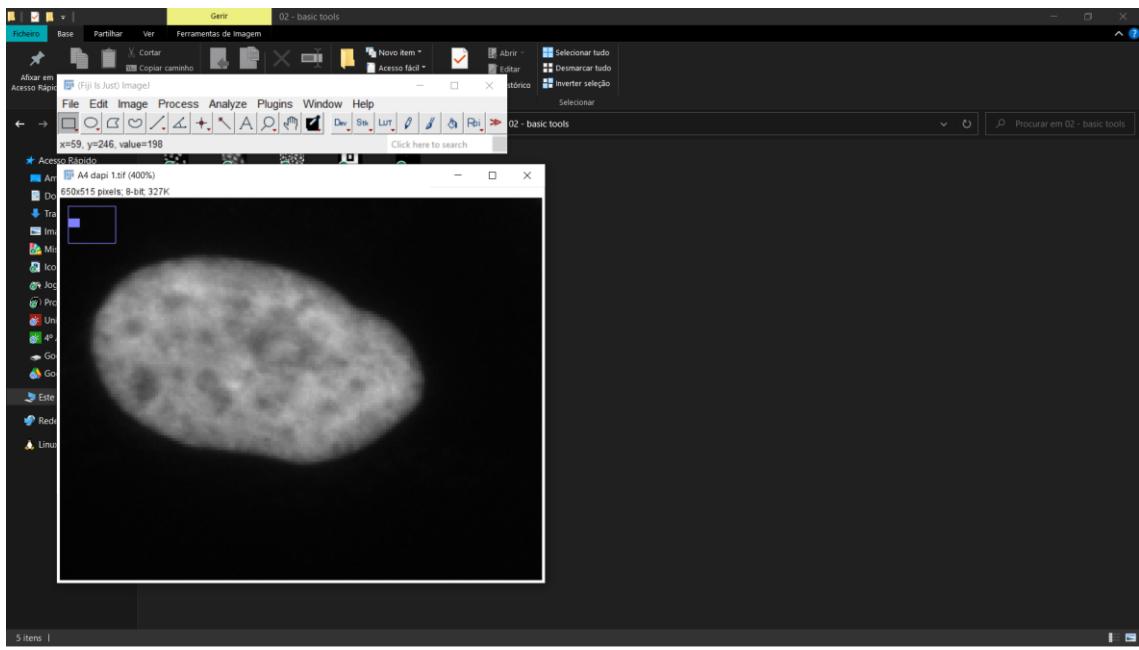
- a) What is the maximal possible zoom? What is the minimal zoom?



O valor máximo do zoom é de 3200% e o mínimo é 1.4%

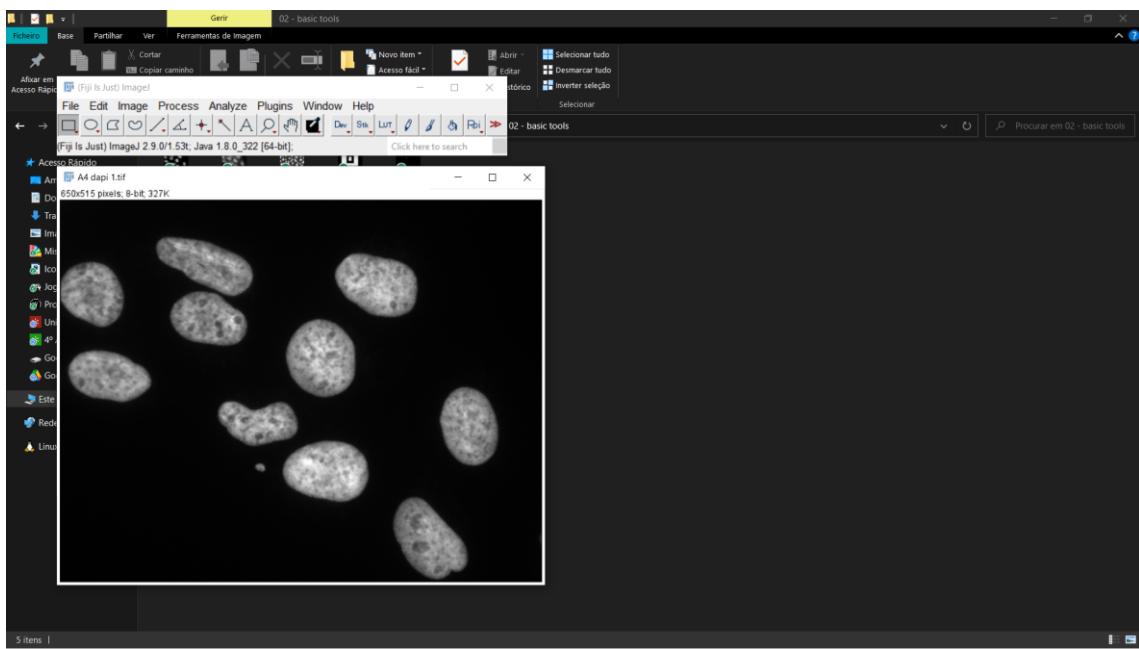
- b) What is the grey-value of the pixel with the coordinates  $x=549$  and  $y=305$ ? What is the grey-value of the pixel with the coordinates  $x=58$  and  $y=246$ ?





O valor das coordenadas  $x=549$  e  $y=305$  é 199, e o das coordenadas  $x=58$  e  $y=246$  é de 198.

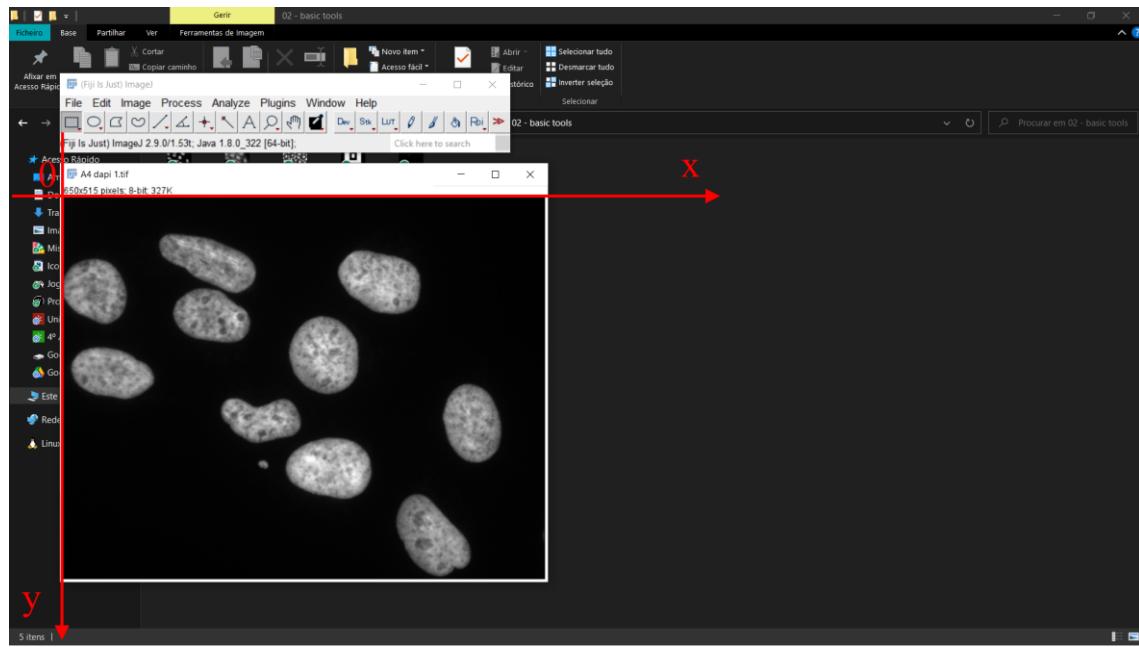
- c) What is the width and height of the image? What is its type? What is its size in the memory?



A imagem tem as dimensões 650x515, é do tipo 8-bit e ocupa 327KB na memória.

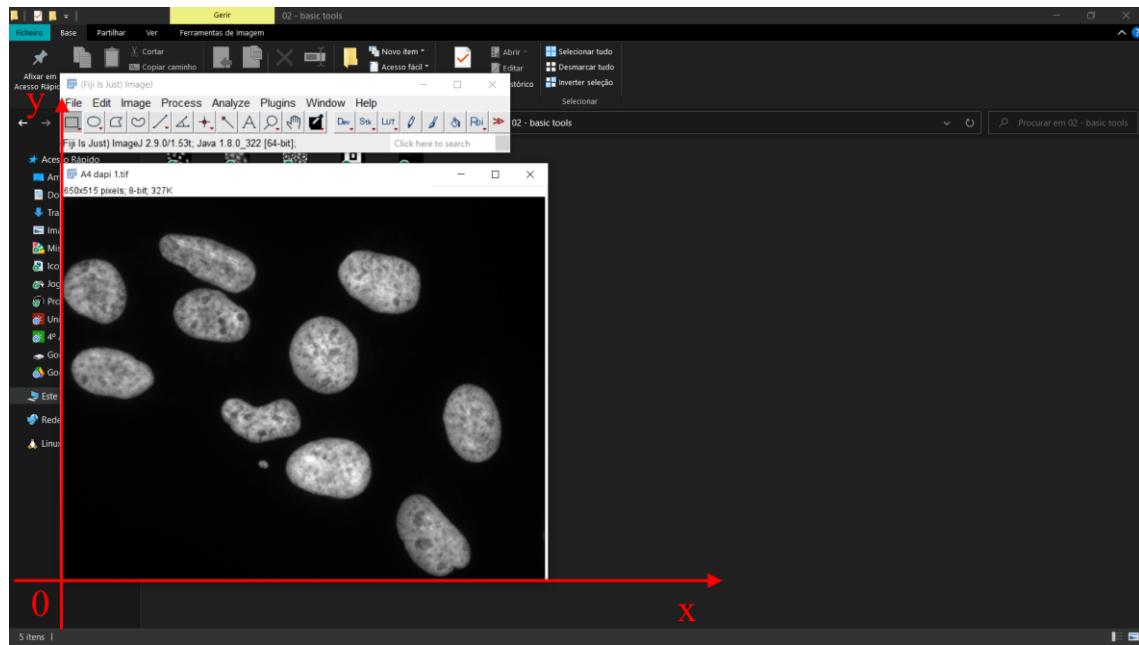
- d) Move the mouse over the image. In which direction does the x-coordinates of the pixels become bigger. In which direction does the y-coordinates become bigger? How does the coordinate-system look like?

As coordenadas  $x=0$  e  $y=0$  estão no canto superior esquerdo, o  $x$  aumenta para a direita, e o  $y$  para baixo, assim, o sistema de coordenadas fica da seguinte forma:



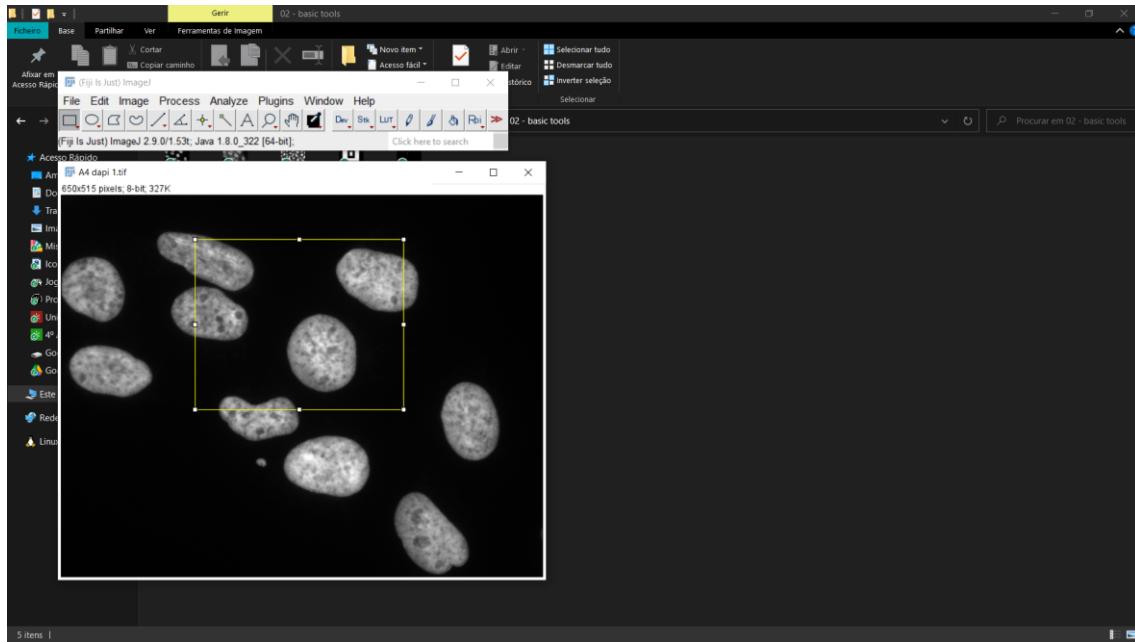
- e) You can change the orientation of the  $y$ -axis. Open the dialogue Analyse>Set Measurements... and select Invert Y coordinates. Move the mouse over the image again. How did the coordinate-system change?

Alterou o ponto de coordenadas  $x=0$  e  $y=0$  para o canto inferior esquerdo, ficando o sistema de coordenadas da seguinte forma:

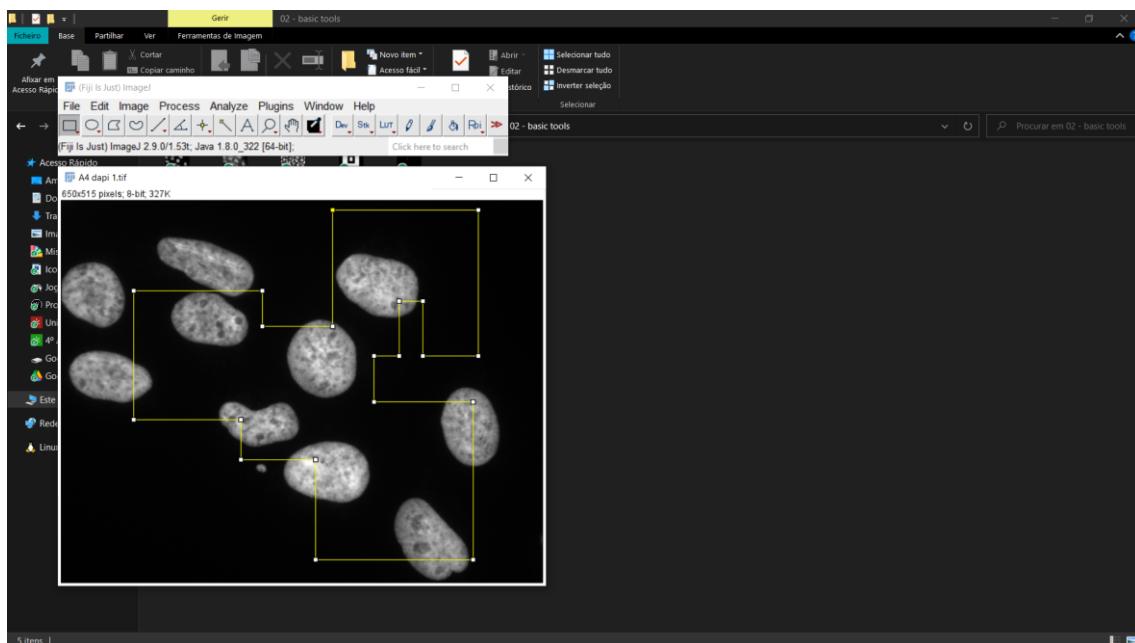


## T2.2 Area Selection Tools

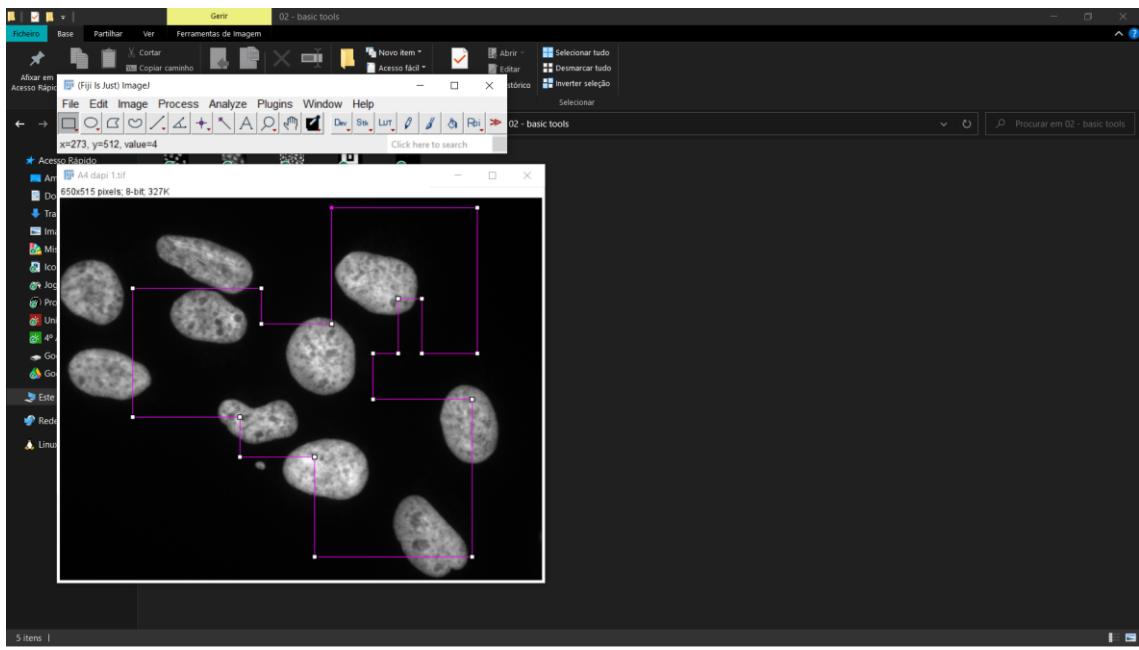
- a) Make a rectangular selection on the image. Move it around. Change its size. Find out in the ImageJ documentation (ImageJ User Guide) which modifier keys exist for the rectangular selection tool and what they do. Try them.



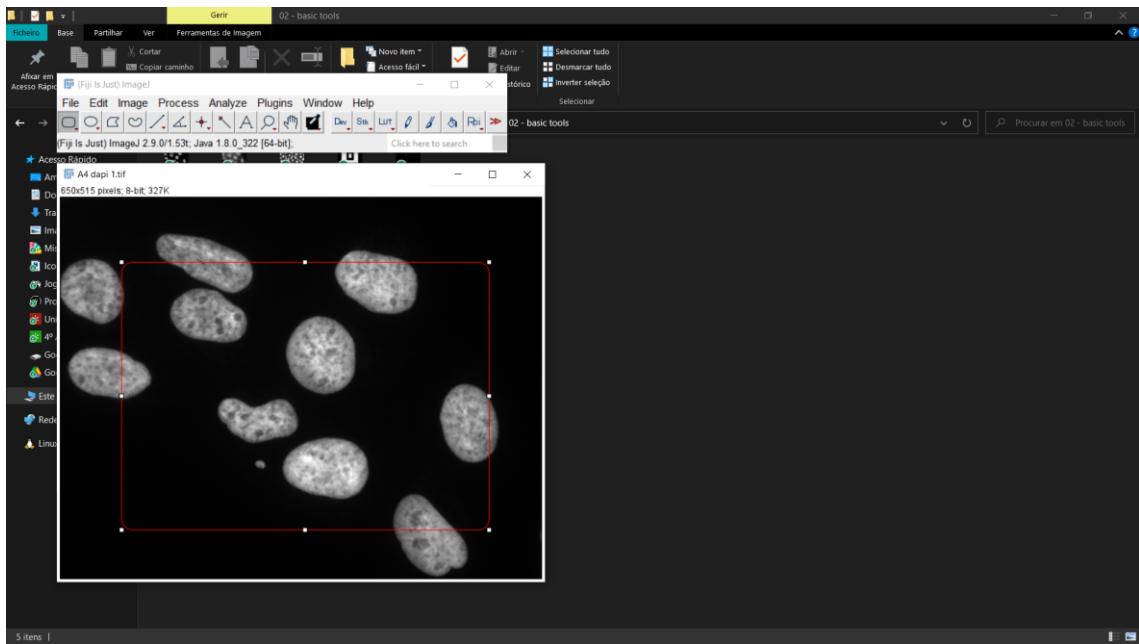
- b) Note that you can create complex selections by using the shift and the alt key. Shift adds to a selection, alt removes from a selection. Try it. Move the complex selection around. Note that you create one selection, even if the parts may be separated.



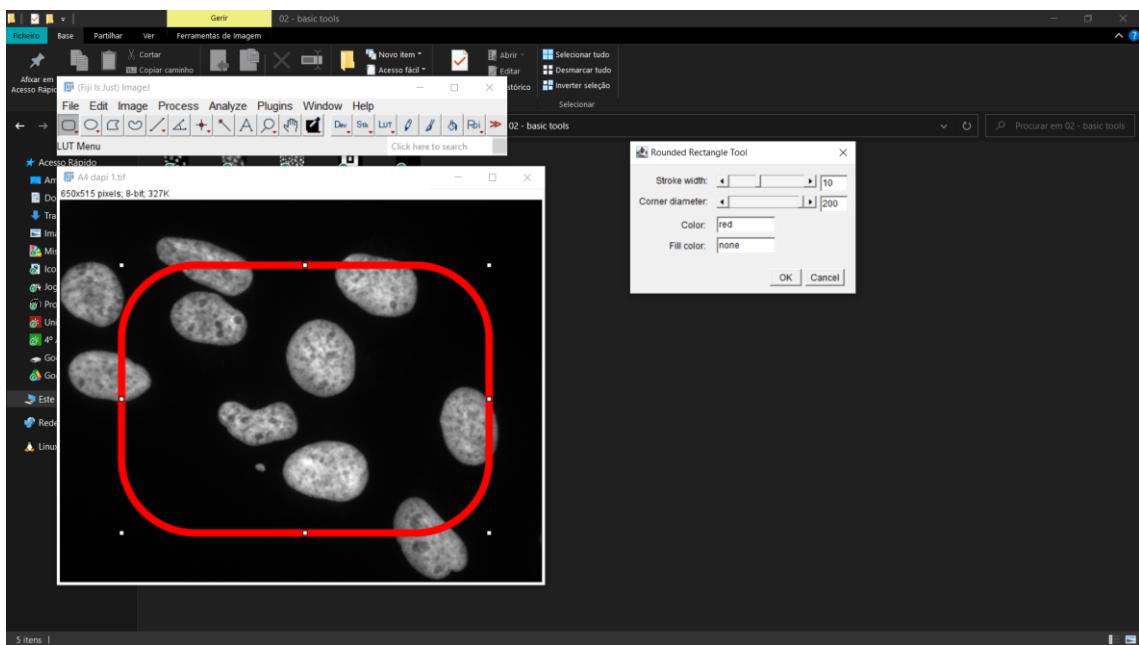
- c) Change the selection colour to magenta (Edit>Options>Colors...).



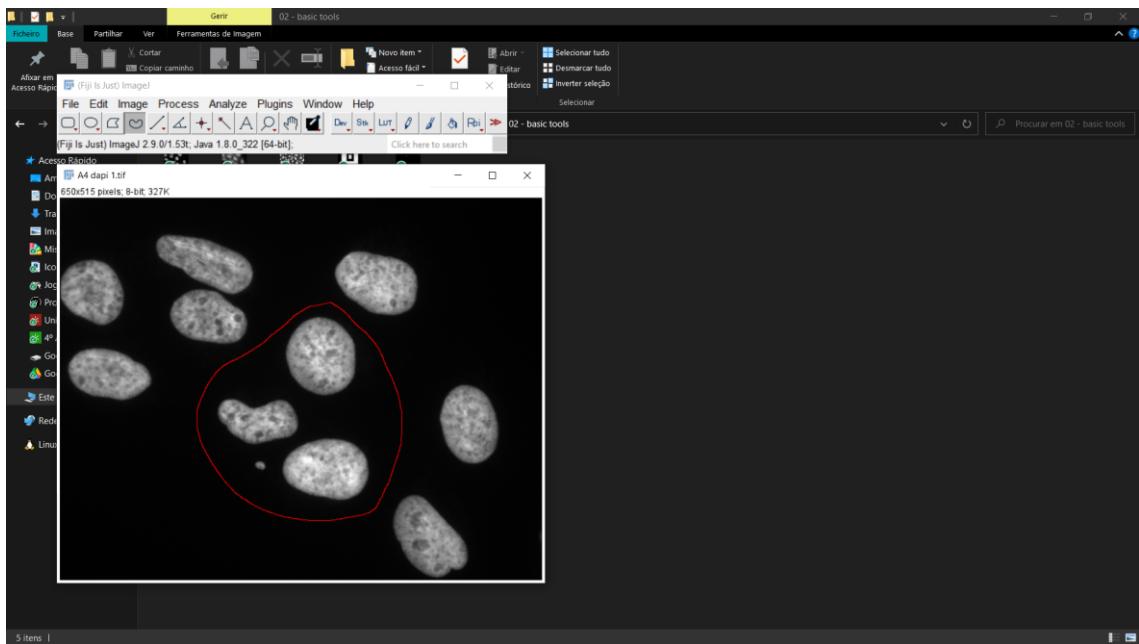
- d) Right-click on the rectangular-selection tool-button and select the rounded-rectangle-tool. Make a selection. You can change its colour and style by double-clicking on the tool-button.



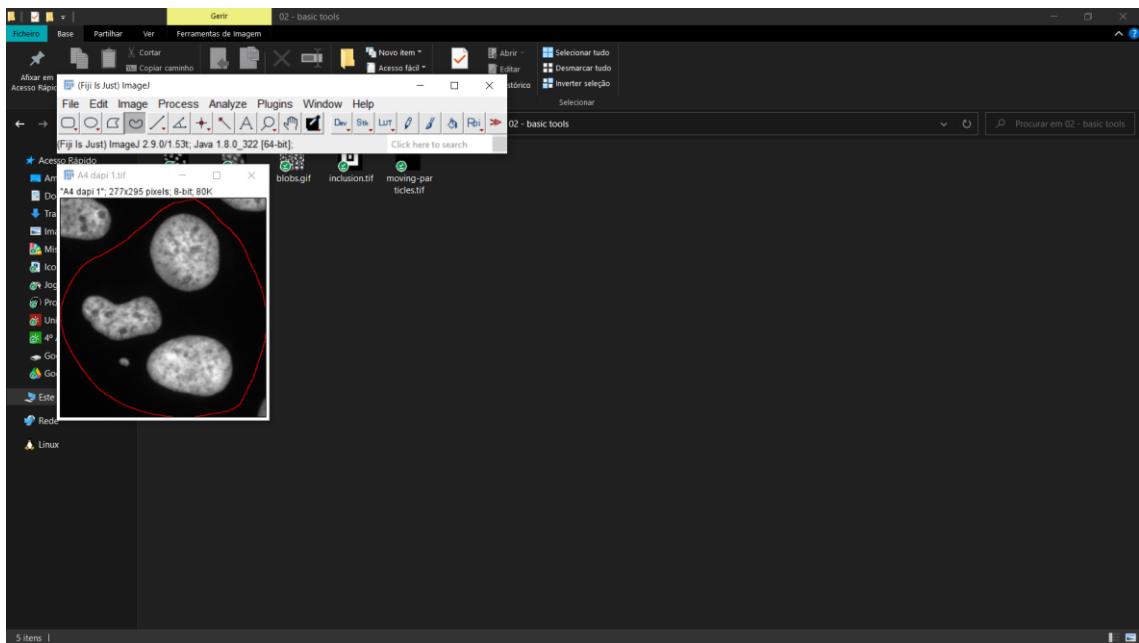
- e) Double-click on the rounded-rectangle-tool-button. Change the value of the corner-arc size and draw a new selection.



- f) Try the other area-selection tools up to and including the freehand-selection tool.  
 Note that when using the freehand-selection tool you should not try to close it yourself. Just go near to the beginning and release the mouse button. The selection will be closed automatically.

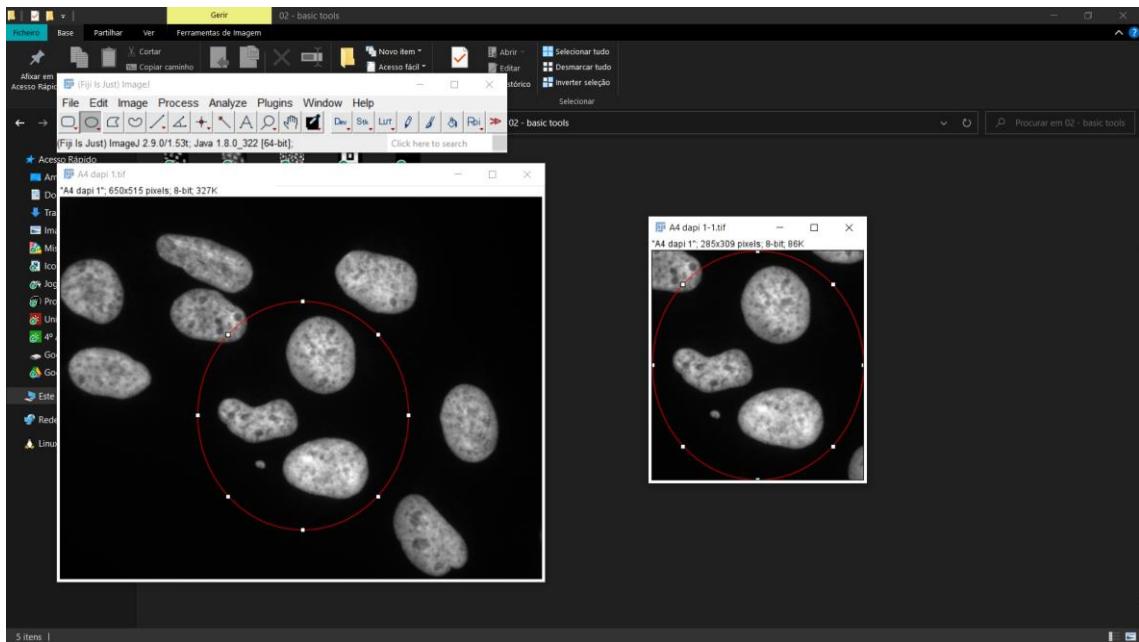


- g) Make a selection and run the command Image>Crop. What happens?



O comando crop “corta” a imagem segundo os eixos de forma a incluir toda a parte selecionada, mas a excluir a parte não selecionada, a não ser que a remoção desta implique a remoção da parte selecionada.

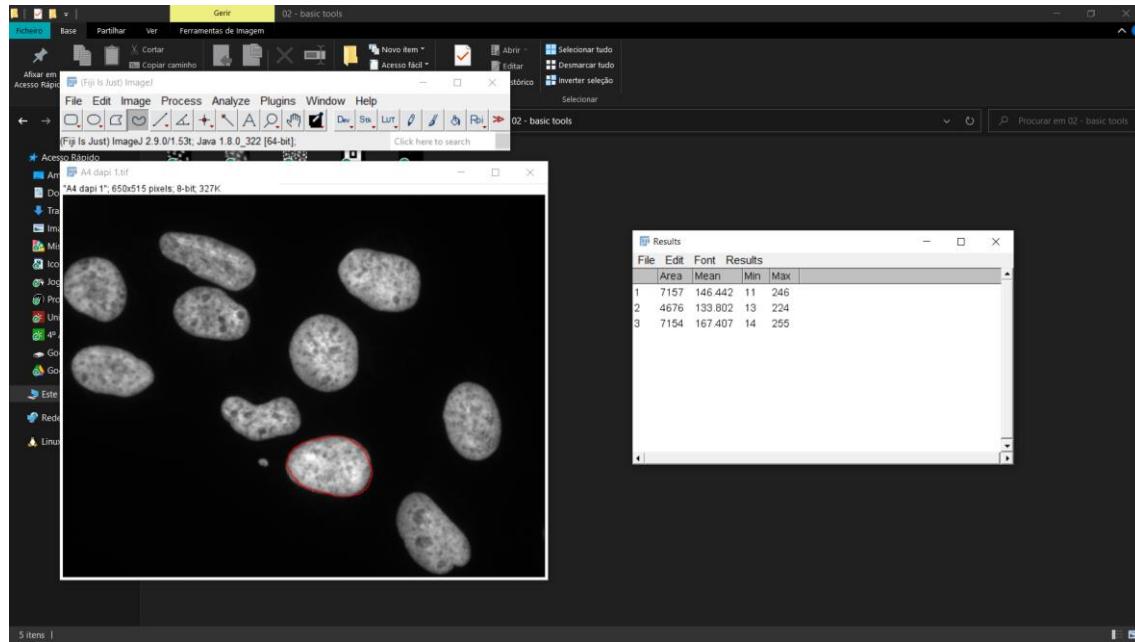
- h) You can reload the original image using File>Revert (ctrl+r). Make a selection again and run the command Image>Duplicate (shift+d). What happens?



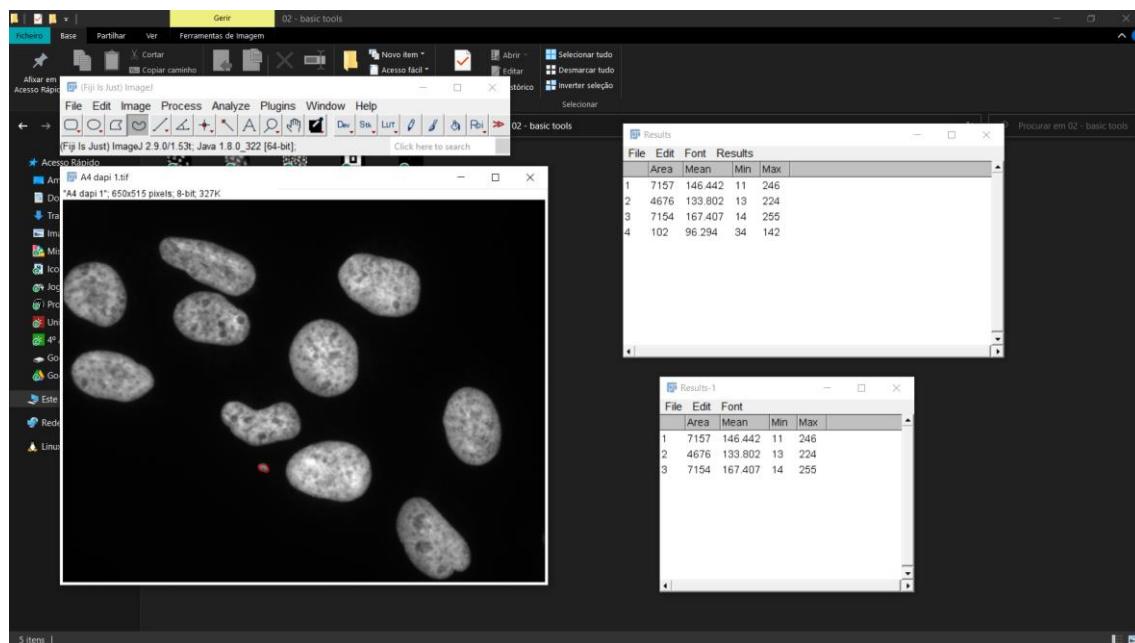
O comando duplicate, quando está selecionado uma secção da imagem, duplica a imagem, mas, de forma semelhante ao crop, apenas na parte selecionada.

## T2.3 Measuring and The Results-Table

- a) Use the freehand-selection to select a nucleus, then measure its area and mean grey-value. Do the same thing for multiple nuclei. Zoom into the image to make a precise selection.

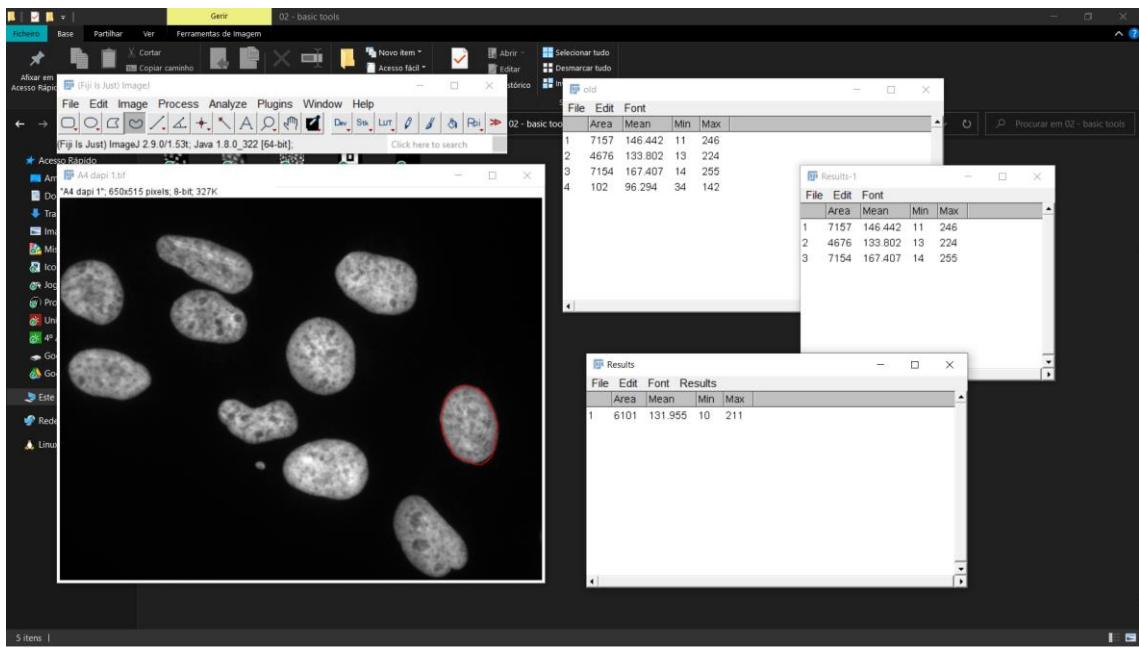


- b) Call File>Duplicate from the results table to make a copy of the results. Measure a new selection. To which table is it added?



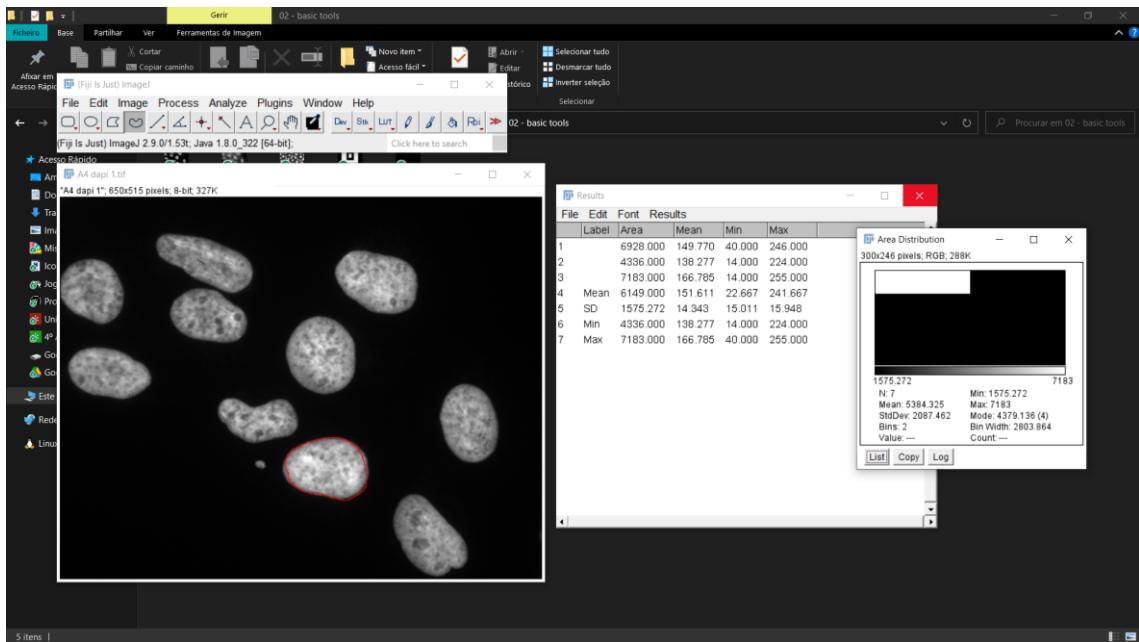
A nova medida é adicionada à tabela original.

- c) Use File>Rename and rename the original results table (Results) to “old”. Make a new measure. Into which results-table is it added?



O novo resultado é adicionado a uma nova tabela com o nome original.

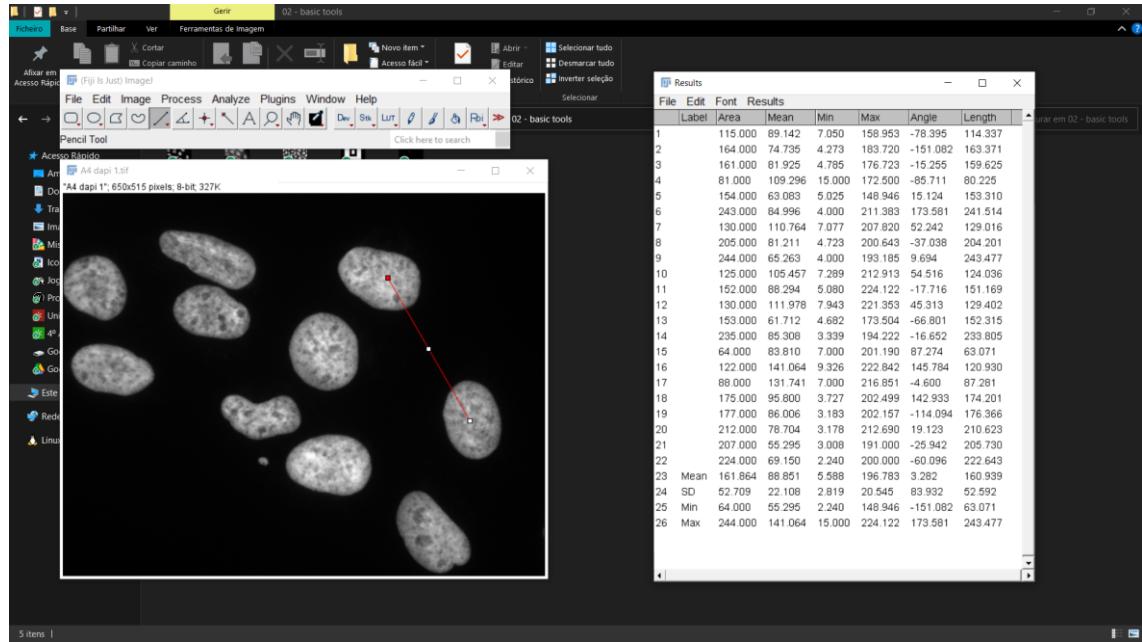
- d) Clear the results table using Results>Clear Results and measure at least 3 nuclei again. What is the mean, the standard deviation, the min, the max and the mode of the distribution of the area values? Use Results>Summarize and Results>Distribution... to get the answers.



A média das áreas é de 6149, o desvio-padrão de 1575.272, o mínimo 4336 e o máximo 7183. Quanto à moda, é de 4379.136.

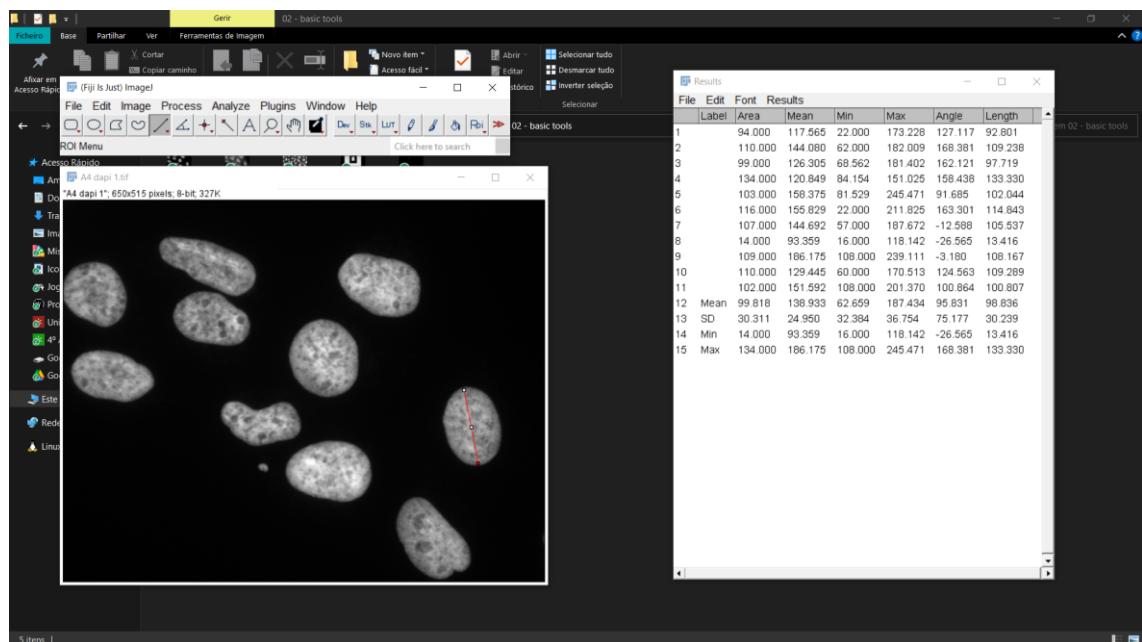
## T2.4 Line Selection Tools

- a) Use the straight-line tool to estimate the distances between the centres of the nuclei.



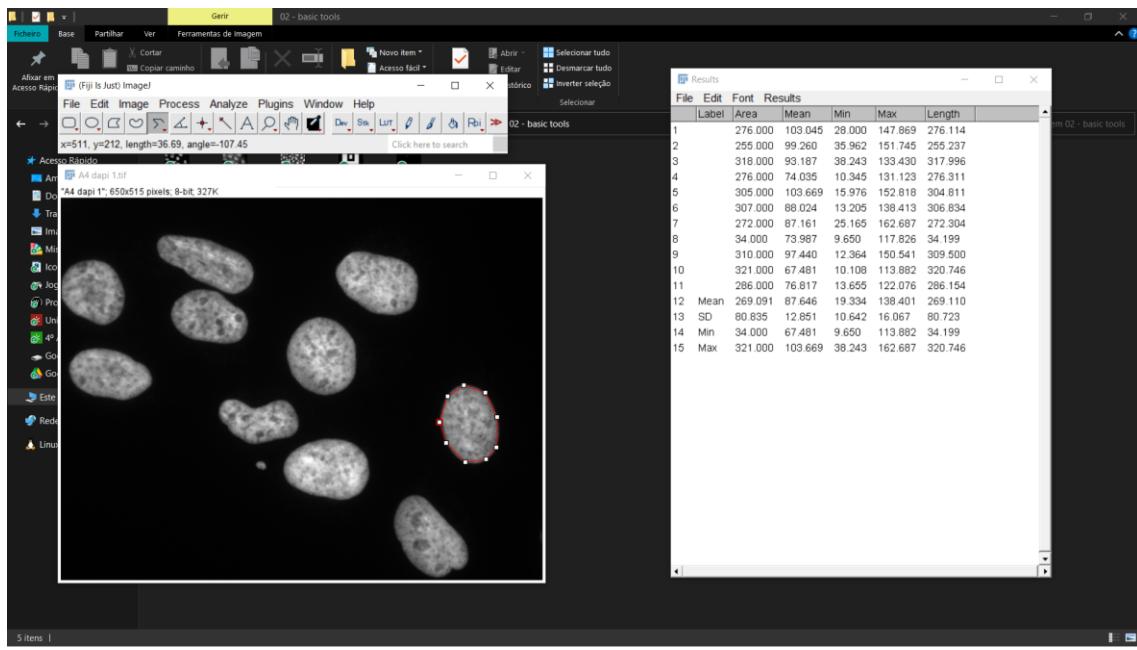
Após 22 medições, a média da distância entre o centro dos núcleos é de 160.939.

- b) Use the straight-line tool to estimate the diameters of the nuclei.



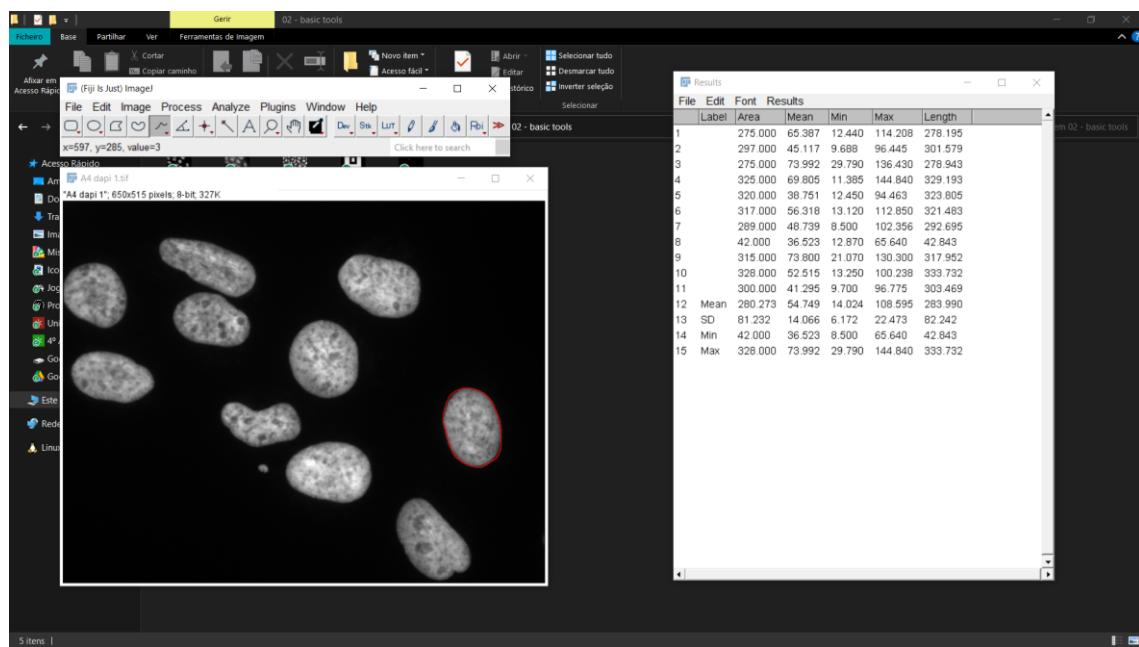
Após 11 medições, a média do diâmetro dos núcleos é de 98.836.

- c) Use the segmented line tool to estimate the perimeters of the nuclei.



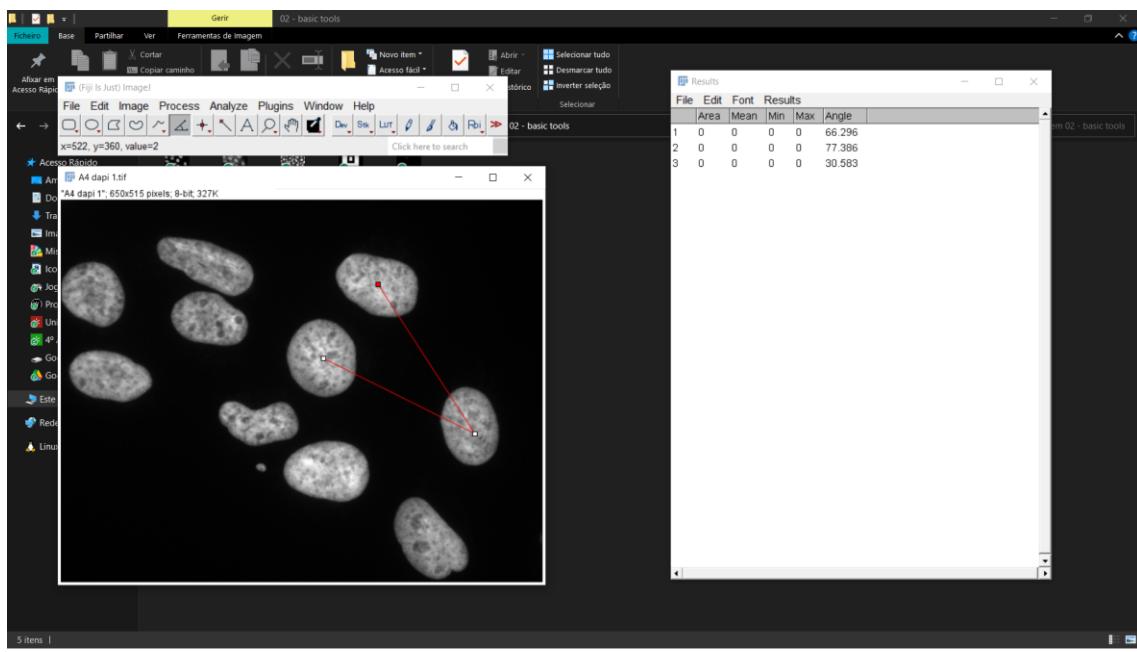
Após 11 medições, a média do perímetro das células é de 269.110.

d) Use the freehand-line tool to estimate the perimeters of the nuclei.



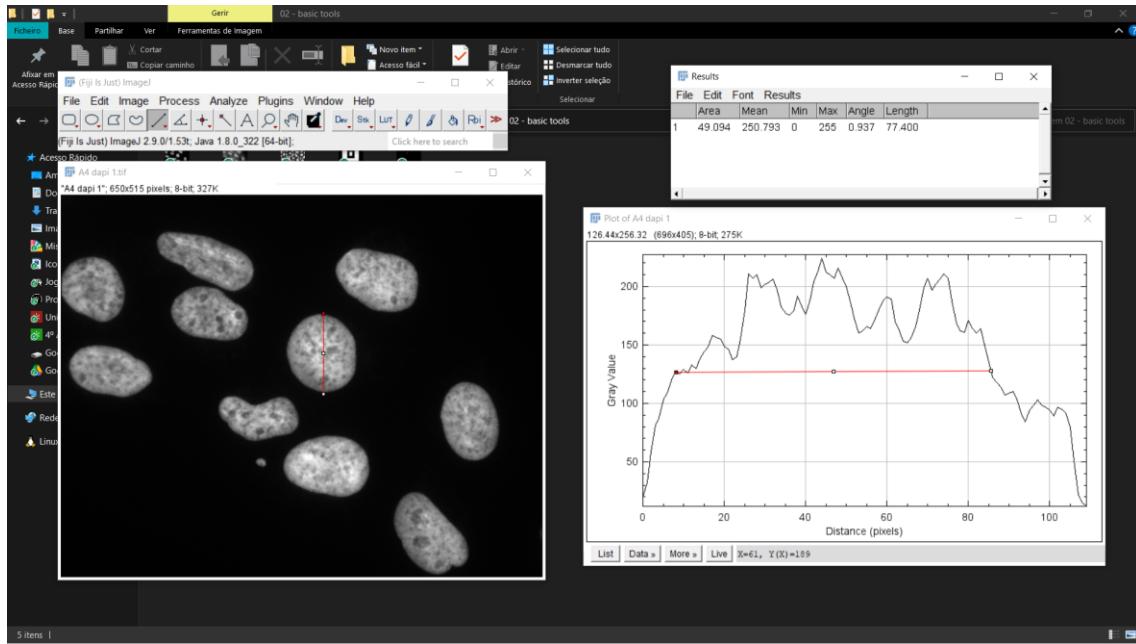
Após 10 medições, a média do perímetro das células é de 283.990.

e) Use the angle tool to estimate the angles between three nuclei.



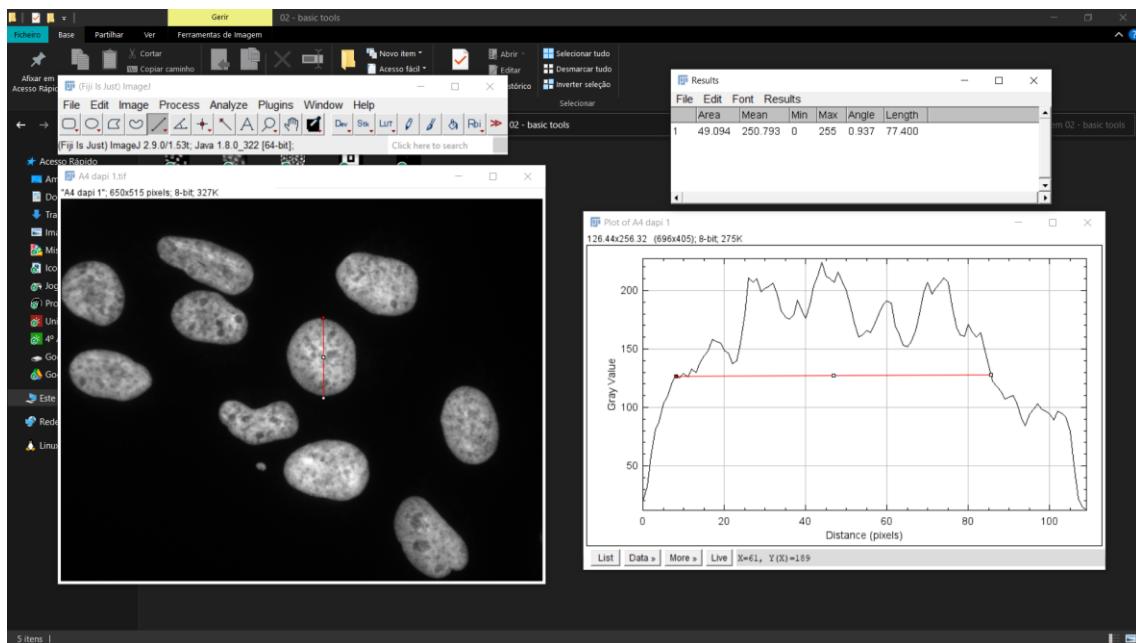
## T2.5 Profile Plots

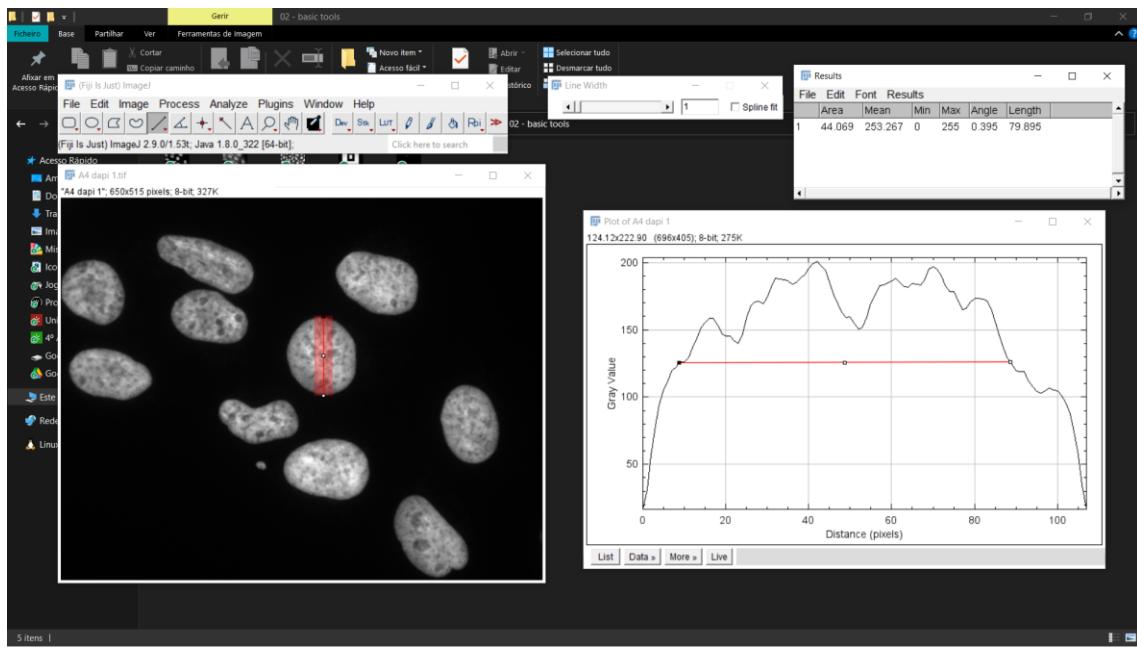
- a) Use a profile plot to estimate the diameter of a nucleus. Note that the plot is an image itself so that you can use ImageJ tools on the plot. Is there an advantage of using the profile plot rather than measuring the diameter in the original image?



Como o plot tem os valores das cores, conseguimos ser mais exatos na deteção das fronteiras no núcleo da célula.

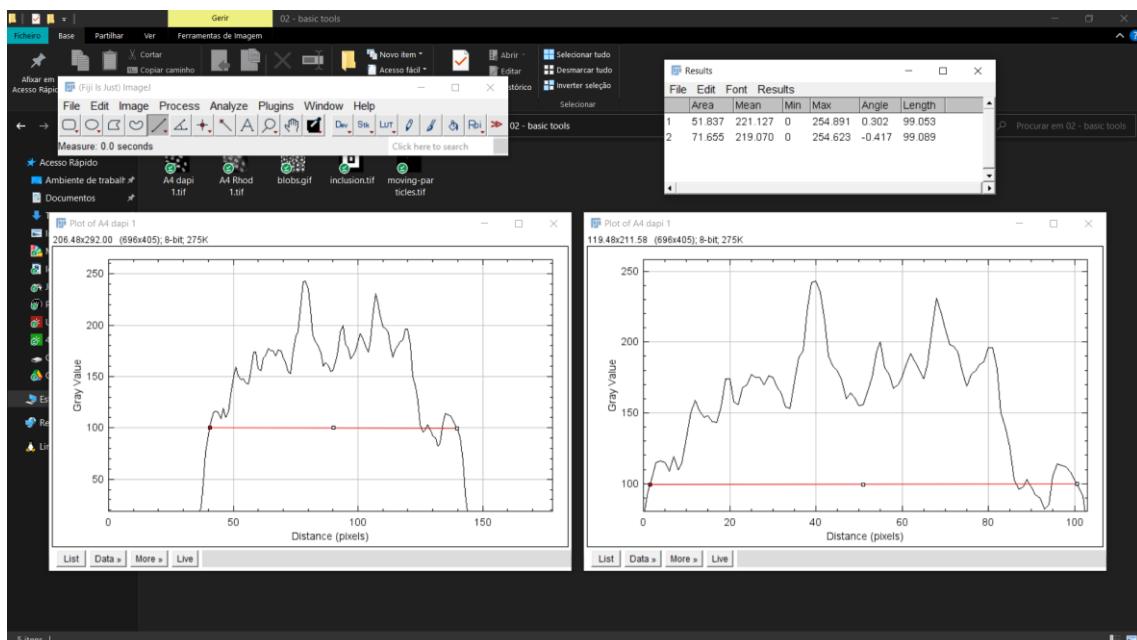
- b) Create a profile plot across a nucleus. Without changing the selection, change the line-width and create a new plot. What has changed?





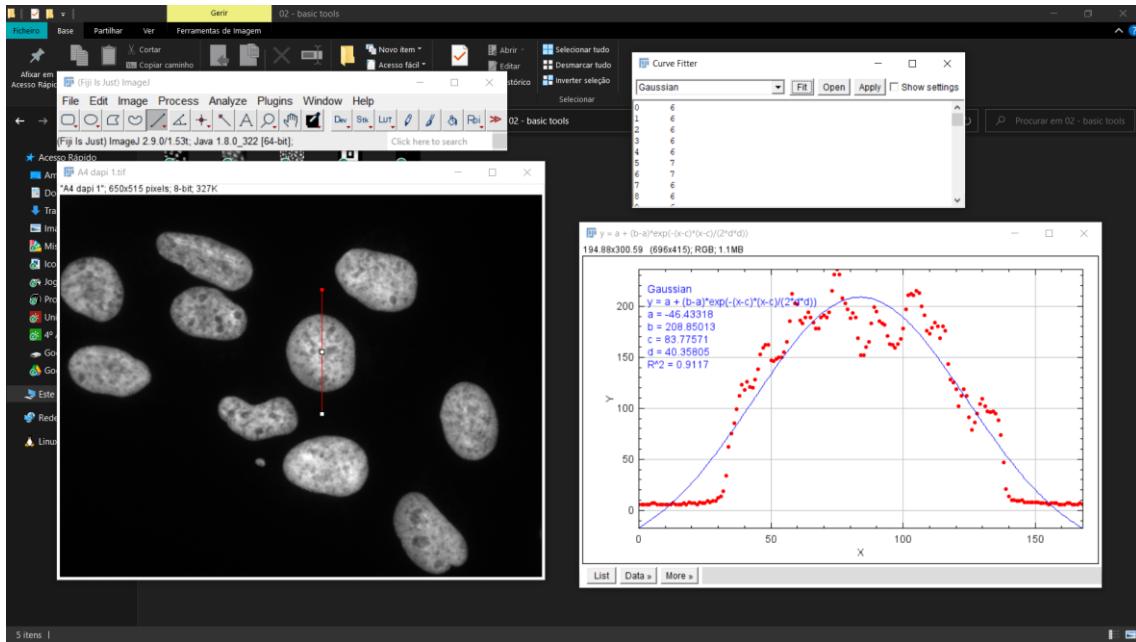
O aumento da espessura da linha faz com que o plot tome valores diferentes, provavelmente a média dos valores ao longo da linha.

- c) Create a profile plot across a nucleus, then make the line longer and create another profile plot (The easiest way to do this without changing the direction is using a horizontal or vertical line with shift pressed.). What is the difference between the plots? Measure the distances between the same two interesting points in the two plots. Do you get the same result in the two plots? How is this possible? Hint: Look at the pixel width and height in the properties of the two plot images (Image>Properties...).



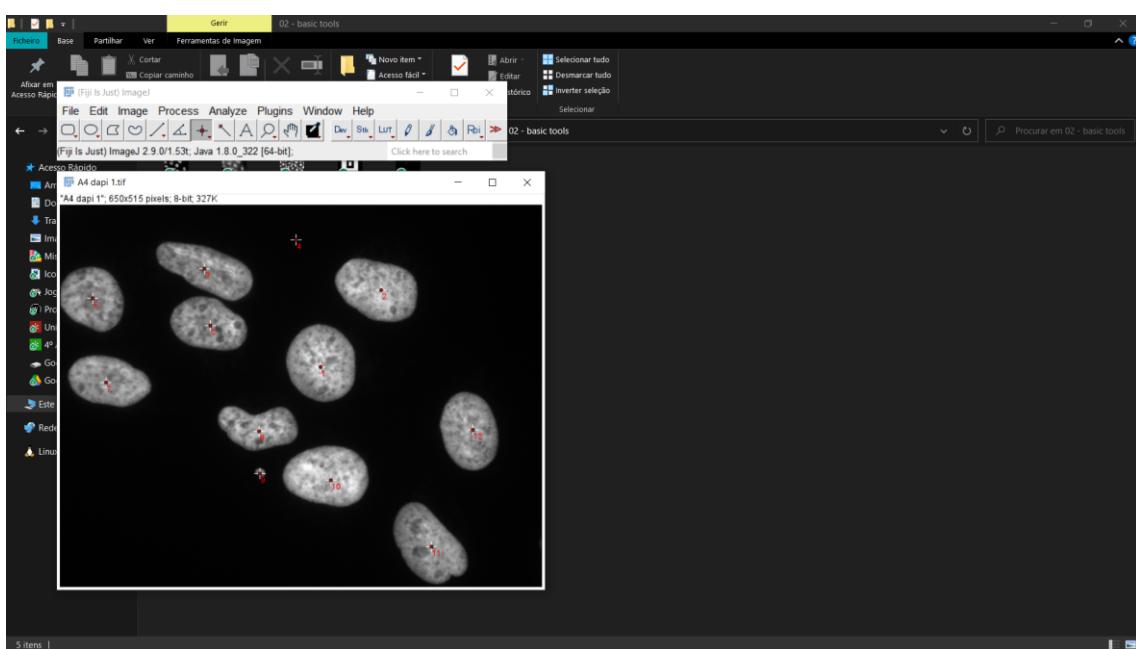
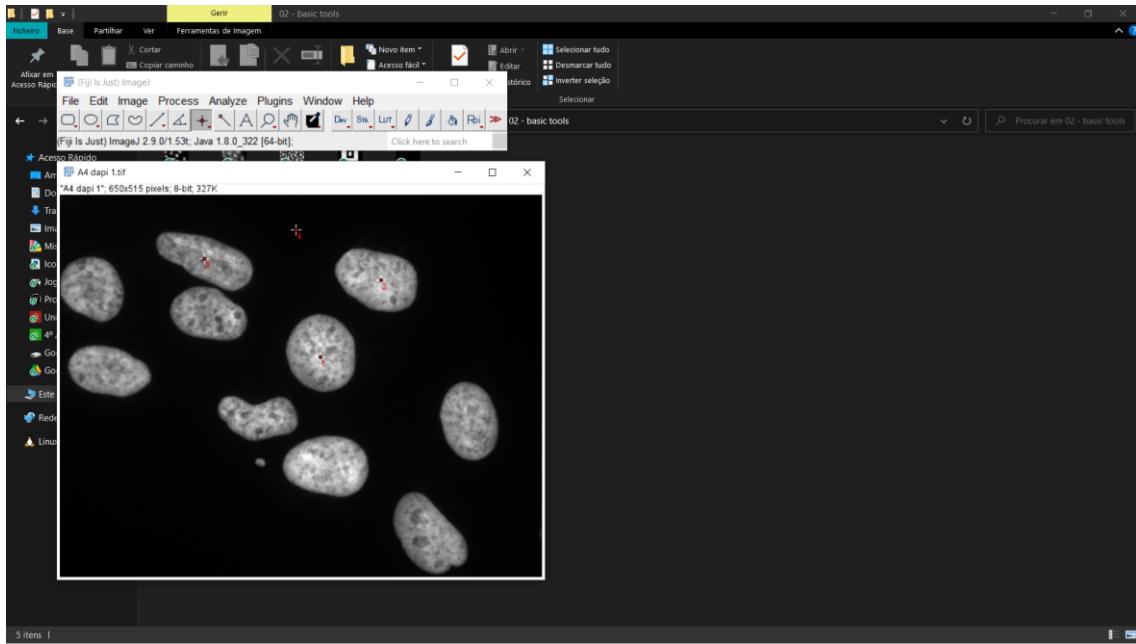
A única diferença é que o gráfico com a linha maior tem mais valores, mas o gráfico da linha menor está contido na íntegra no gráfico de linha maior.

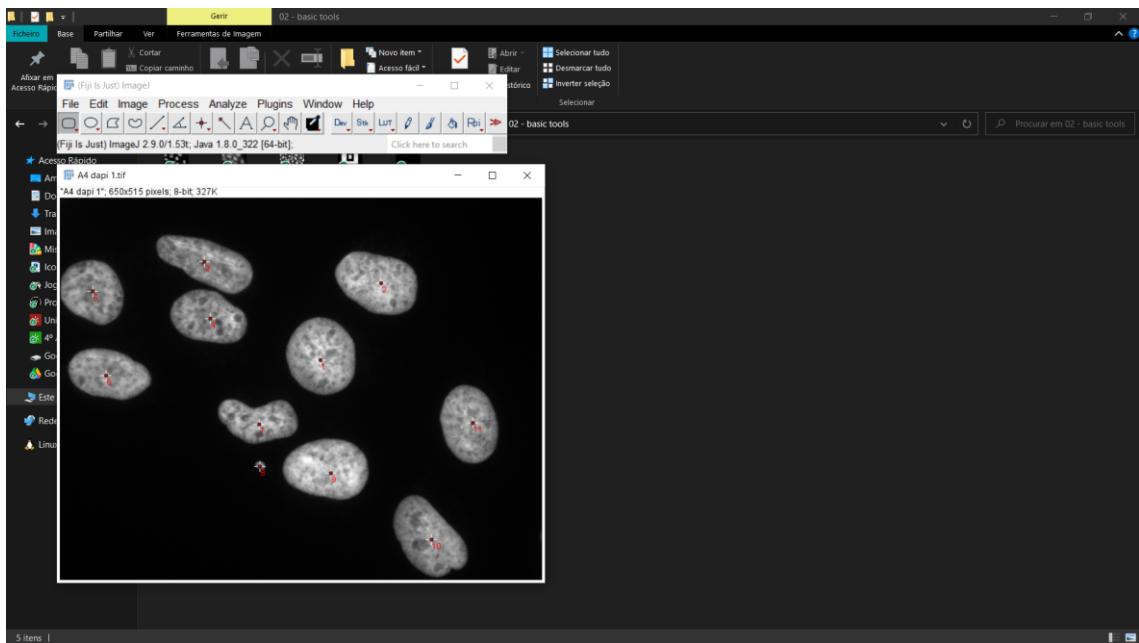
- d) Create a profile plot across a nucleus. Click the List button on the profile window. Copy the values into the clipboard (ctrl+a to select all and ctrl+c to copy). Open the Curve Fitter from Analyze>Tools>Curve Fitting... Delete the values in the curve fitter and paste the values from the clipboard into it (ctrl+v). Make a gaussian-fit of the measurement-points.



## T2.6 Point Selections

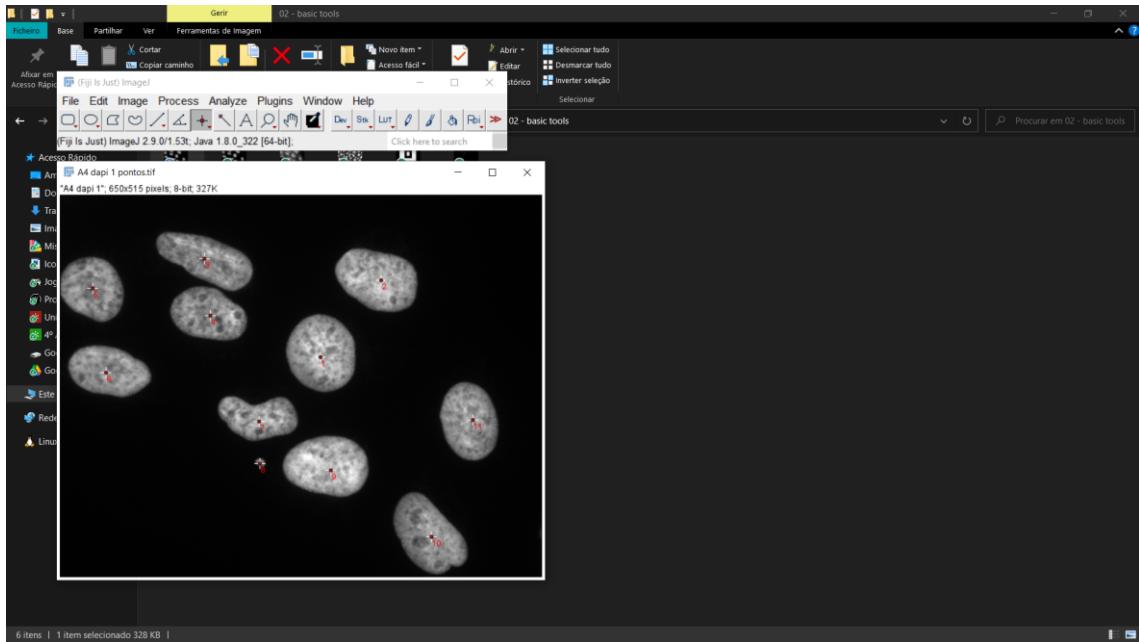
- a) Make a point selection on three nuclei, then make a point somewhere in the background and make point selections on the remaining nuclei. Delete the point on the background using alt-click. What happens to the numeration of the points?

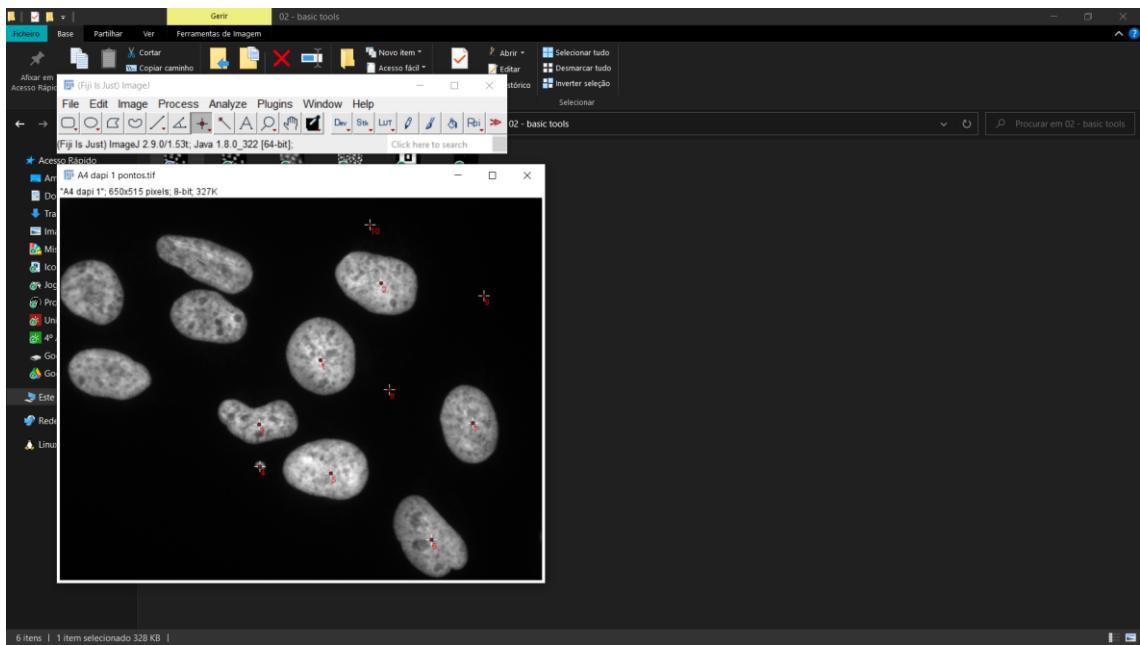




Após eliminar um dos pontos, a numeração deles ajustou-se, subtraindo um a todos os números que fossem maiores do que o número eliminado.

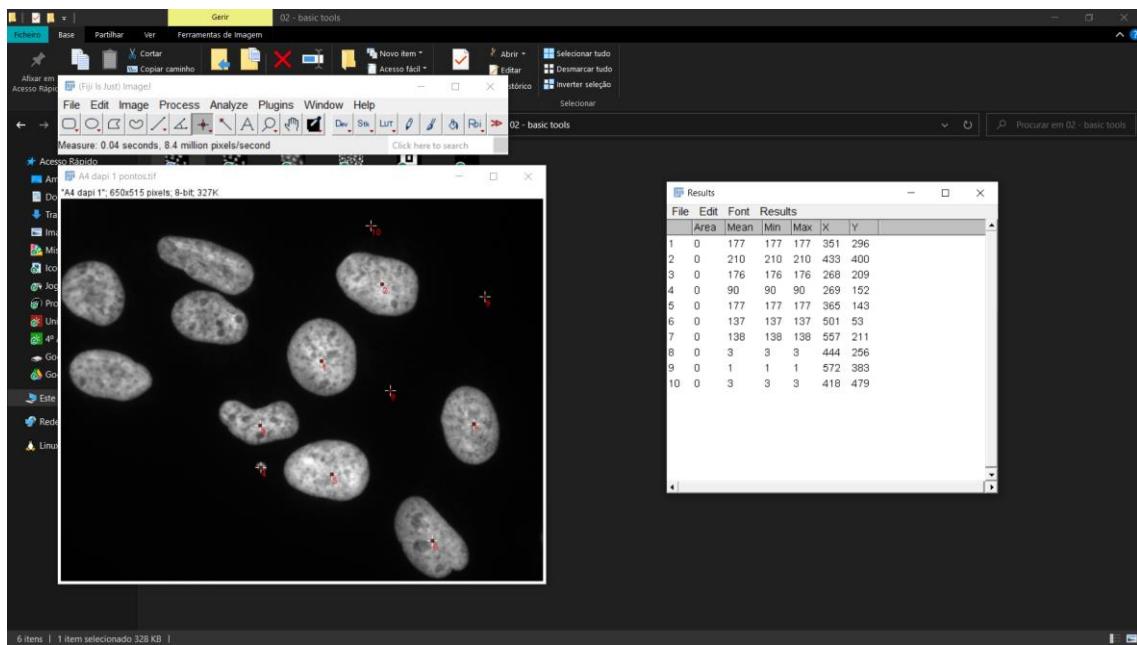
- b) Save an image with a point selection somewhere in the format tif (File>Save As>Tiff...). Close the image and reload the saved image (File>Open...). What happened to the selection? Can you still modify it?





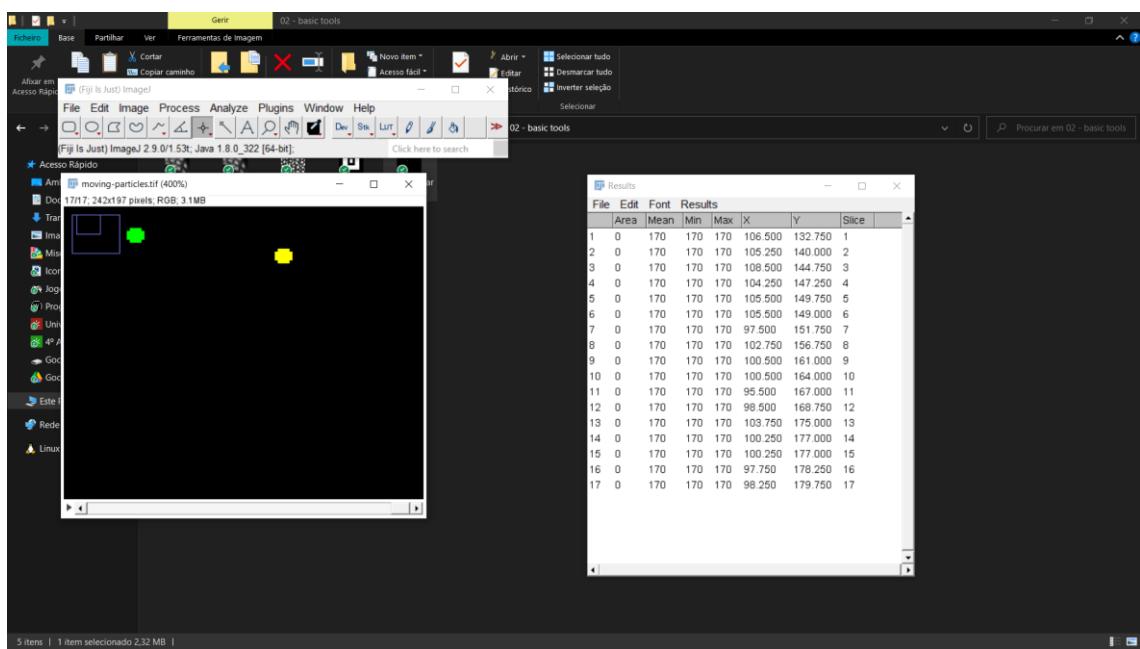
Depois de guardada a imagem, e reaberta, os pontos continuam no sítio onde estavam, e é possível eliminar os pontos existentes e adicionar outros.

- c) Check the option Auto-Measure of the point tool. What does it do? Hint: Look at the results table.



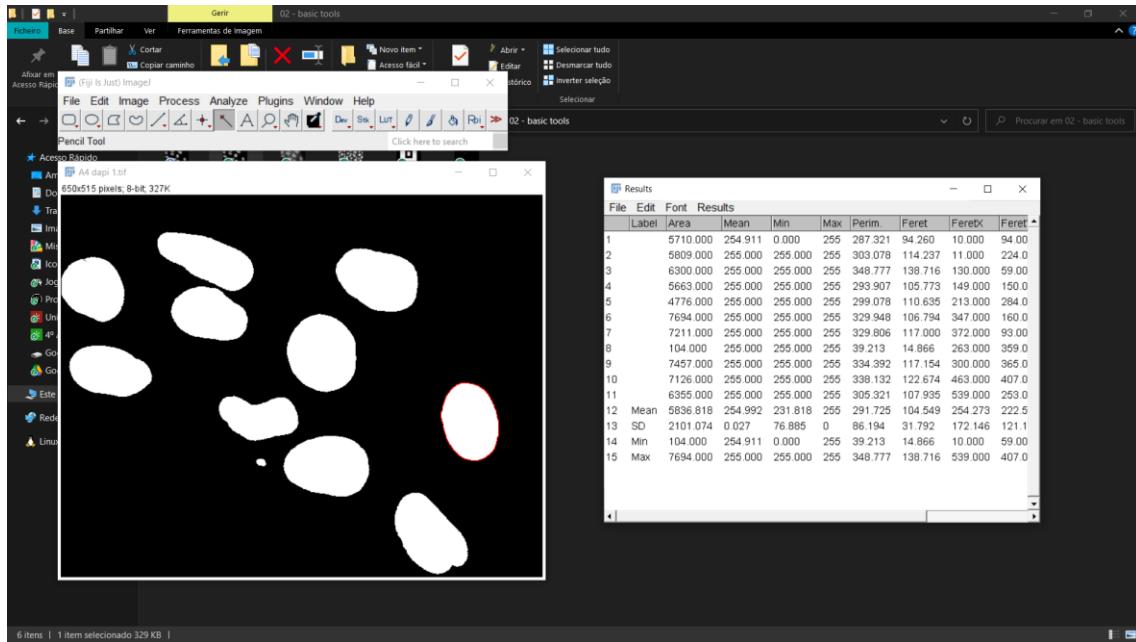
A opção de auto-measure faz com que sejam apresentadas as coordenadas x e y de todos os pontos, bem como o seu valor.

- d) Can you use the point-selection tool with the Auto-Measure and Auto-Next Slice options to trace the yellow particle in the time series image moving-particles.tif from folder 02 - basic tools?



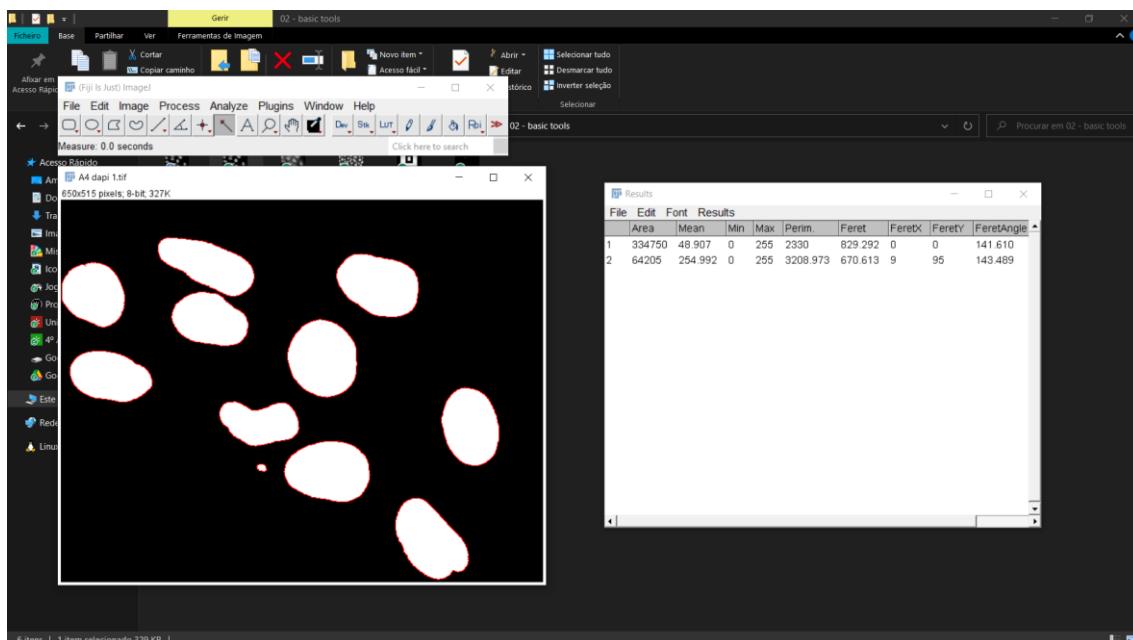
## T2.7 Thresholding and The Magic Wand Tool

- a) Use the wand-tool in combination with the threshold adjuster to select nuclei. Measure the nuclei. How long are the biggest and the smallest perimeters and how long are the biggest and smallest diameters?



O maior perímetro é 348.777 e o menor é 39.213, o maior diâmetro é 138.716 e o menor é 14.866.

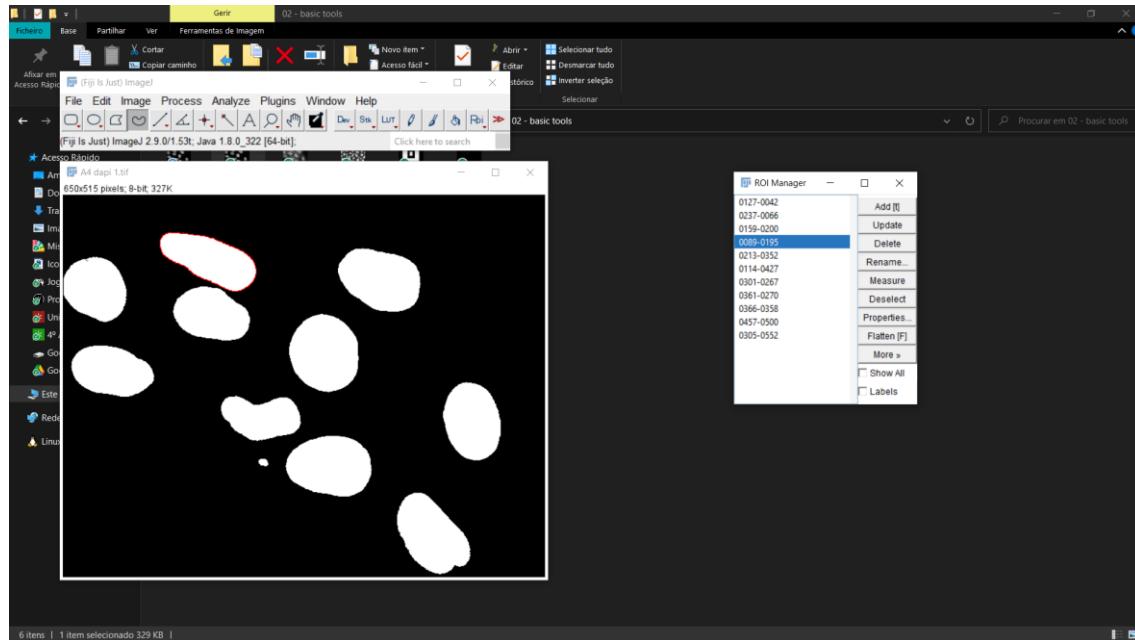
- b) Use the wand-tools to select all nuclei as one selection (shift-click). Find out what percentage of the image area is covered by nuclei. Hint: you can use ctrl+a to select the whole image.



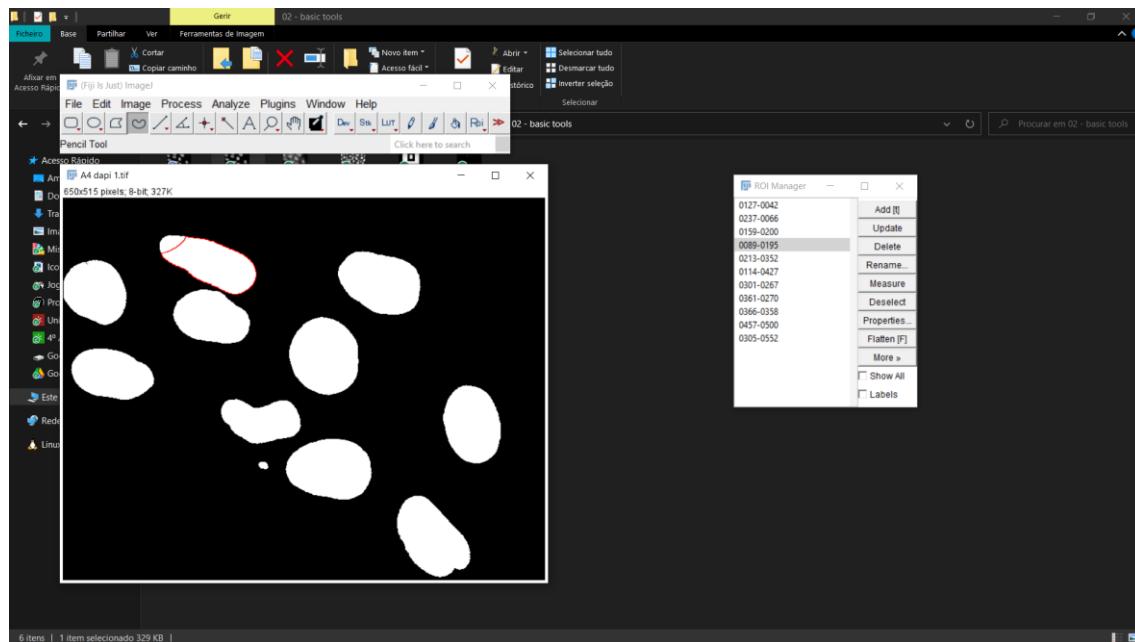
Aproximadamente 19% da imagem é coberta por núcleos.

## T2.8 The ROI-Manager

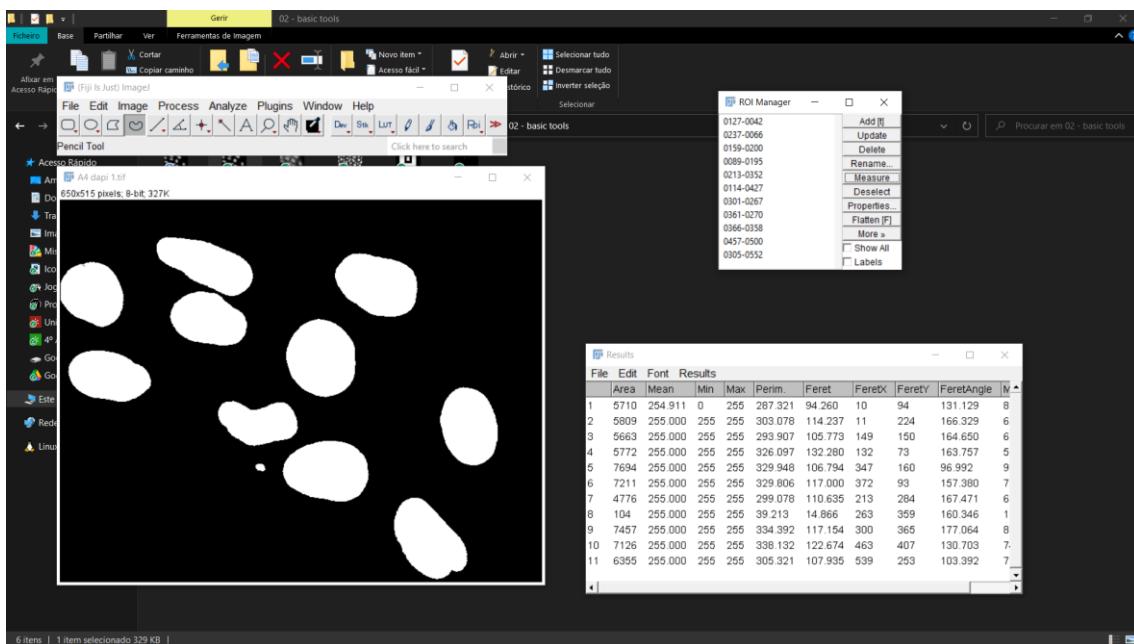
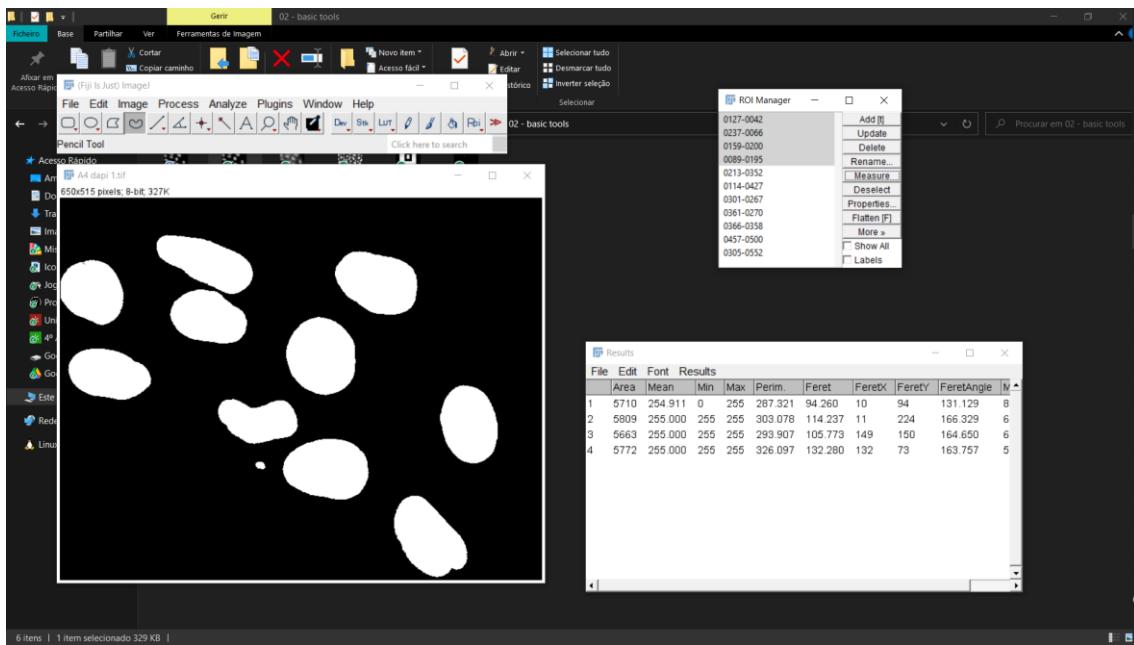
- a) Use the wand-tool to select a nucleus, then press t (shortcut for add to ROI-manager, you find the ROI-manager under Analyze>Tools>ROI Manager...). Select the Show All option on the ROI-manager. Select the other nuclei and them to the ROI-manager.



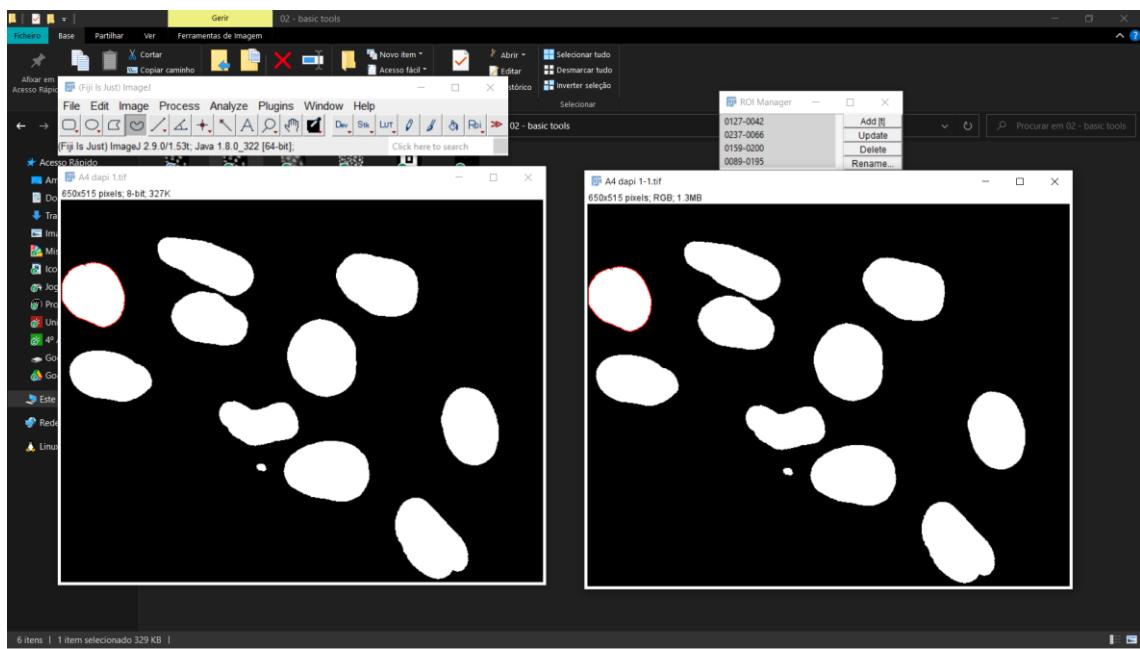
- b) Click on one of the labels (for example 5) in the image. Note that the corresponding line in the ROI-manager is selected. Modify the selection (remove a part using the freehand-selection tool with alt pressed). Press the update button in the ROI-manager to make the change permanent.



- c) Select one or multiple lines in the ROI-manager (you can use ctrl/shift) and press the measure-button. Press the deselect-button, close or clear the results-table and press the measure button again.

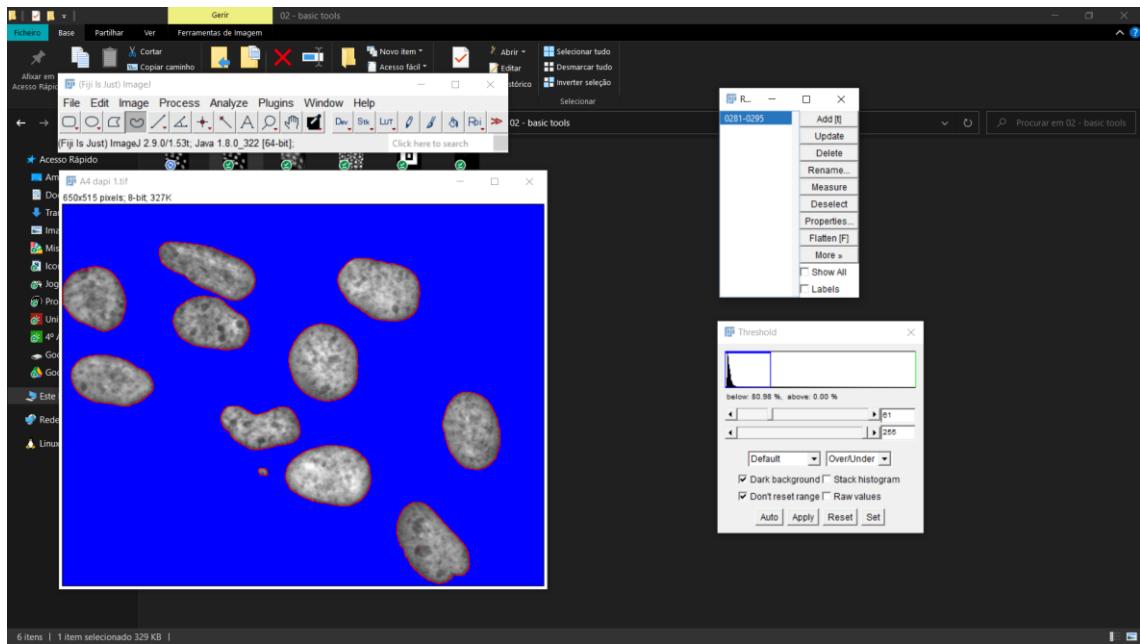


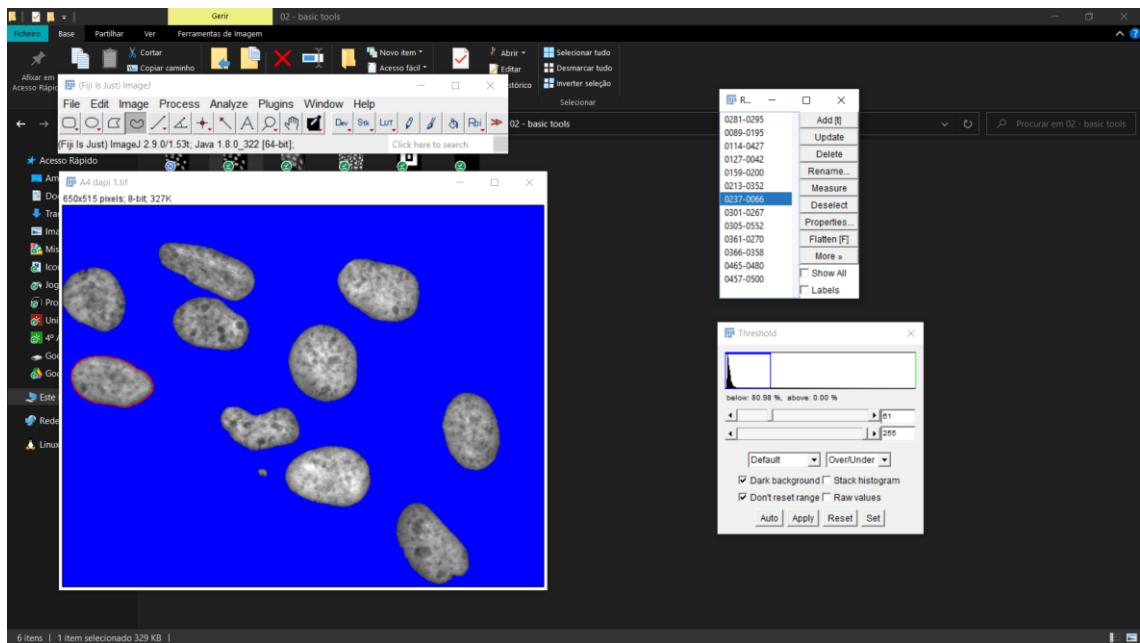
- d) What does the flatten button do? Hint: Have a look at the image type of the resulting image.



O flatten criou uma cópia da imagem, mas em vez de ter do tipo 8-bit, é do tipo RGB.

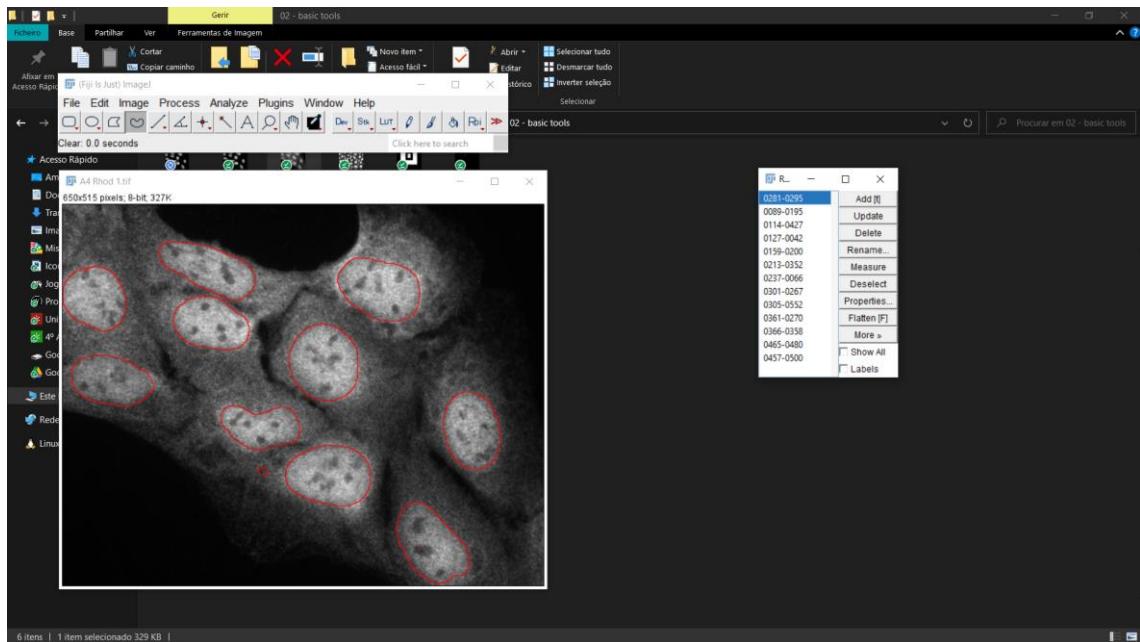
- e) Set the lower threshold so that the background becomes blue and there are no holes in the nuclei. You can create a selection from the thresholded values by using Edit>Selection>Create Selection. Add the ROI to the ROI-manager and run the split command. What happens?





O comando split divide o ROI em vários ROI cases estes não estejam conectados.

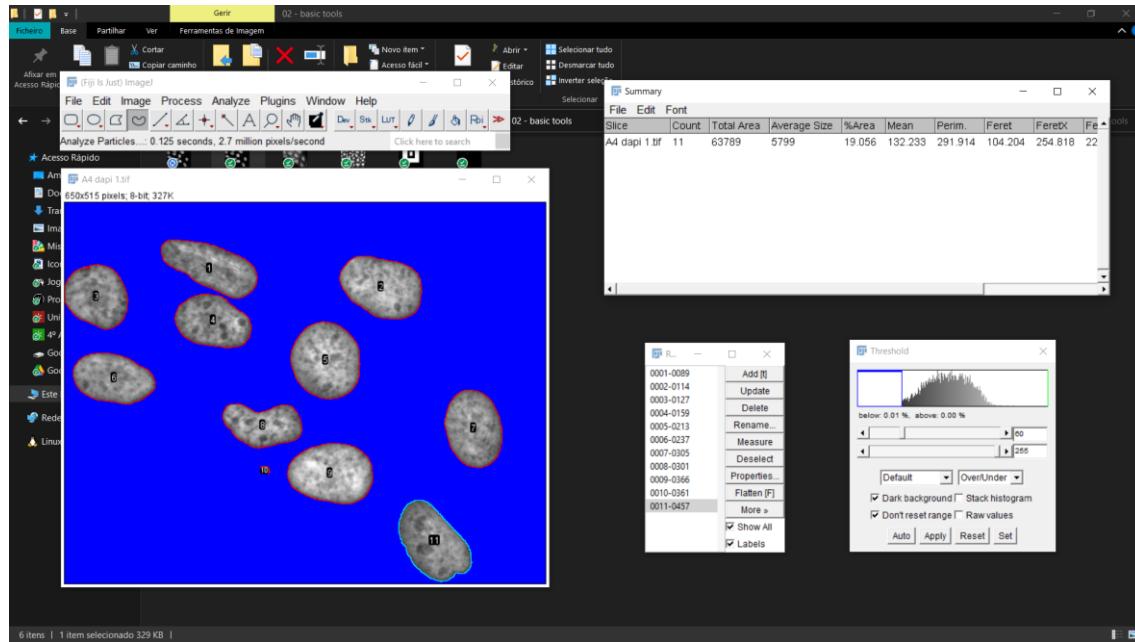
- f) Add the selections of all nuclei to the ROI-manager. Now open the image A4 Rhod 1.tif from folder 02 - basic tools. With the newly loaded image active, deselect Show All from the ROI-manager and select it again. Press the measure button. What happens?



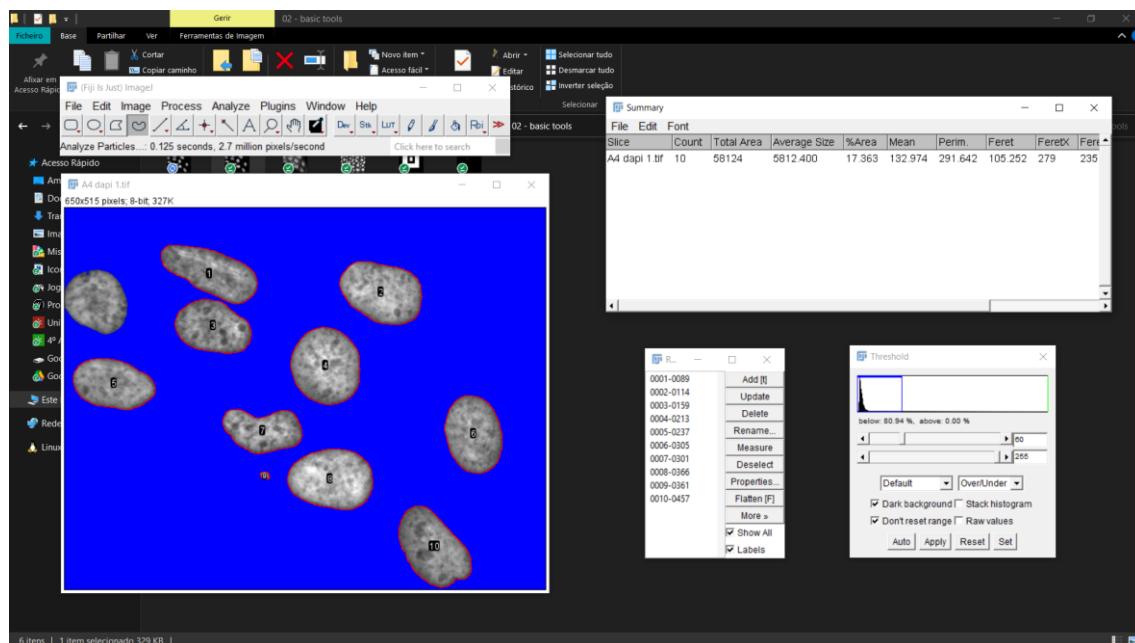
Ao abrir outra imagem os ROIs são aplicados à mesma, preservando tanto a forma como a posição dos ROIs, realçando assim os núcleos das células.

## T2.9 Measuring Objects With The Particle Analyzer

- a) Use the threshold-adjuster (Image>Adjust>Threshold or shift+t) and the particle-analyzer to add the nuclei to the ROI-manager (deselect everything on the particle-analyzer except Add to Manager).

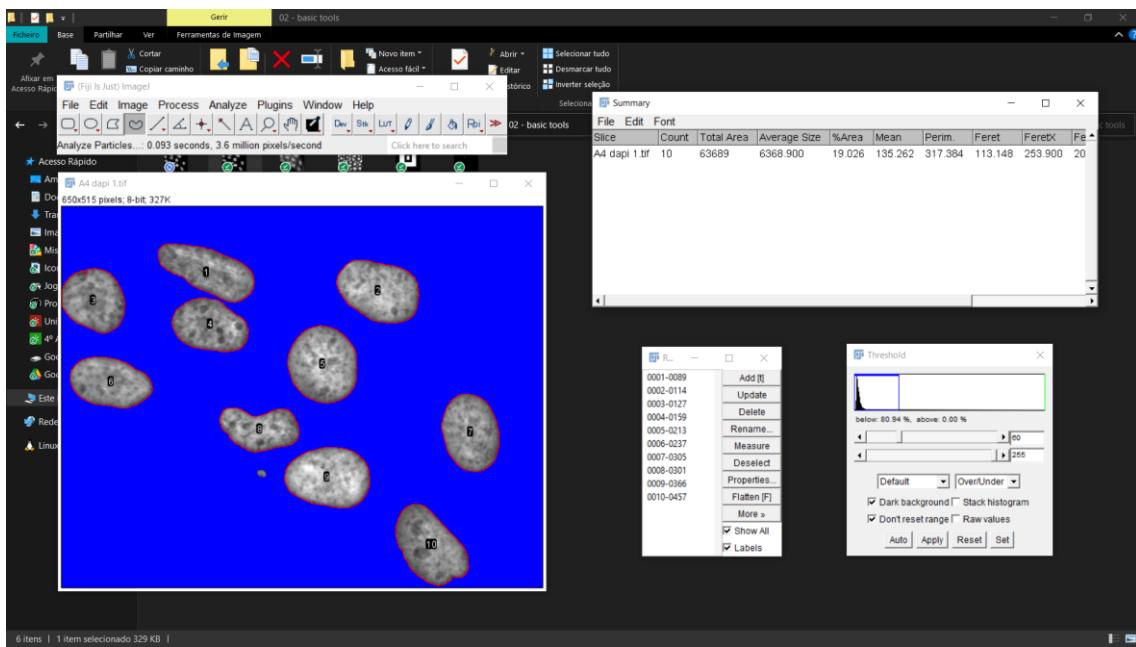


- b) Delete all from the ROI-manager and use the particle-analyzer again. This time select the Exclude on edges option. Can you explain what this option is good for and when it should be used?

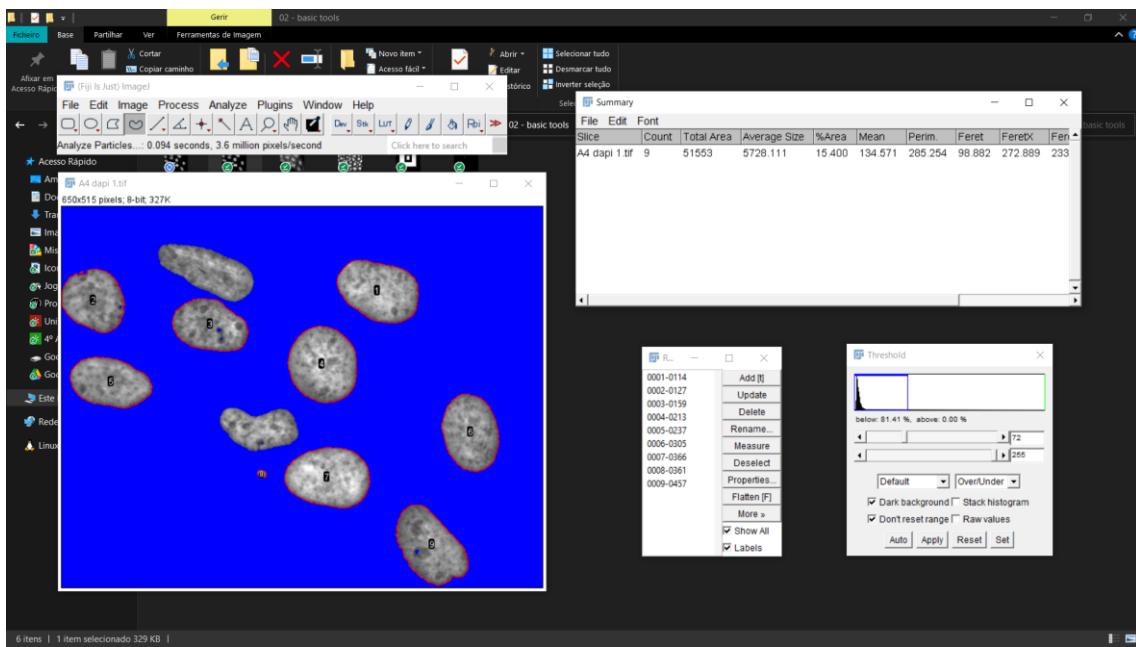


Esta opção exclui os ROIs que estivessem em contacto com o limite da imagem. Isto seria interessante no caso de, por exemplo, a imagem cortasse um núcleo, pois a inclusão deste iria afetar negativamente os resultados, visto que não temos toda a informação dele.

- c) Use the particle-analyzer again. This time try to exclude objects that are too small to be nuclei by setting a minimum size in the dialogue of the particle-analyzer.



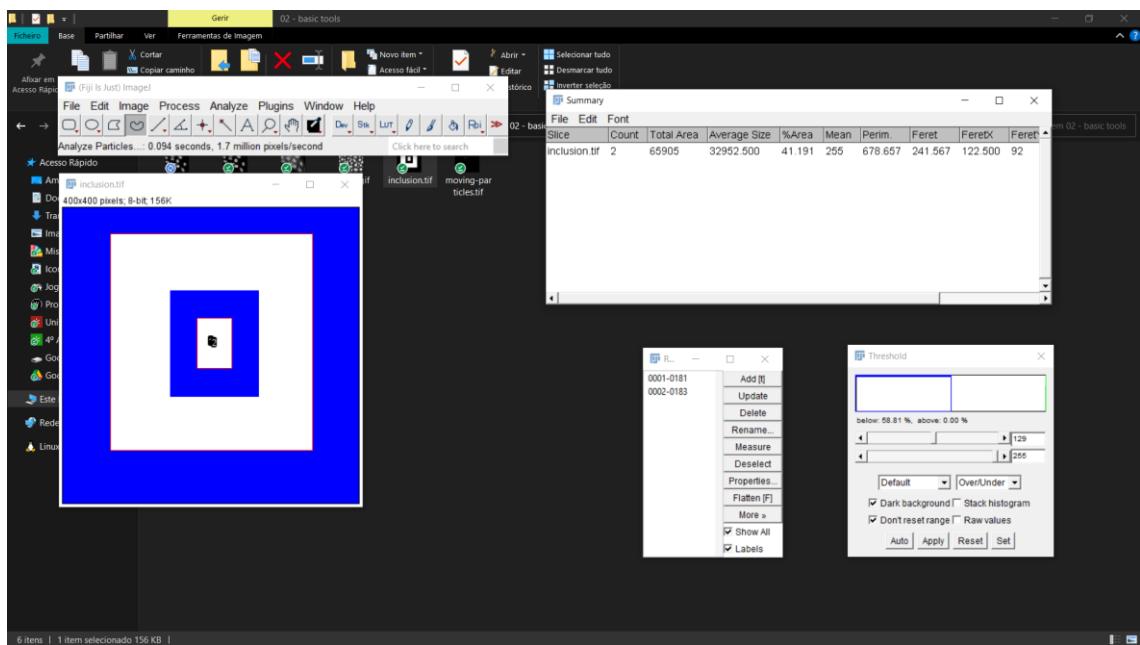
- d) Use the particle-analyzer to select only nuclei that have a high circularity value. What is the value that excludes the two nuclei with the lowest circularity?



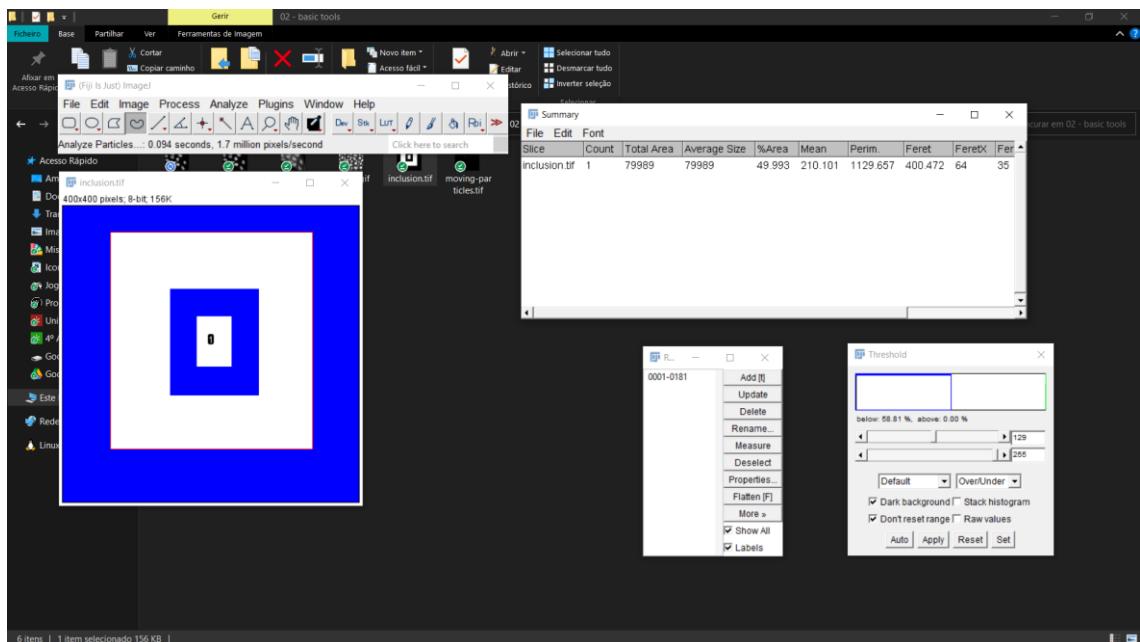
0.7 é o valor que exclui os dois núcleos com menor circularidade

- e) Open the image inclusion.tif. Imagine that the inner and outer white rectangles are particles. Use the particle-analyzer with and without the Include holes option. For this comparison deselect Add to Manager and select Display results. What is the difference?

Sem include holes:

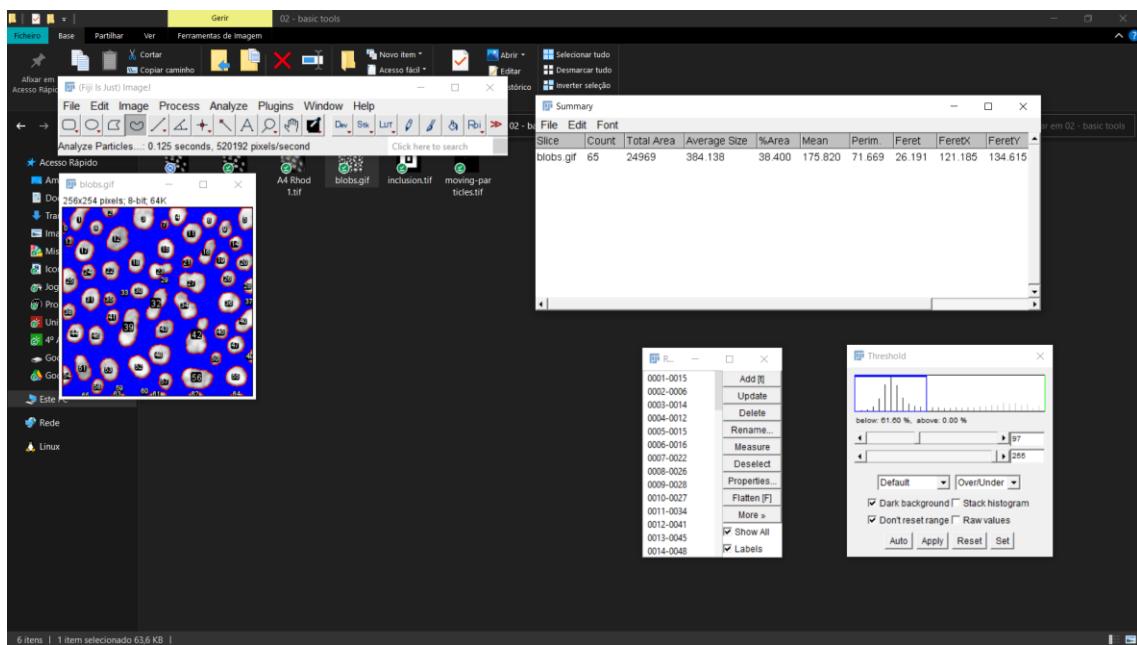


Com include holes:



A opção faz com que os buracos existentes dentro das ROIs sejam incorporados nelas, podendo, ou não, resultar em menos ROIs, caso exista uma ROI que esteja completamente cercado por outra.

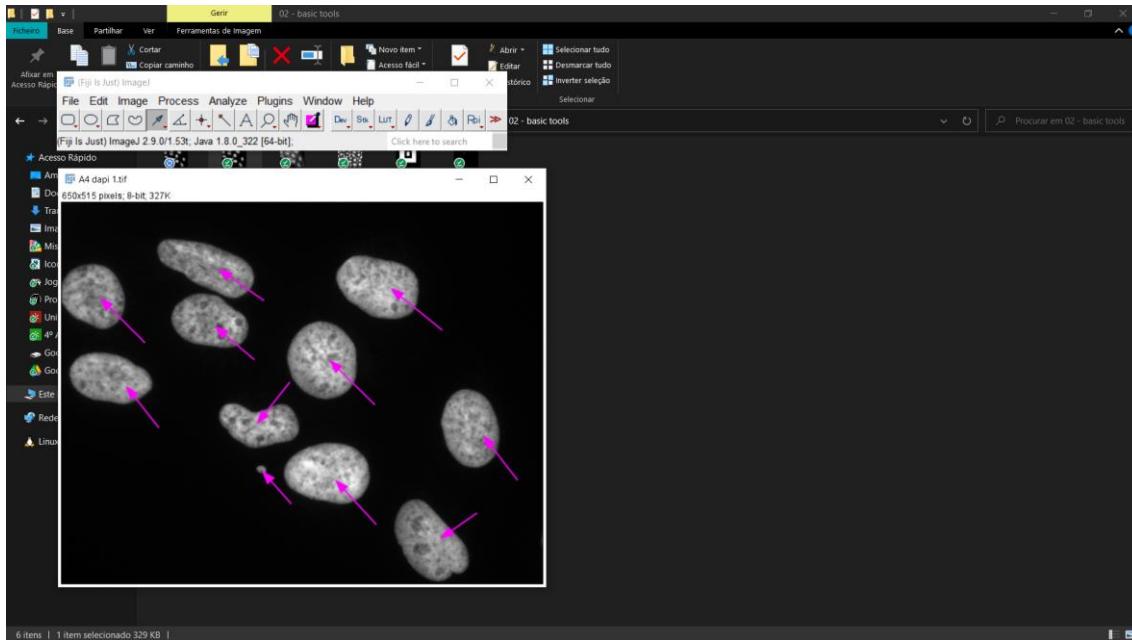
- f) Use the particle-analyzer to find out how many bright blobs there are in the image blobs.gif from folder 02 - basic tools. What is the average area of the blobs?



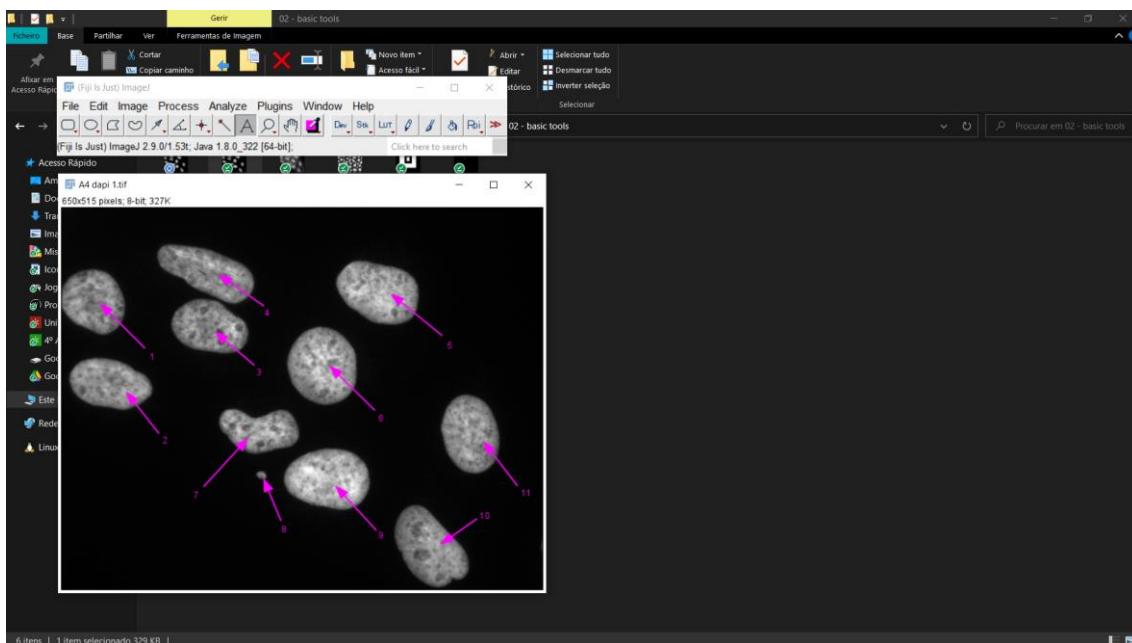
No ficheiro “blobs.gif” existem 65 blobs, com uma área média de 384.138.

## T2.10 Arrows, Annotations and Overlays

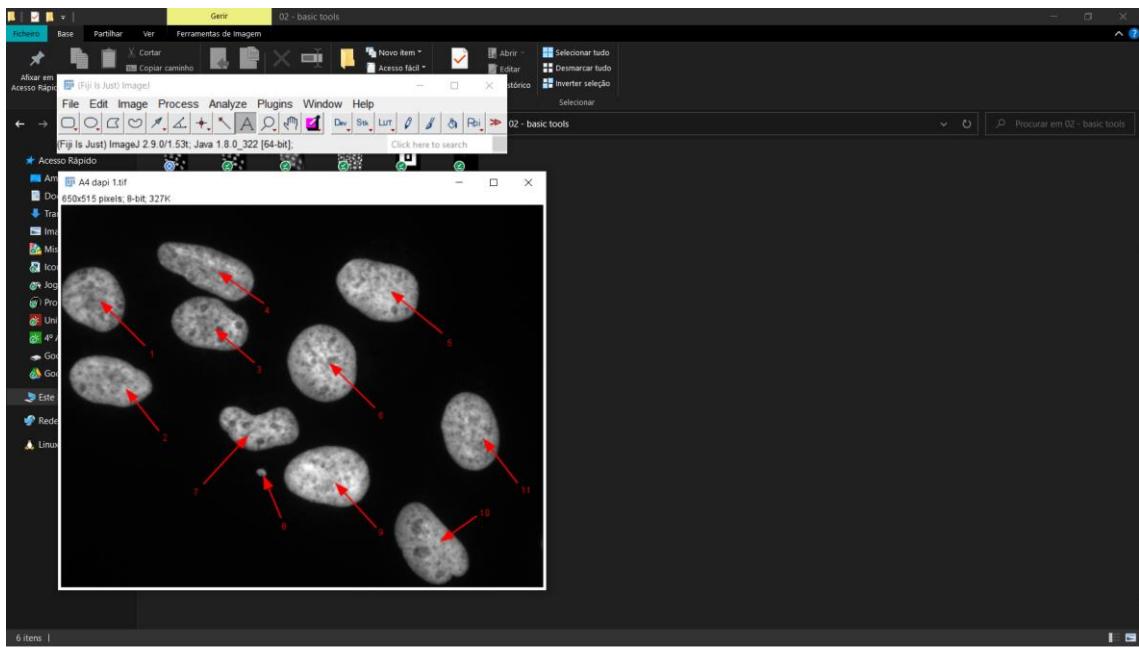
- a) Use the arrow tool (right-click on the line-selection tool-button) to create arrows that point to a nucleus each. Before creating the arrows, use the options of the arrow-tool to set the colour of the arrows to magenta.



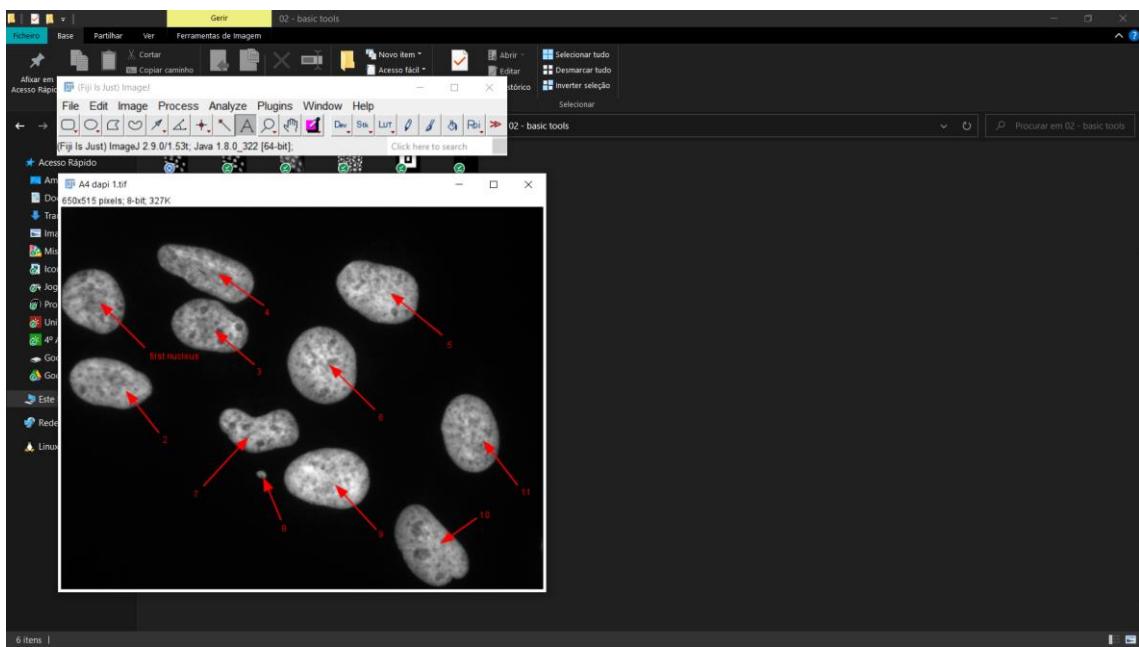
- b) Use the text tool to write an annotation for each arrow (nucleus one and nucleus two). Use **ctrl+b** to add the text to the overlay as it is or **ctrl+alt+b** to it to the overlay with different options.



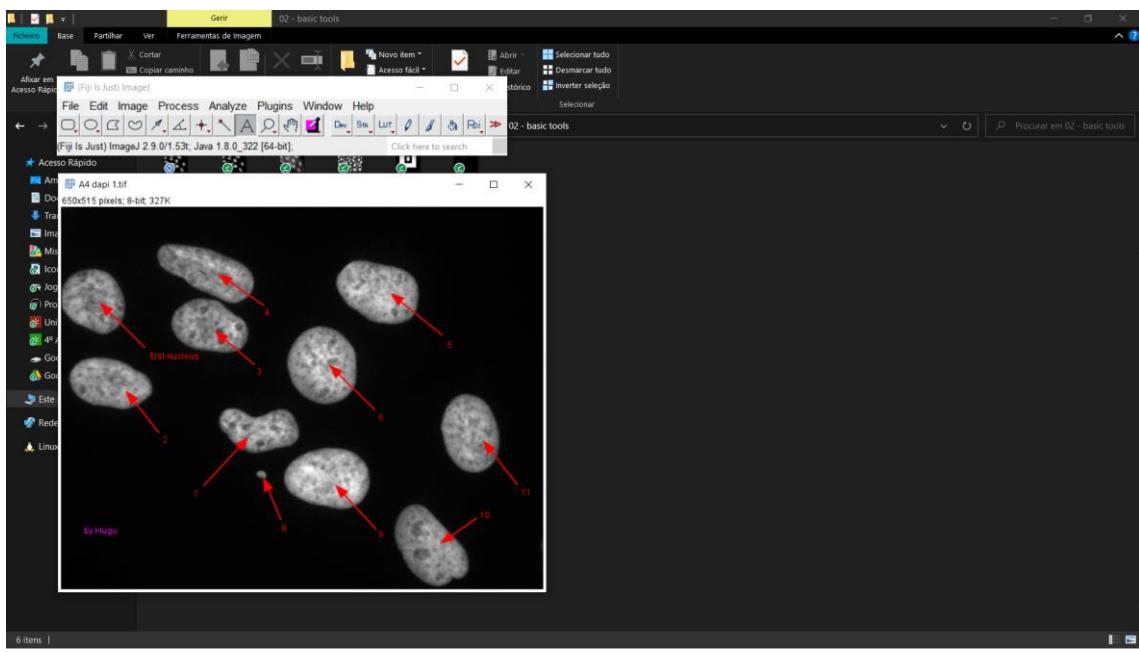
- c) Modify the arrows. Hint: Call Use Image>Overlay>To ROI Manager first. Don't forget to click the Update-button on the ROI-manager after modifying an arrow.



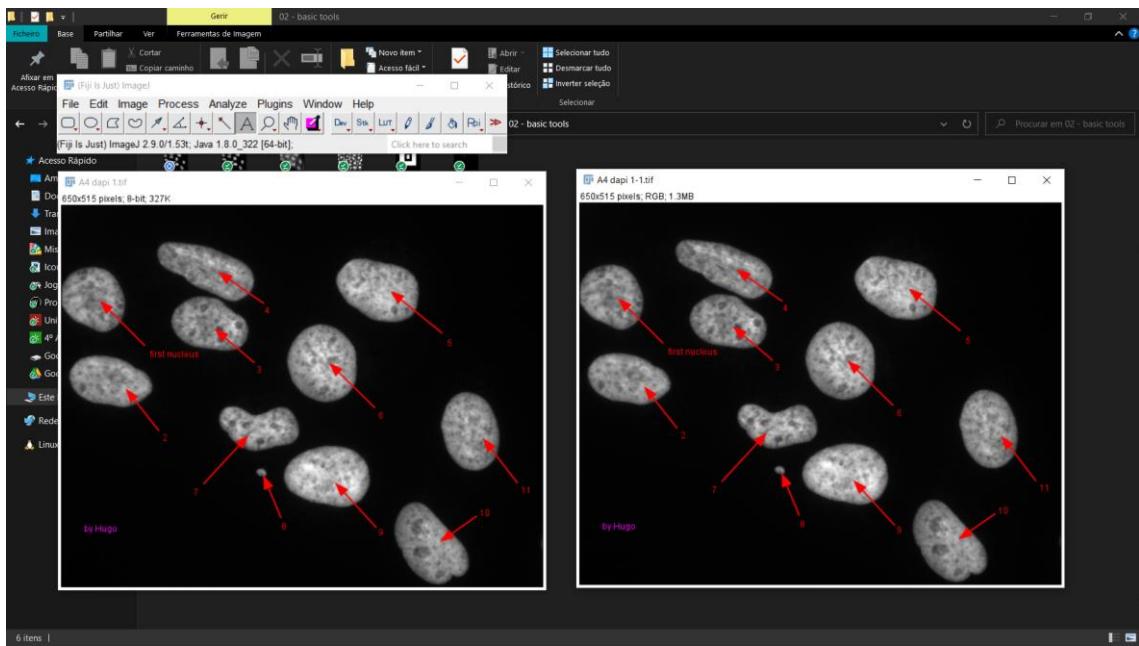
- d) Replace the text nucleus one by another text (first nucleus). Hint: You cannot modify the text itself. Select the text in the ROI-manager, write a new text with the text-tool and press the update button.



- e) Add a third annotation. Hint: Convert the selections back into an overlay, add the new annotation to the overlay.



- f) Use **Image>Overlay>Flatten** (ctrl+shift+f) to create an image containing the annotations that you can copy into your presentation.

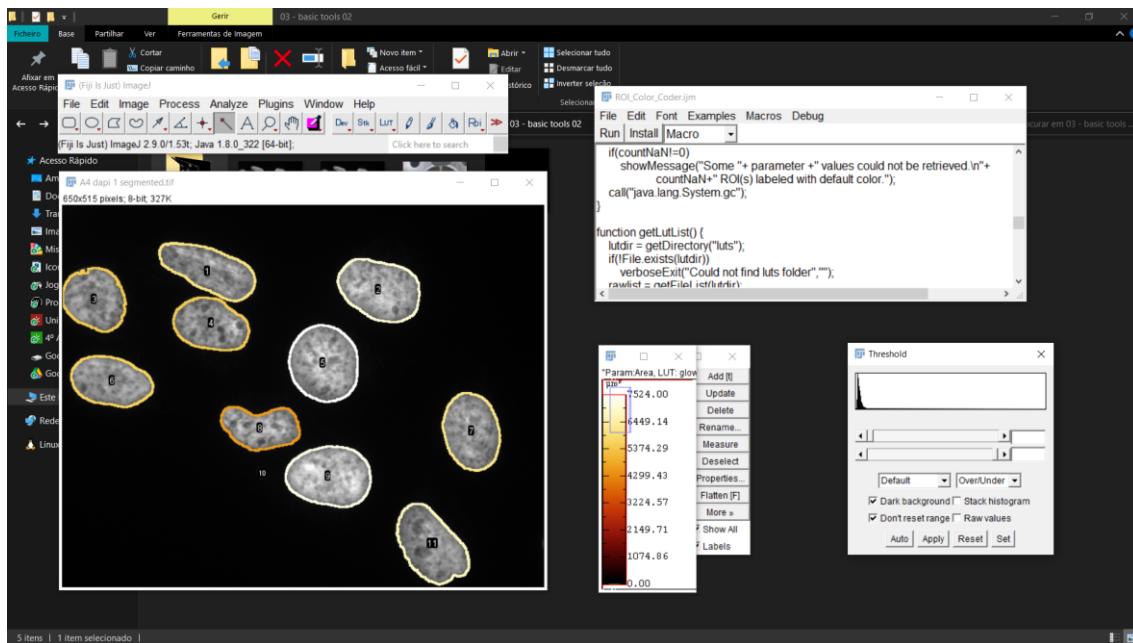


# T3

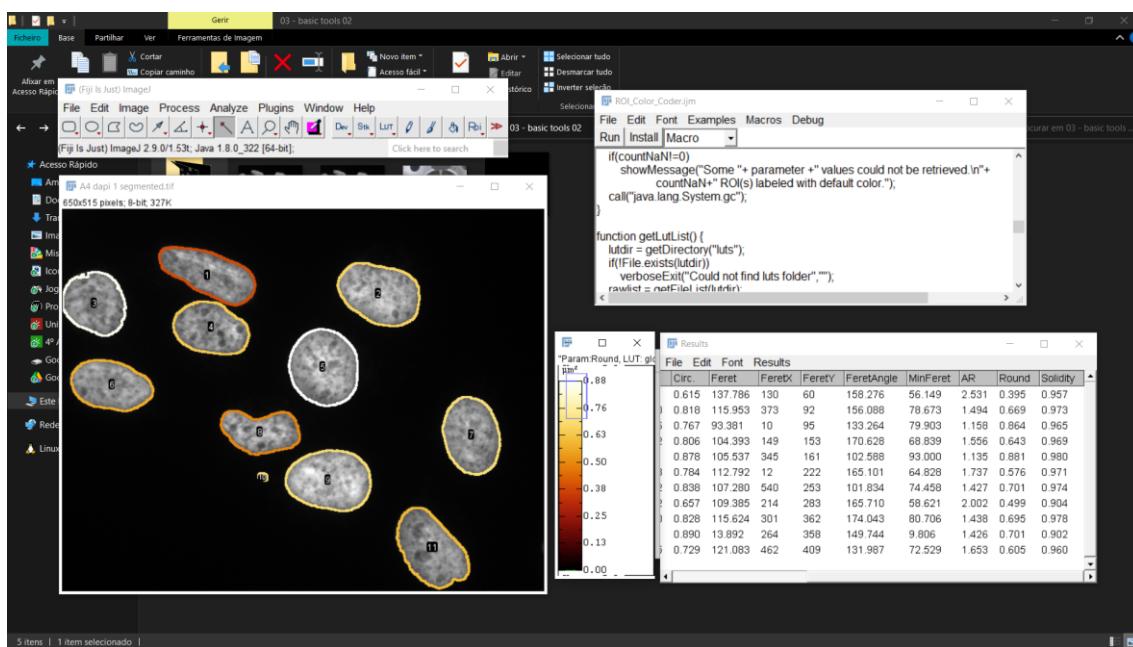
## T3.1 Using Macros

- a) Open the image A4 dapi 1.tif from the folder 01 opening images. Use the Help menu of ImageJ to open the macros web-page. Look for the macro and drag it onto the ImageJ-launcher window. The macro needs the selections in the ROI-manager and the corresponding measurements in the results-table. Color-code the nuclei by area. Try again and colour-code them by roundness. Hint: Use the menu Macros>Run from the macro-editor window to run the macro.

Area:

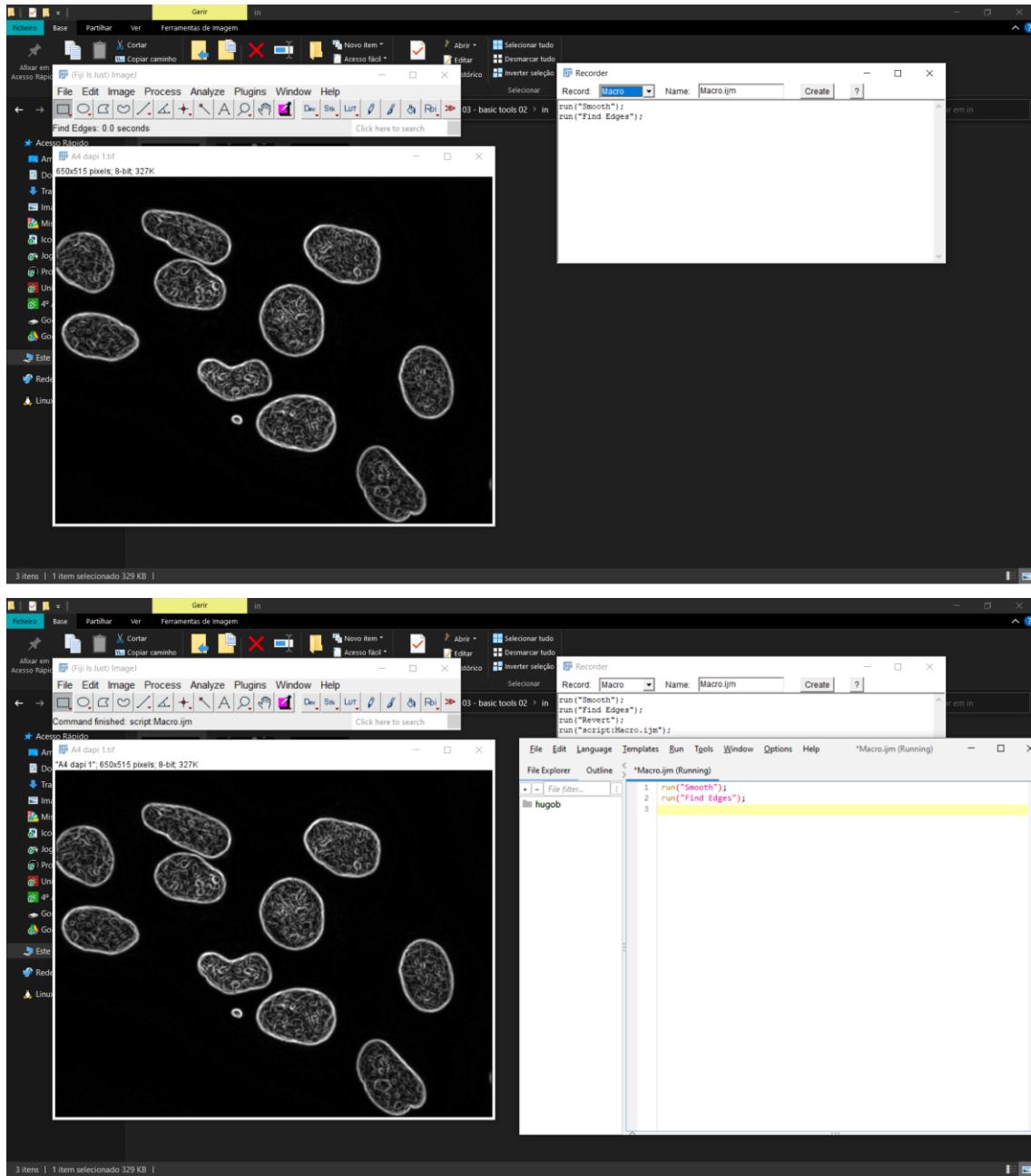


Roundness



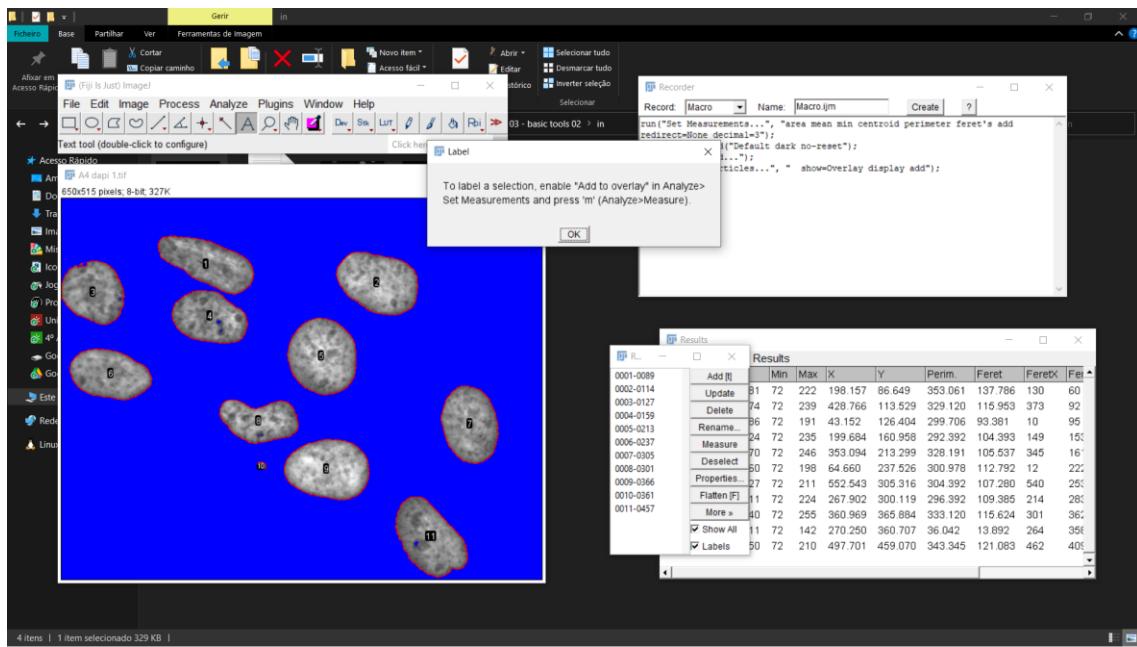
## T3.2 Recording Macros and Batch Processing

- a) Run some commands (smooth, find edges, ...) and record them with the macro-recorder. Press the create button to create the macro. Revert the image (File>Revert or ctrl+r) and run the macro from the macro-editor.

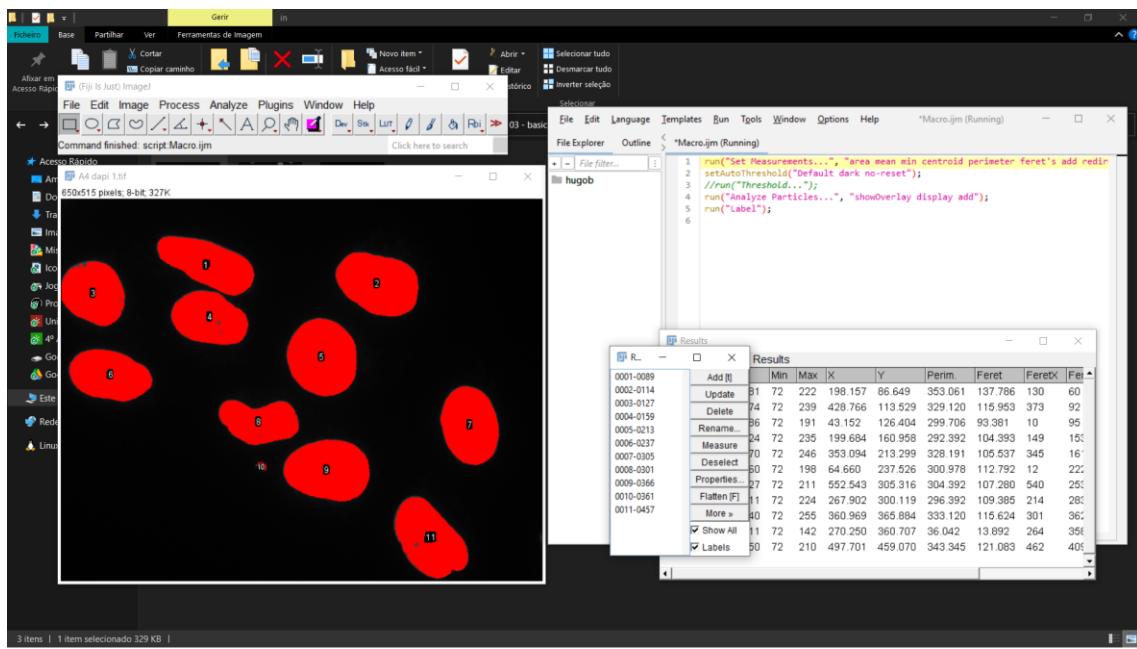


- b) Record a macro to measure the features (area, mean intensity, etc) of the nuclei in the image A4 dapi 1.tif from the folder 03 - basic tools 02/in. It should contain the following steps:

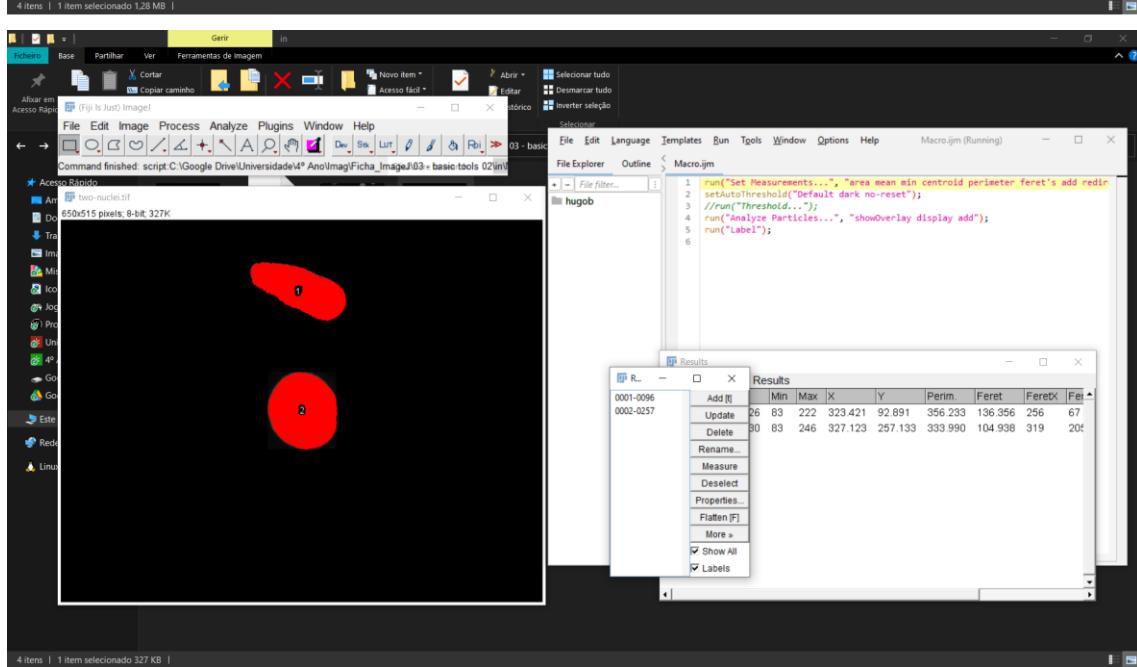
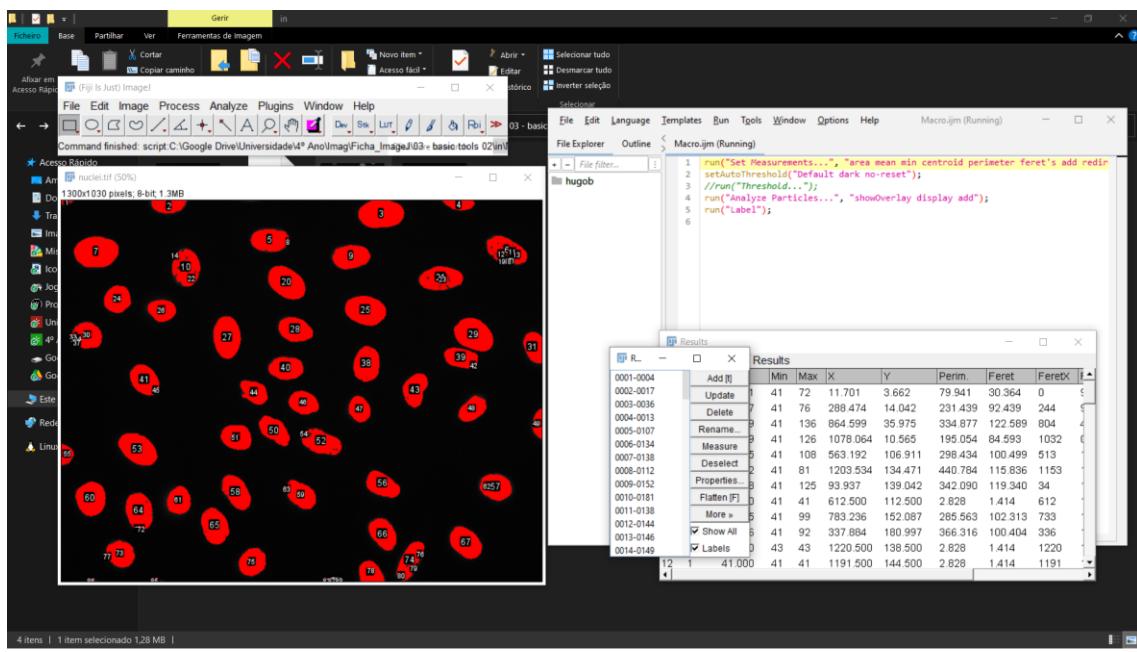
- set the measurements you want to have in the result
- set an auto-threshold on the image
- run the particle-analyzer (with the display result option)
- run the command Analyze>Label to show which objects have been found



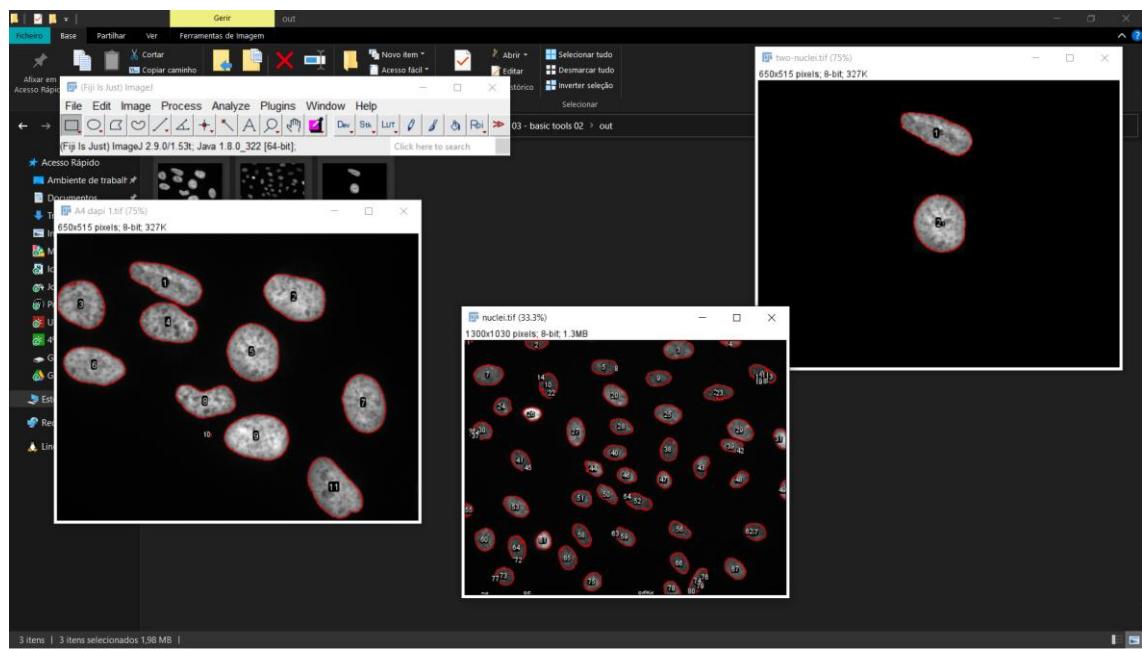
Está a dar erro quando corro o comando label (a solução proposta pelo programa não funciona). Mas correndo a macro na imagem original tem-se este resultado:



- c) Apply the macro to all the images in folder 03 - basic tools 02/in, one after the other.

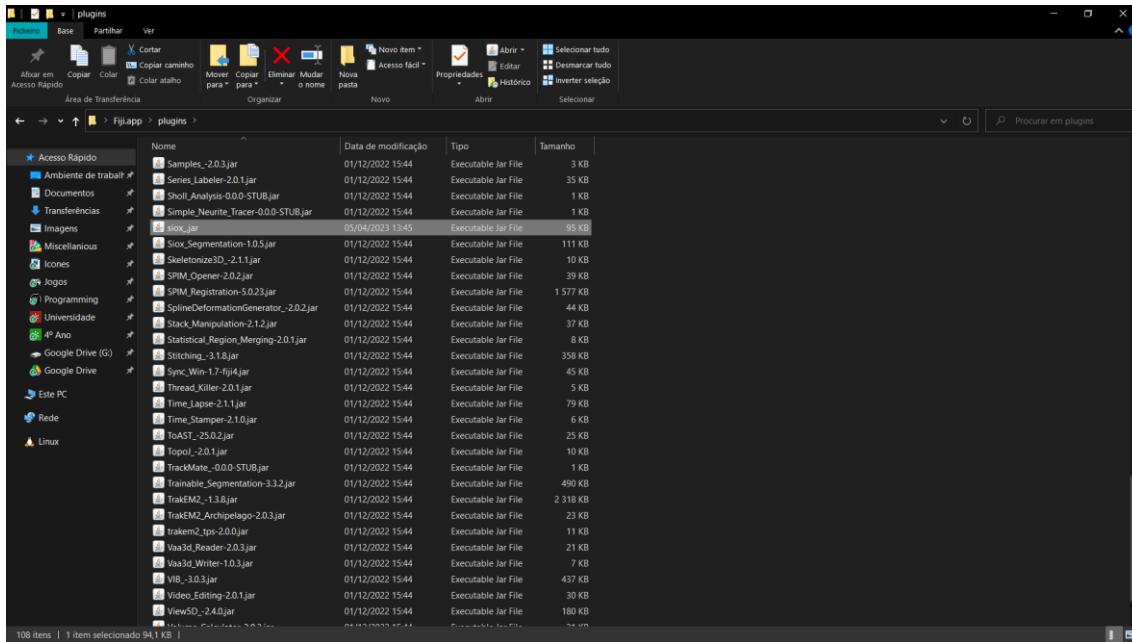


- d) Run the macro on the whole folder using Process>Batch>Macro... Copy and paste the macro into the window, select the input and output folder and press Process. What do you get in the output folder? What do you get in the results-table?

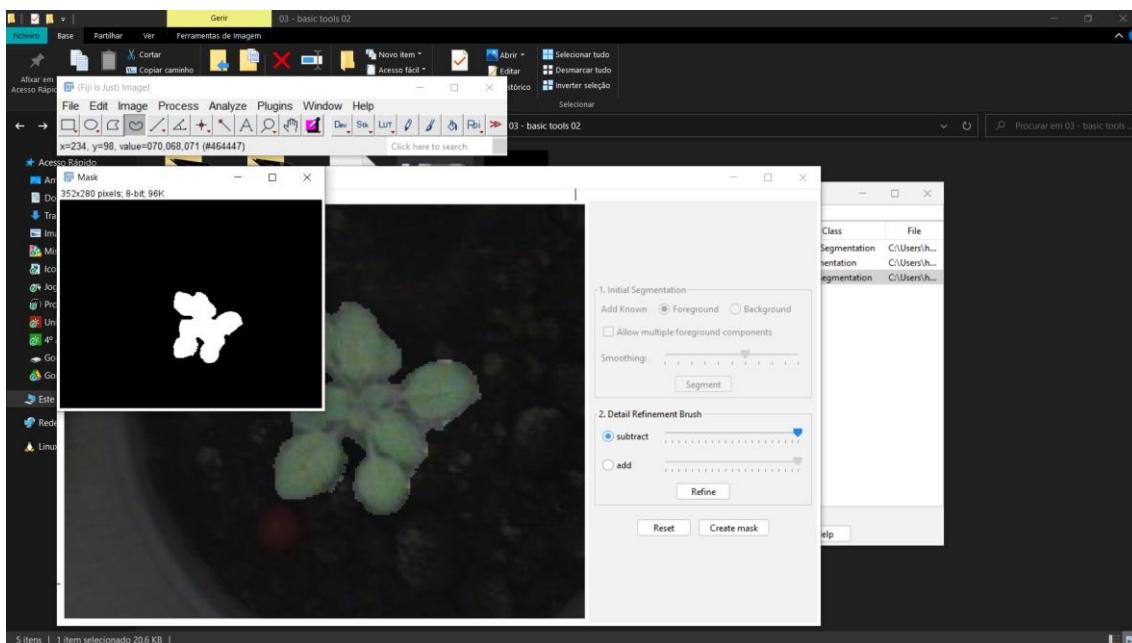


### T3.3 Using Plugins

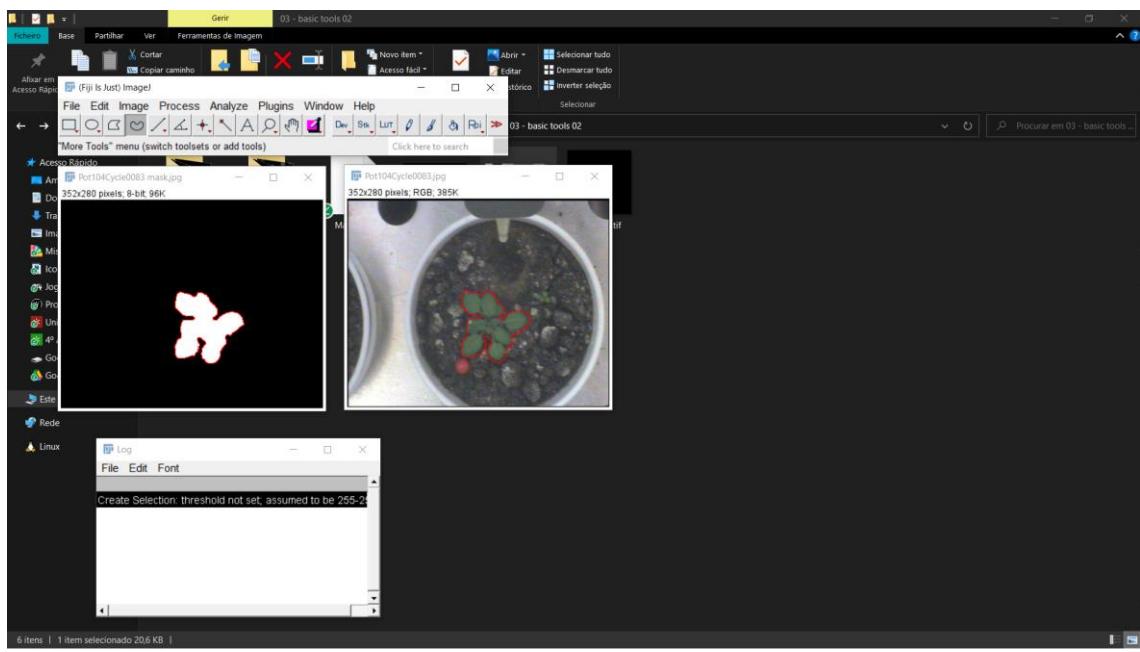
- a) Install the SIOX plugin from the ImageJ website. Open the plugins page from Help>Plugins. Search for SIOX and click on the link. Drag the link siox\_.jar onto the ImageJ launcher. Save the plugin into the ImageJ plugins folder.



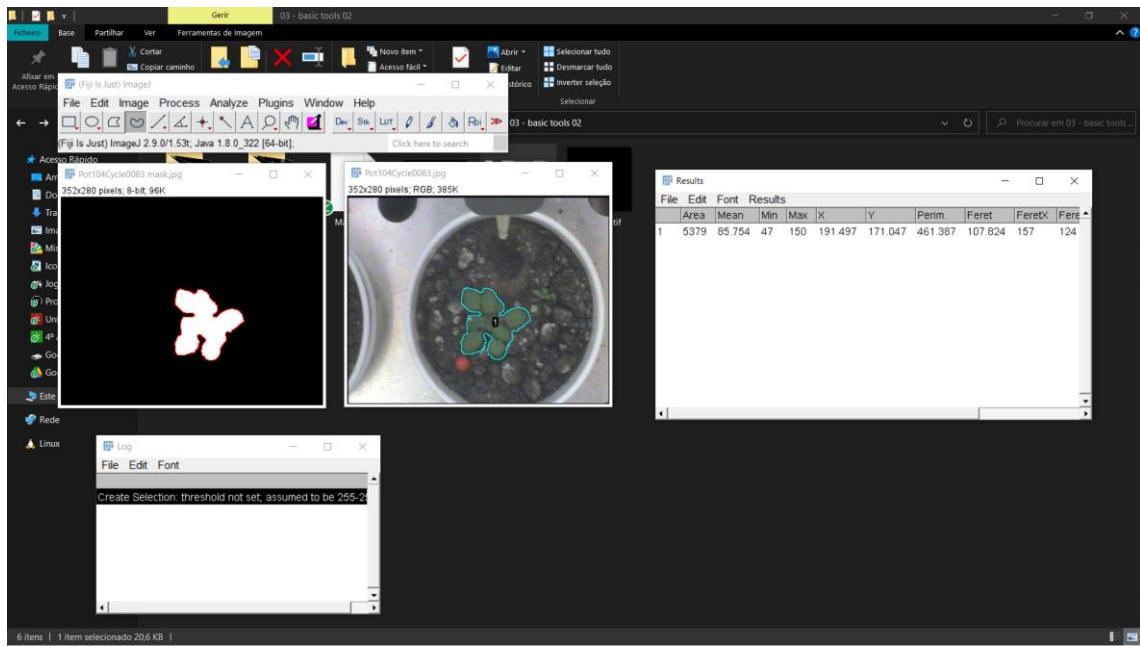
- b) Open the image Pot104Cycle0083.jpg and try to use the SIOX plugin to segment (separate from the background) the plant.



- c) Create a mask using the SIOX plugin. From the mask create a selection (Edit>Selection>Create Selection). Put the selection onto the original image (Edit>Selection>Restore Selection) and control the quality of the segmentation.



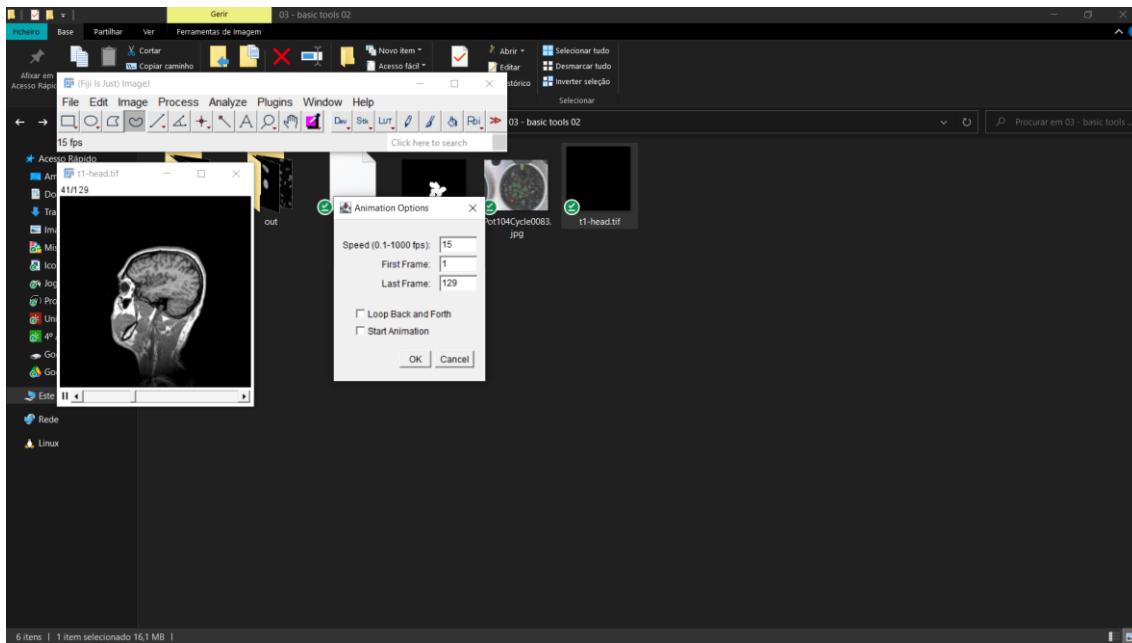
d) Measure the area of the plant.



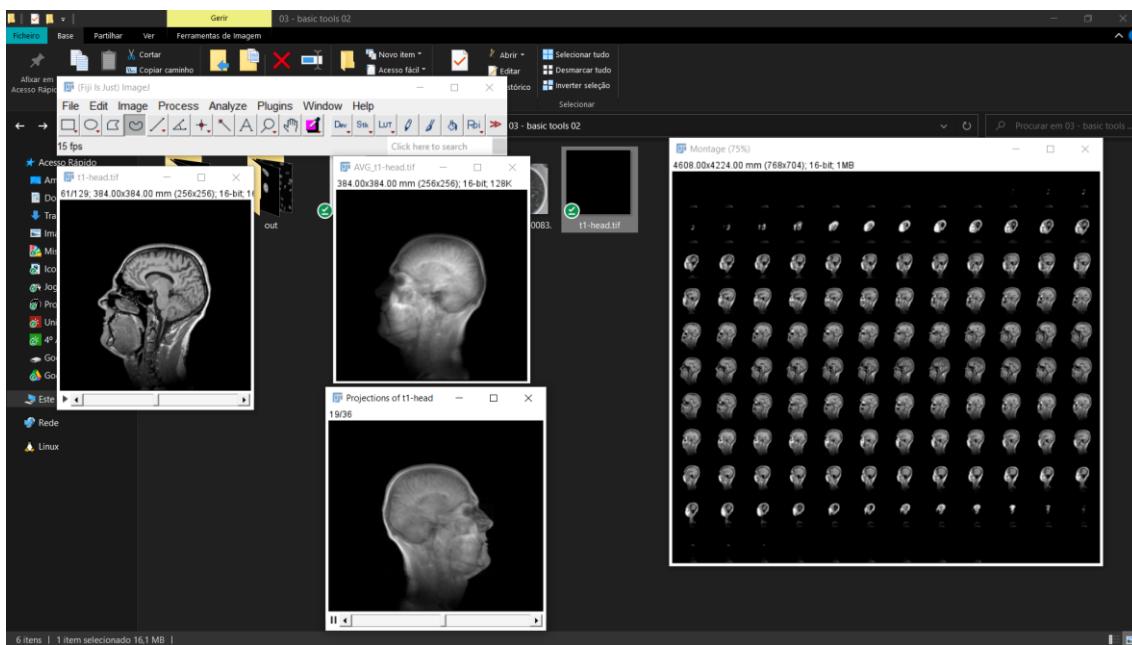
A área da planta é 5379.

## T3.4 Working With Stacks

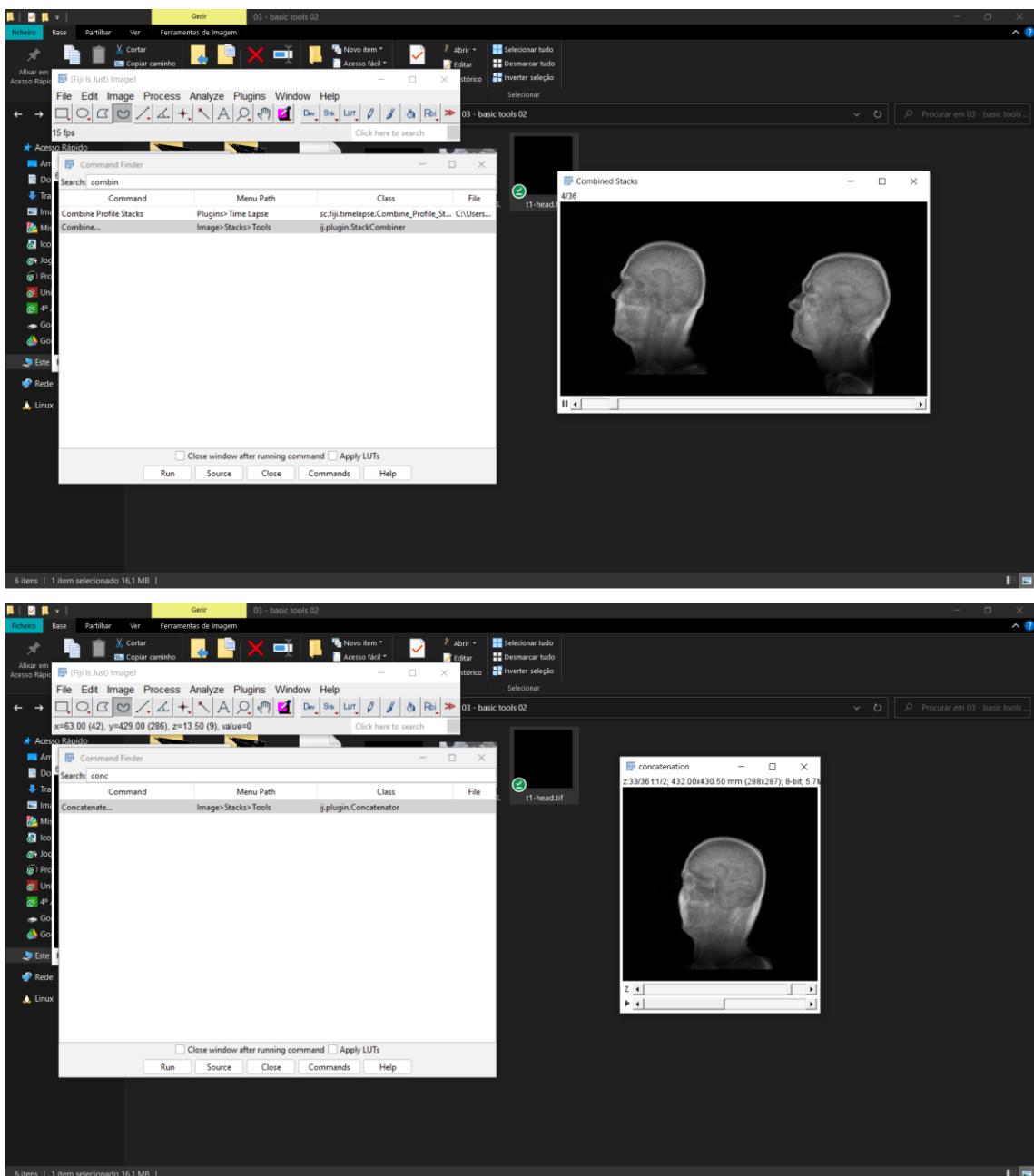
- a) Start the animation. Change the animation speed.



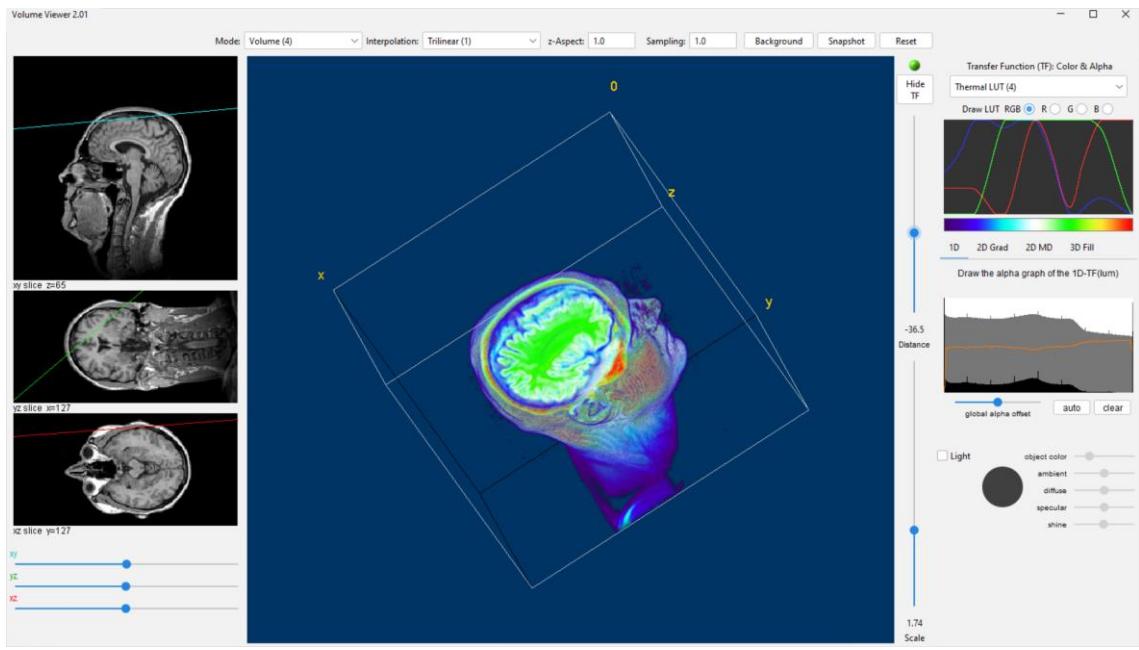
- b) Try the commands Z Project..., 3D Project... (select Mean Value as projection-method) and Make Montage... from the stk tool-button.



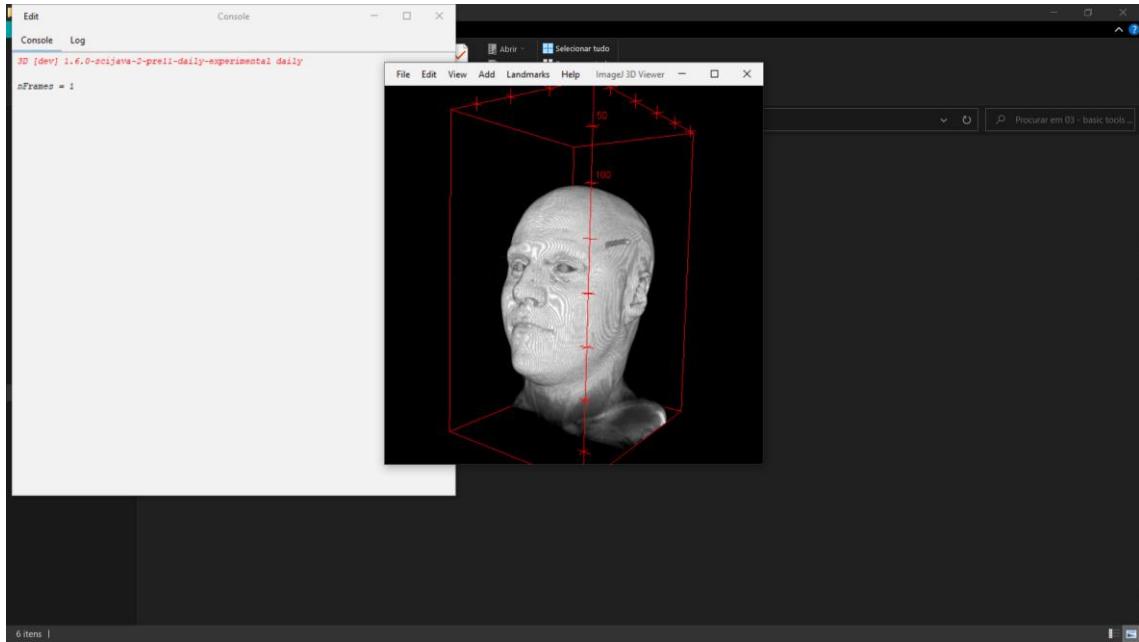
- c) Make one 3d-projection around the x-axis and one around the y-axis using the mean-value projection-method. Rename the resulting images, otherwise, they will both have the same name, which can lead to problems. Try the commands combine and concatenate from the stk tool-button. Hint: To concatenate the images must have the same size in the x and y dimensions. You can adjust the canvas size of the image using Image>Adjust>Canvas Size...



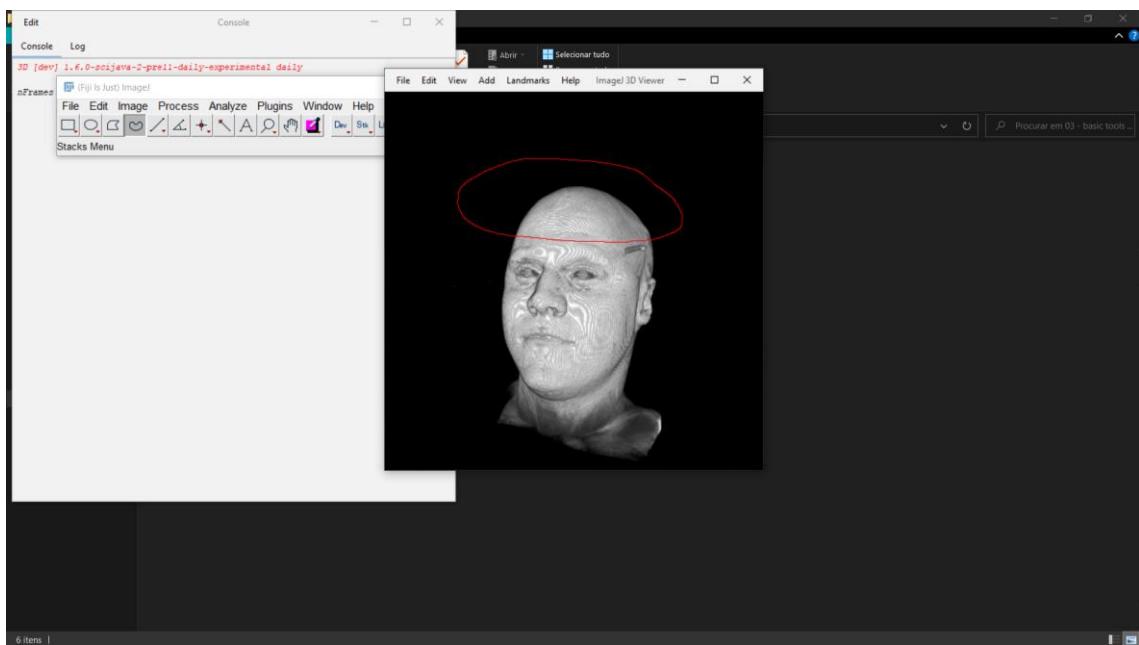
- d) Try the Volume Viewer (Plugins>3D>Volume Viewer). Set Volume as mode and select a Thermal LUT. Zoom in, change the distance and turn the volume view around by dragging it with the mouse.



- e) Try the ImageJ 3D Viewer. Set the resampling factor to 1 and press ok when you are asked if the image should be converted to 8 bit. As long as the scrolling tool is selected, you can turn the object in the image. You can zoom in with the magnifying glass tool. If you lose the object call View>Reset View from the 3D viewer window.



- f) Make a freehand 2d selection across the head. Select the object from the Select menu and then call Edit>Fill Selection. Turn the object. What happened?

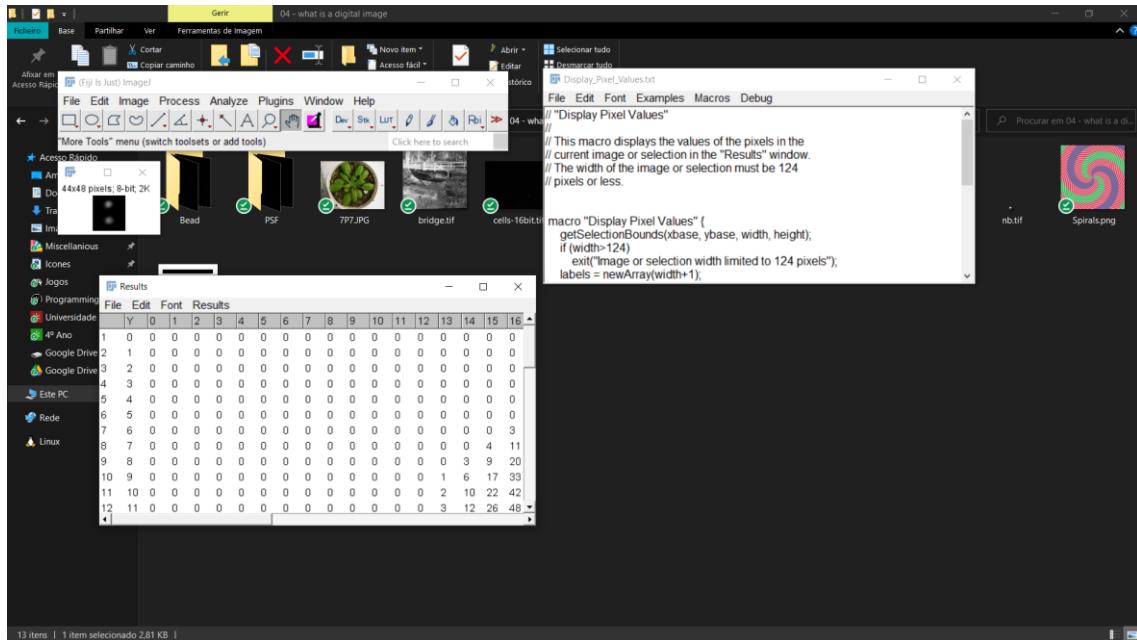


Não parece ter acontecido nada

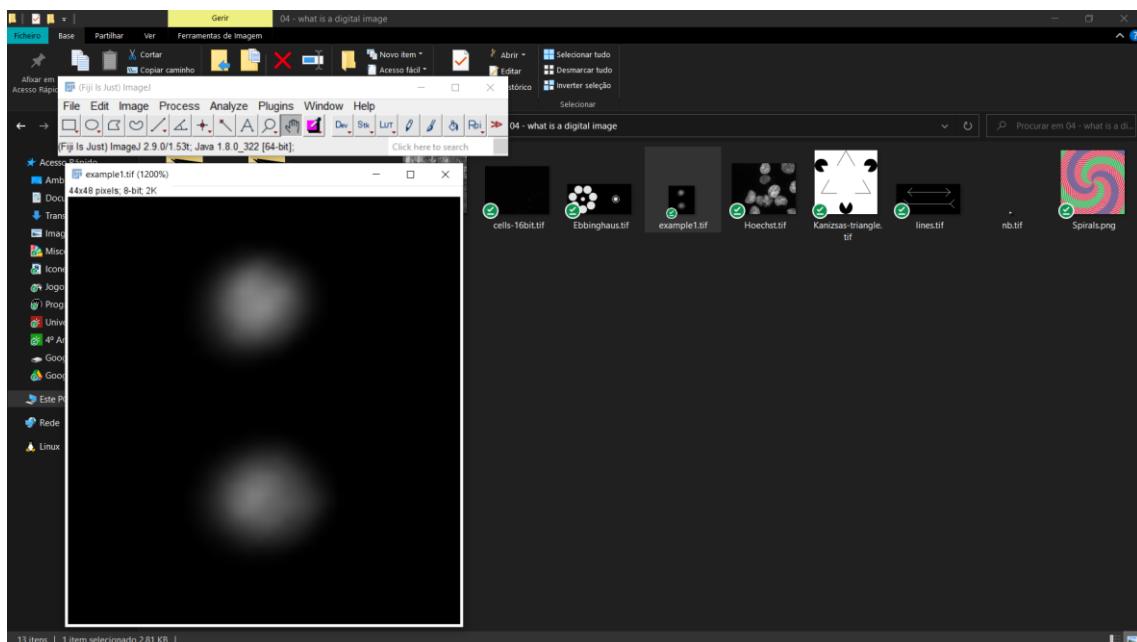
# T4

## T4.1 How Is The Image Represented By The Computer? - Pixels

- a) Open the image example1.tif from folder 04 - what is a digital image. Run the macro Display\_Pixel\_Values from ImageJ's macro web-page.



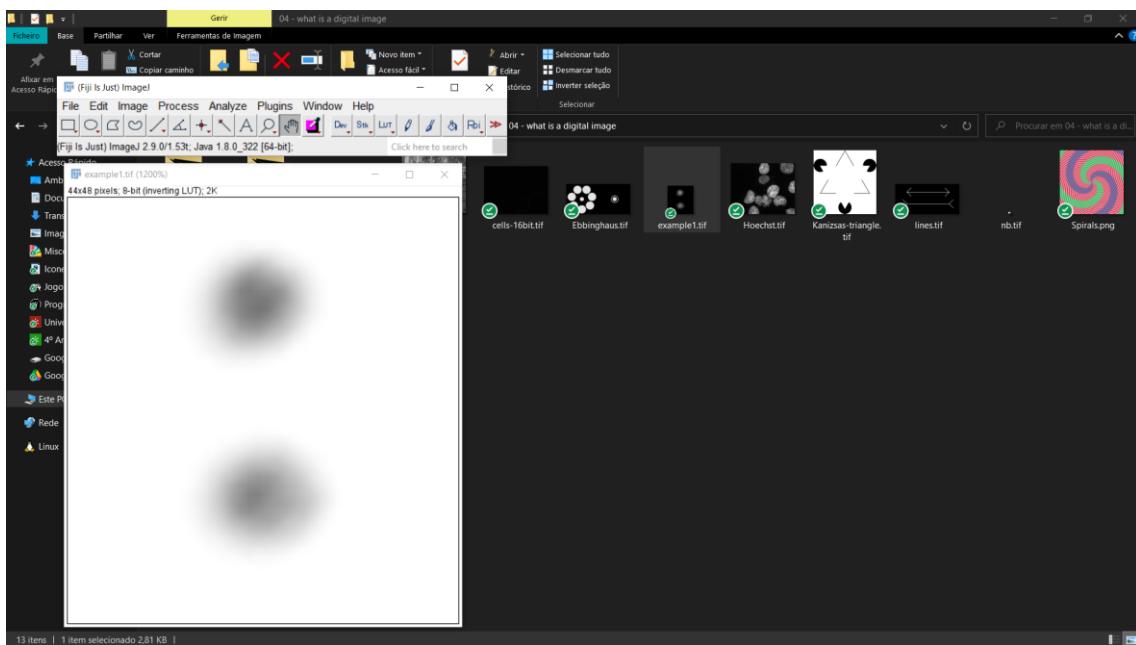
- b) Zoom into the image. You should now see little homogeneous squares. Note that this is just one possible way to display the pixels. As said above we should rather think about them as point samples. Go to Edit>Options>Appearance and select Interpolate Zoomed Images. What happens?



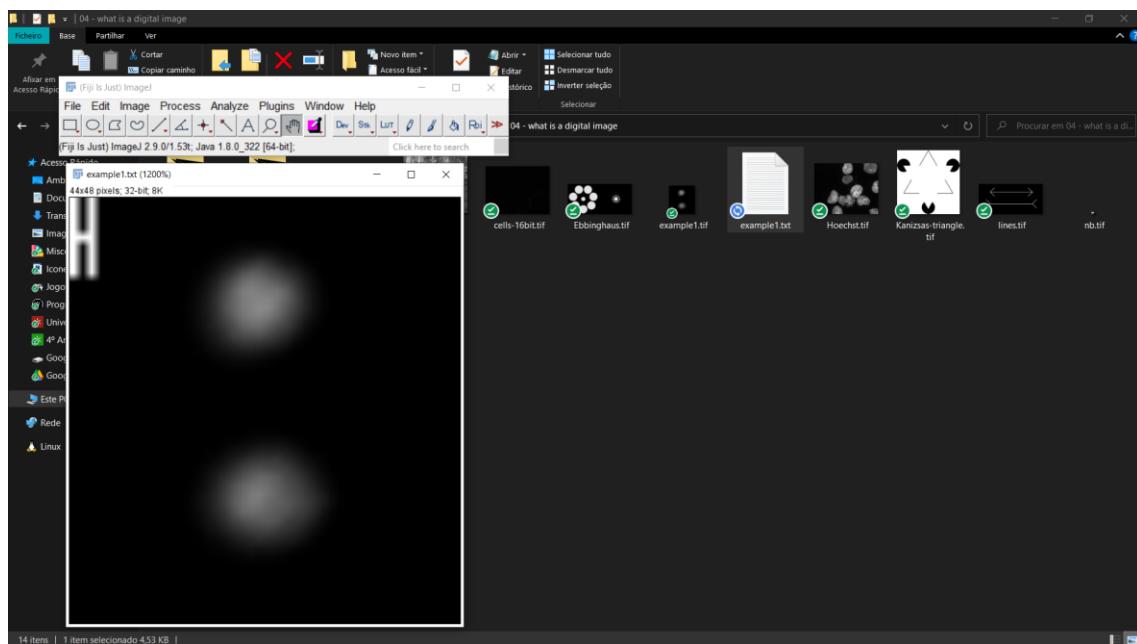
A opção Interpolate Zoomed Images remove a pixelização da imagem quando está ampliada, deixando-a desfocada.

- c) Are higher pixel values displayed brighter or darker than lower ones? Run Image>Lookup Tables>Invert LUT. What is the answer to the question now?

Os pixéis escuros representam valores inferiores do que pixéis claros. Ao inverter a LUT, passa a ser o contrário, os pixéis escuros representam valores superiores.

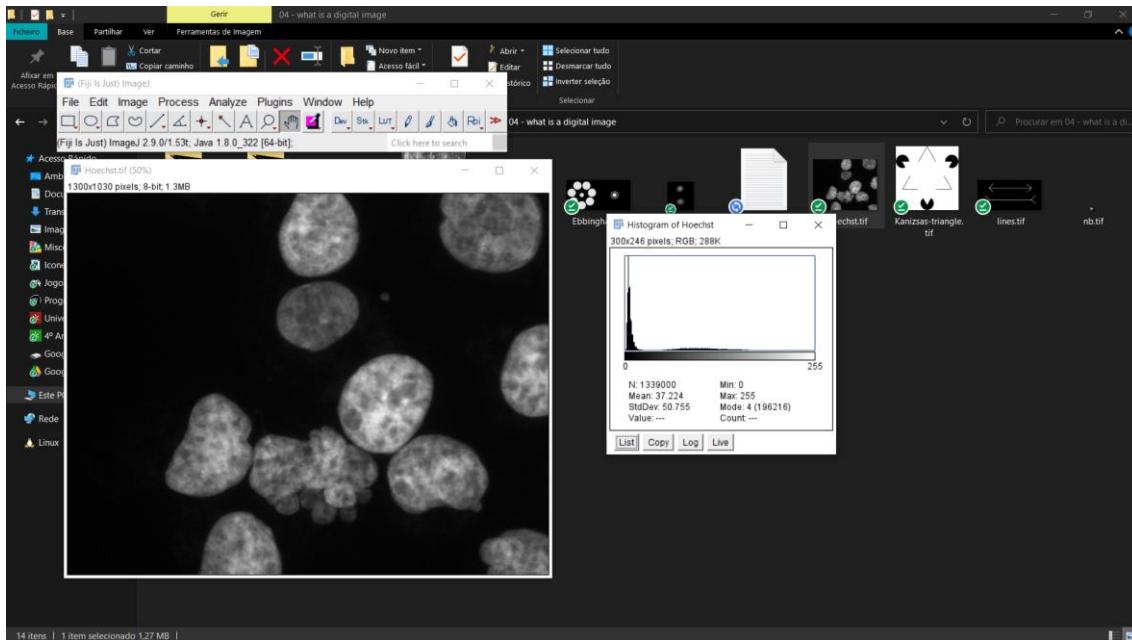


- d) Save the image as text-image (File>Save As>Text Image). Open it with a text editor, change some values to 255 and save the modified file. Reimport the text-image into ImageJ (Import>Text Image).



## T4.2 How Is The Image Represented By The Computer? – In Memory Image Formats

- a) Open the image Hoechst.tif from folder 04 - what is a digital image. Display the histogram. Use the commands from the menu Process>Math to add 100, subtract 100, divide by 2 and multiply by 2. Show the different histograms. Between two operations revert the image (ctrl+r). What are the values you expect for count, mean, min, max and mode (mode is the most frequent intensity in the image – the intensity value with the highest peak in the histogram)?

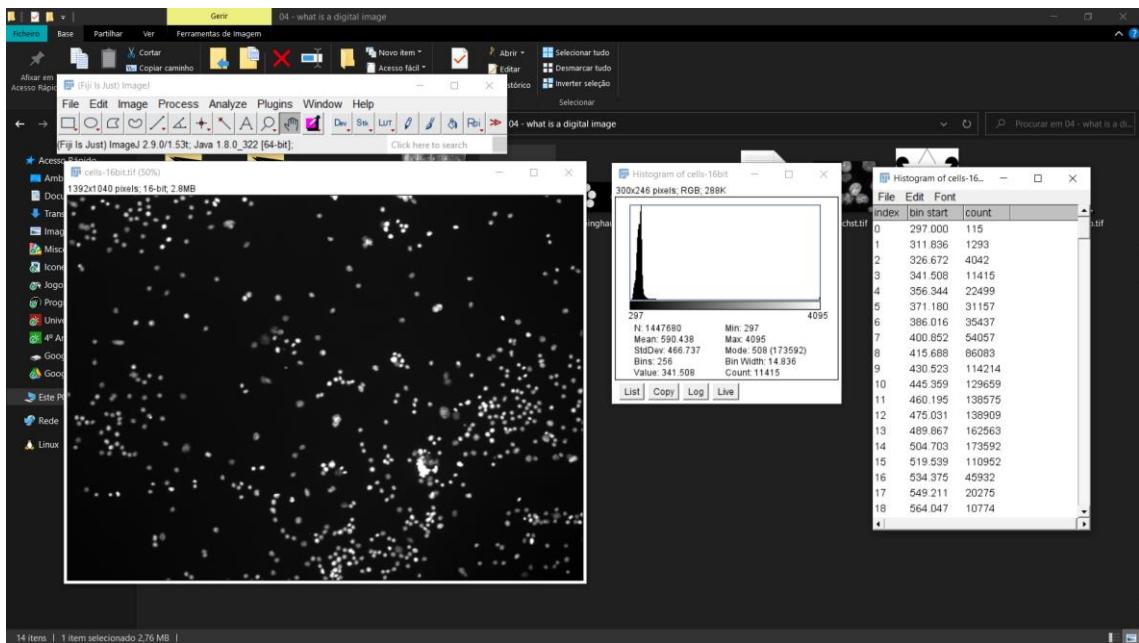


	count	min	max	mean	stdDev	mode
original	1339000	0	255	37.224	50.755	4 (196216)
+100	1339000	100	255	136.583	49.020	104 (196216)
-100	1339000	0	155	5.576	16.285	0 (1119190)
×2	1339000	0	255	70.075	91.291	8 (196216)
/2	1339000	0	128	18.839	25.393	2 (314957)

- b) Press the list button on the histogram of the image multiplied by 2. Each second row has the value 0. Can you explain why?

Como os valores são multiplicados por 2, então os valores resultantes da multiplicação serão todos múltiplos de 2, ou seja, números pares.

- c) Open the image cells-16bit.tif. Display the histogram. What is the maximum value?  
**Hint:** the camera uses 12 bit. Note that the histogram uses 256 bins and not one count for each grey-value. Press the list button to see the start values of the bins.



O valor máximo é  $4095 (2^{12}-1)$ .

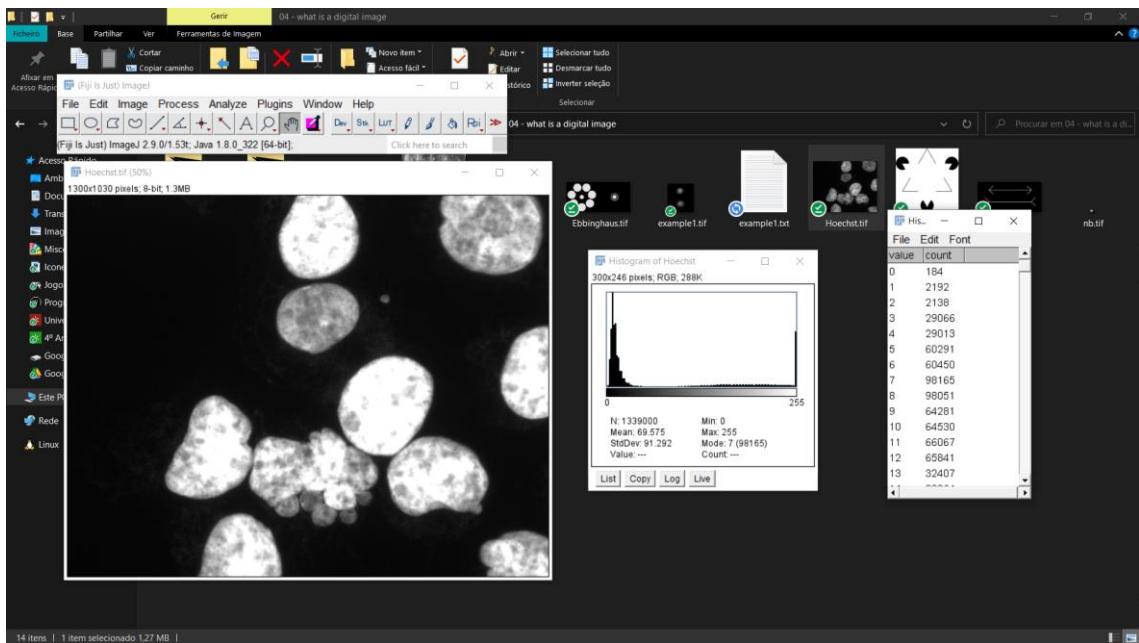
- d) What would you have to do in order to make the image in b) use the full range of available grey-values?

Apply your calculation using Process>Math>Macro... and look at the histogram again.

Existem 256 tons de cinza disponíveis, um para cada valor de 0 a 255. Desta forma, se só tivermos valores pares, só utilizaremos metade dos tons. É necessário fazer com que a alguns valores pares seja subtraído um valor de modo a utilizar toda a escala.

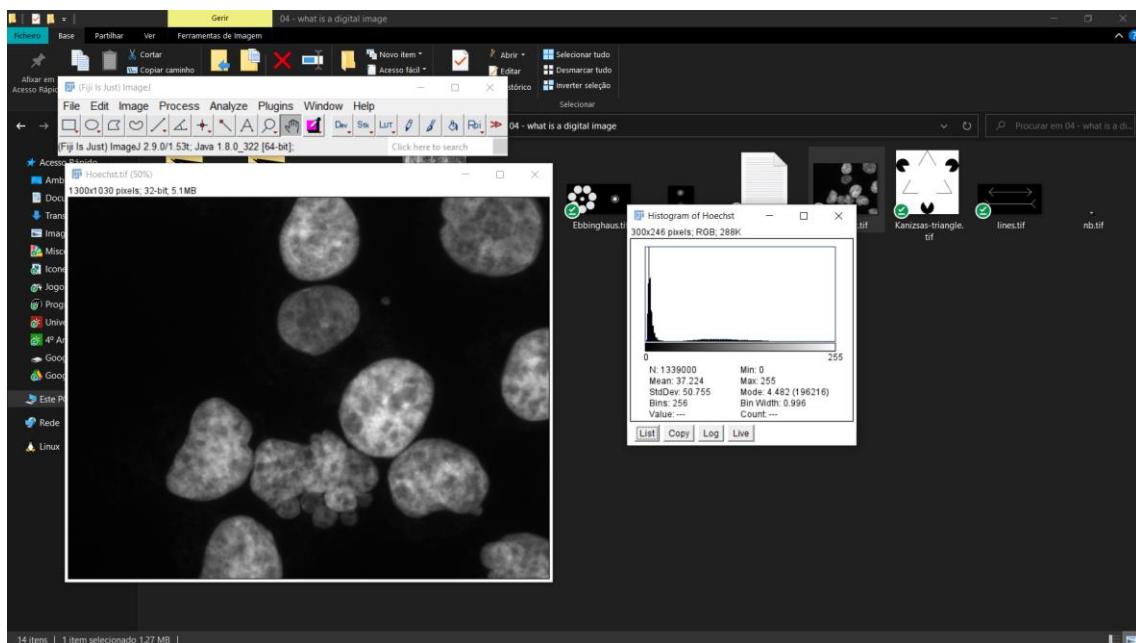
Isto consegue-se aplicando, por exemplo, a macro “v-(x+y)%2” à imagem. Ela calcula o resto da divisão inteira por 2 da soma das coordenadas x e y, ou seja, é 0 para x e y simultaneamente pares ou ímpares, e 1 caso um seja par e o outro ímpar, e subtraí esse resultado ao valor do pixel.

Ou seja, em x e y simultaneamente pares ou ímpares, o valor não é alterado, em x e y diferentes (um par e outro ímpar), o valor passa a ser o número ímpar inferior, preenchendo assim toda a escala de cores.

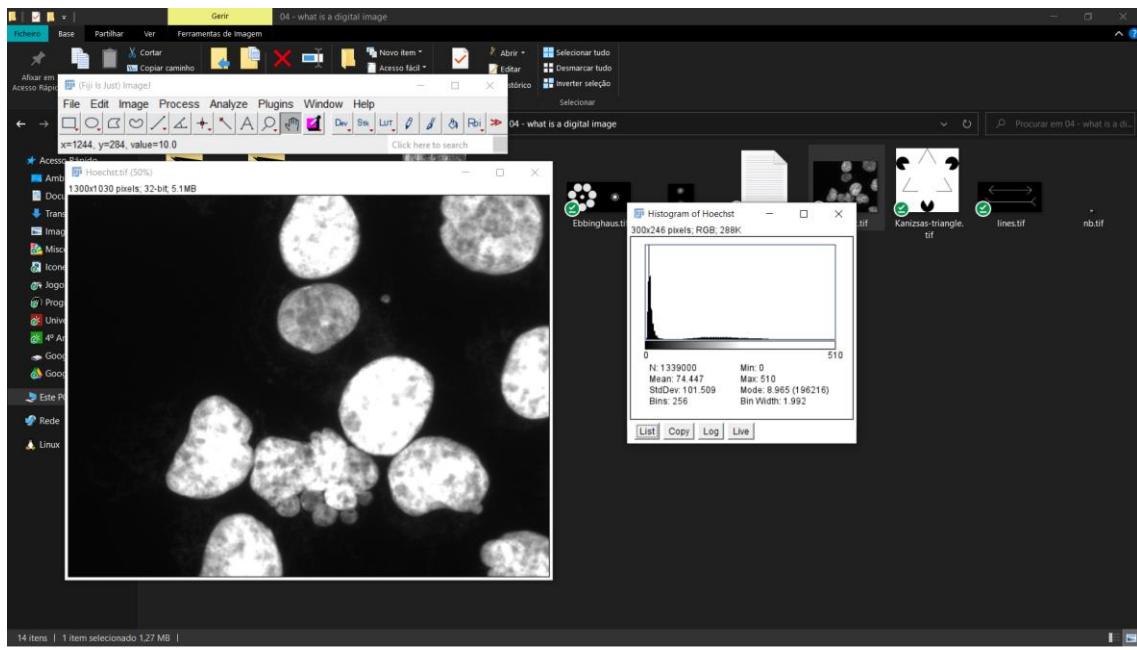


- e) Open the image Hoechst.tif and convert it into a 32bit image (Image>Type>32-bit). Look at the histogram. Multiply the image by 2 and look at the histogram again. Divide the image by zero. What is the result? Multiply it by -1. What are the pixel values now? Divide it by Infinity.

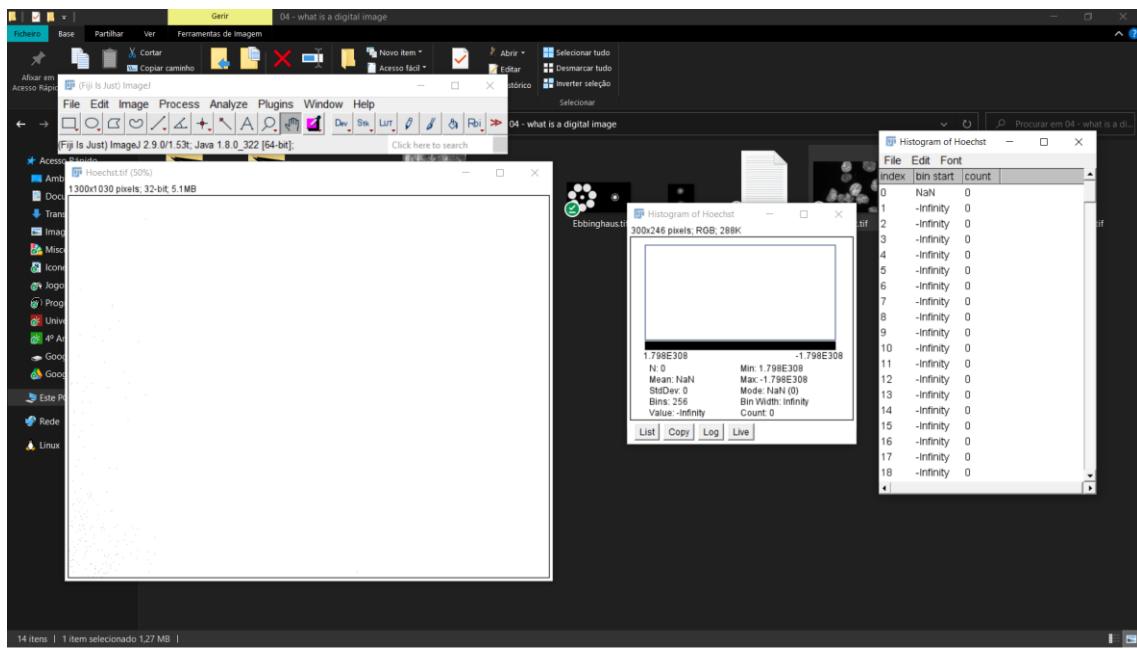
Original



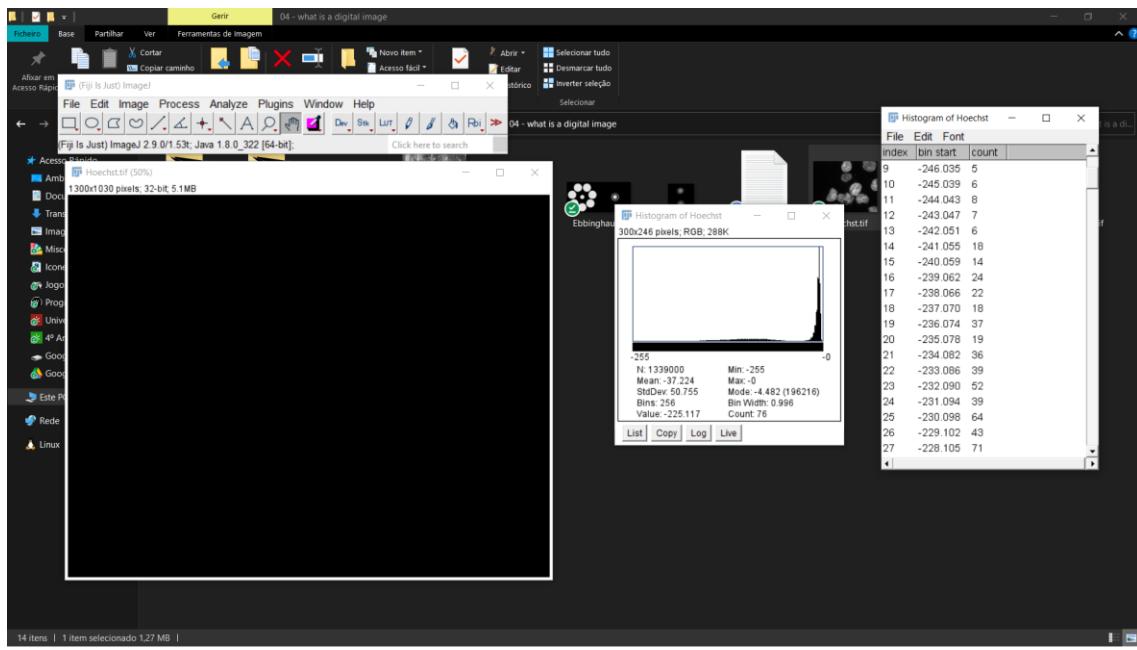
Multiplicado por 2



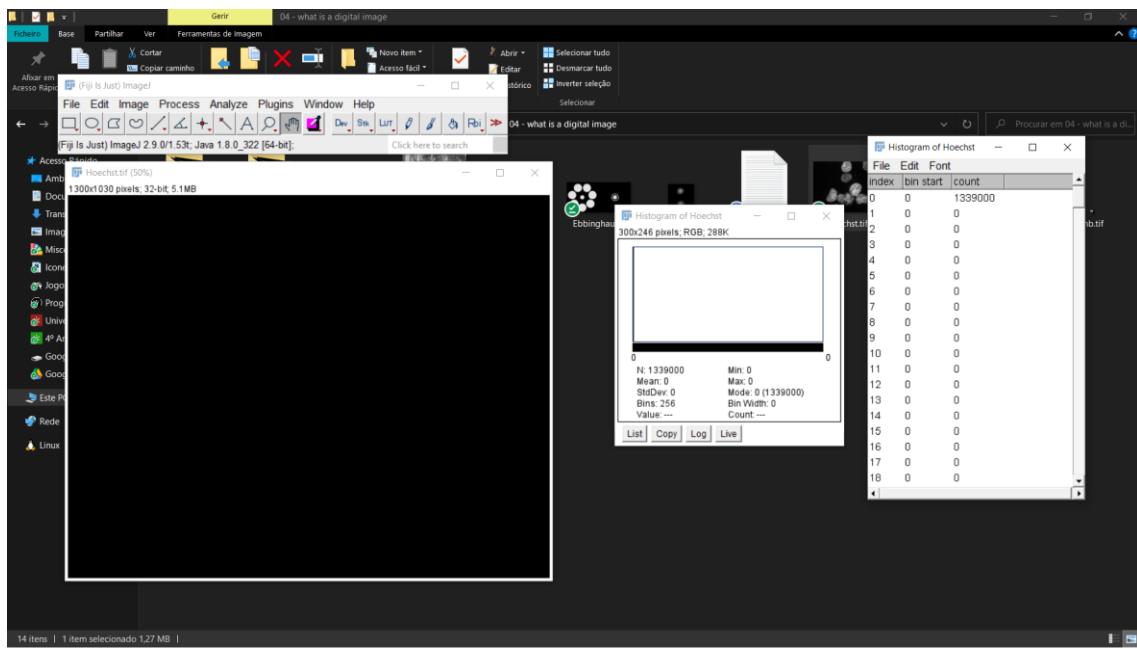
Dividido por 0 – os valores passam a ser -infinito.



Multiplicar por -1 – os valores passam efetivamente a negativos, mas não a imagem fica toda preta, de certo a escala de cores coloca tudo a preto para valores inferiores a 0.

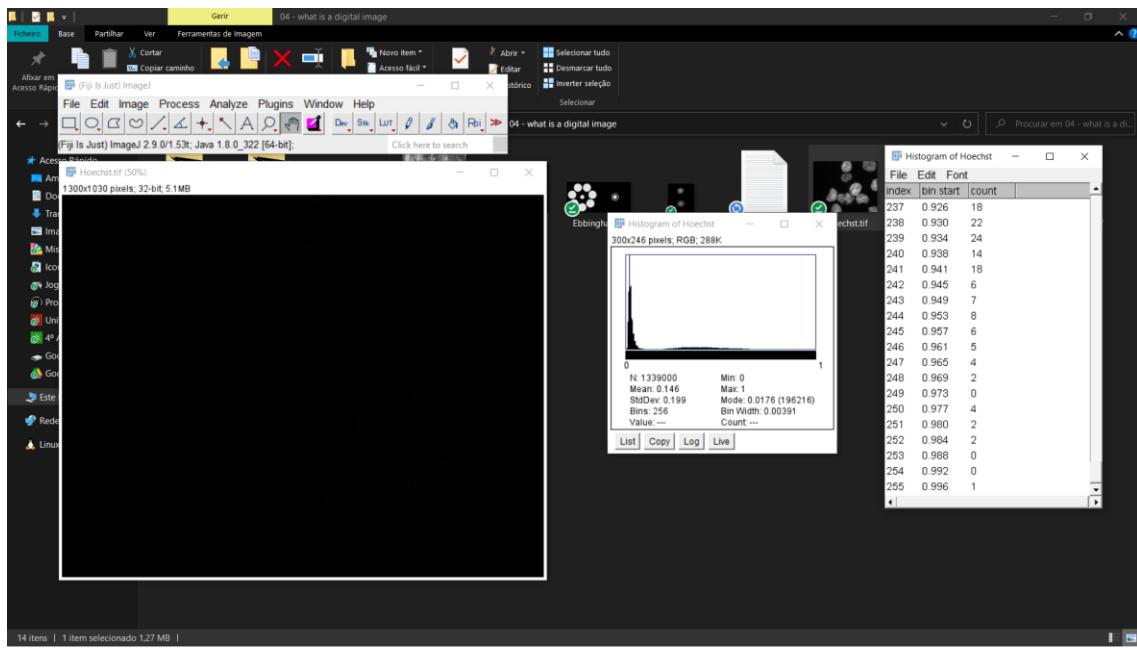


Dividir por infinito – os valores passam todos a ser 0.

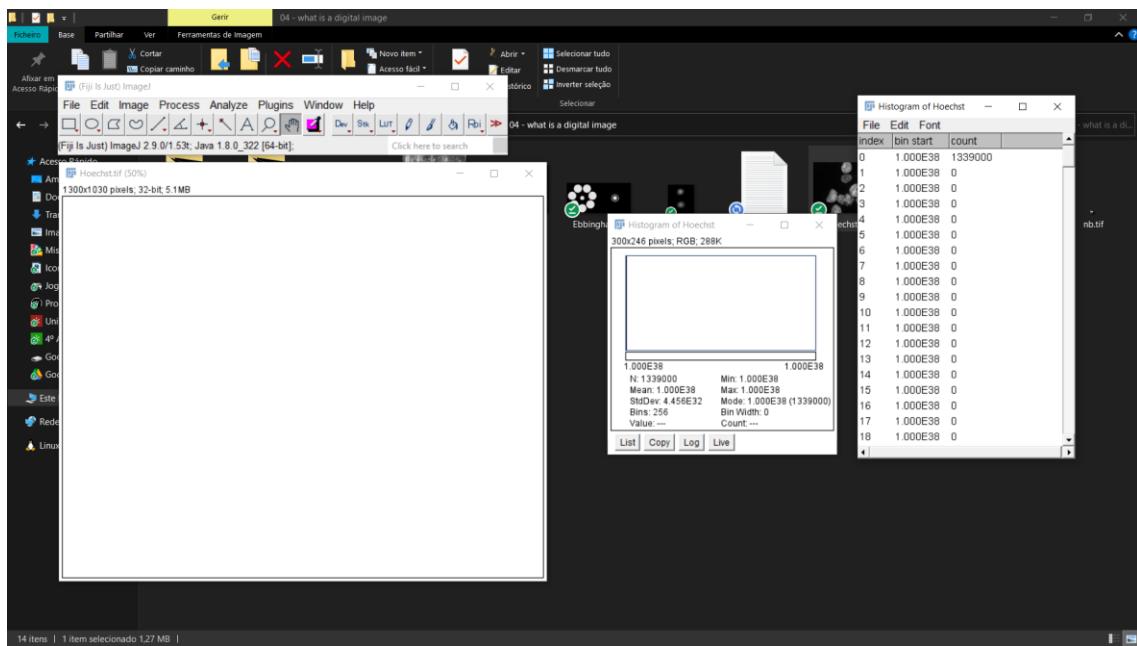


- f) (OPTIONAL) Revert the image Hoechst.tif and convert it to 32 bit again. Divide it by 255. What is the maximum now? Add  $1e+38$  (i.e.  $1*10^{38}$ ). What is the maximum? Multiply by 10. What happens?

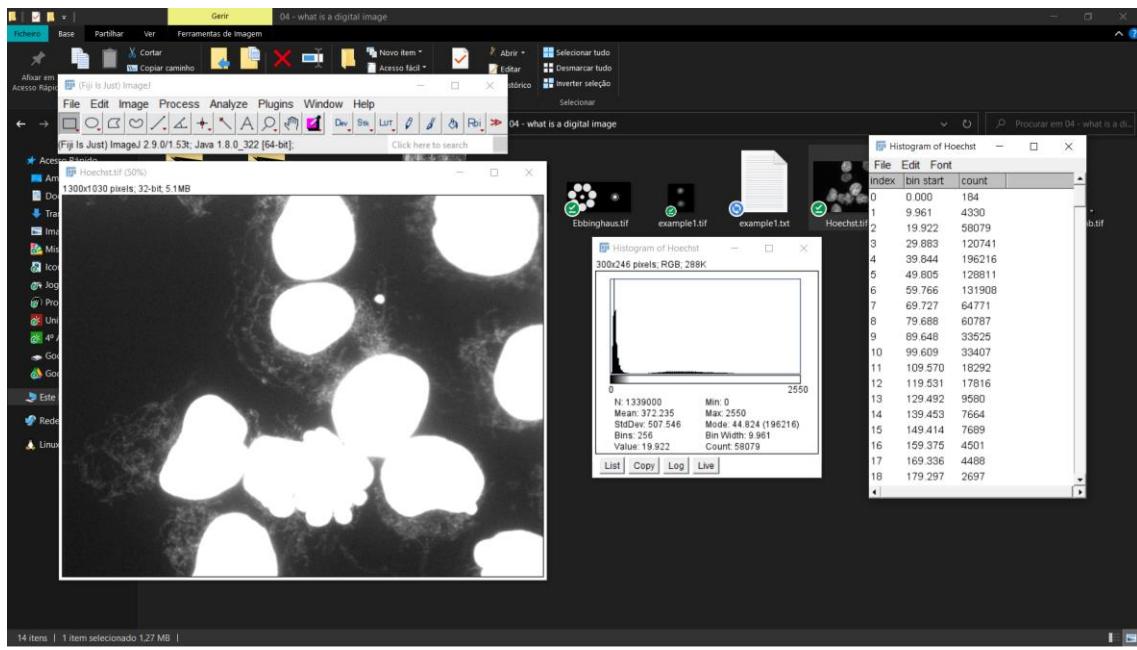
Dividir por 255 – os valores aparecem “normalizados” (entre 0 e 1).



Adicionar  $1e+38$  – os valores ficam todos a  $1e+38$ , visto que estamos a adicionar um valor várias ordens de magnitude superior ao valor do pixel.

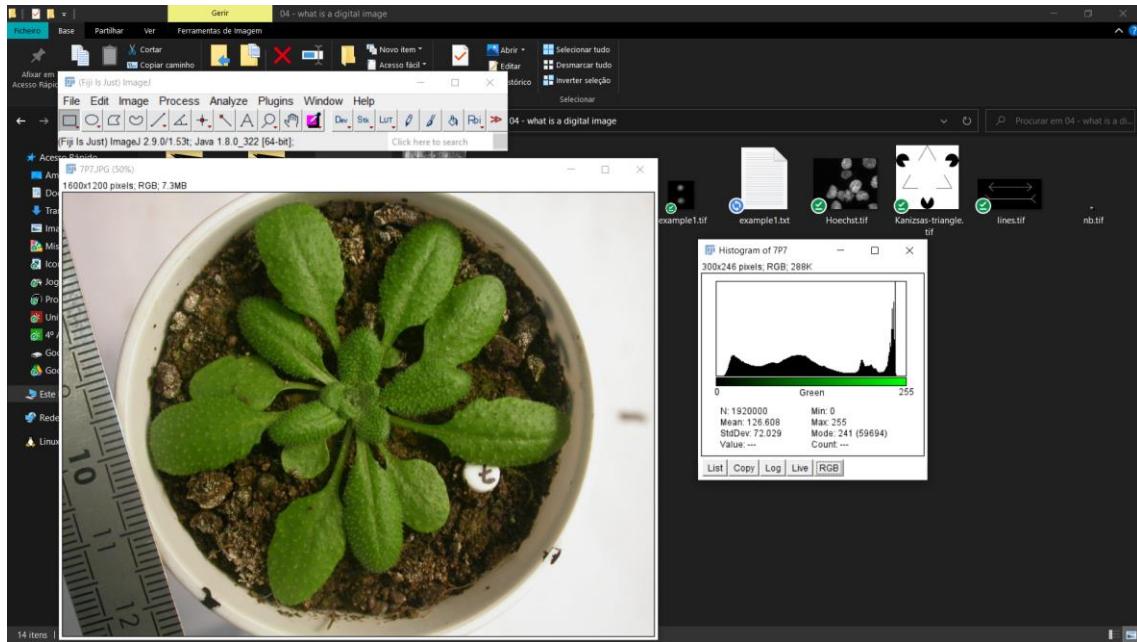


Multiplicar por 10 – os valores ficam multiplicador por 10, as partes anteriormente claras da imagem ficam totalmente brancas, visto que os seus valores depois de multiplicar por 10 passam de 255, e ficam visíveis mais detalhes da parte escura da imagem, possivelmente algumas moléculas das células.



- g) Open the image 7P7.JPG. Move the mouse over the image and look at the status bar. You should see three values for each pixel for the components red, green and blue. Open the histogram by pressing h (Analyze>Histogram). Press the RGB button multiple times. What does it do?

O botão RGB altera a escala que está a ser visualizada, rodando entre “Intensity (unweighted)”, “Intensity (weighted)”, “R+G+B”, “Red”, “Green” e “Blue”.



- h) How do the colours on the screen mix? Create a new hyperstack with 3 channels, 1 slice and 1 frame. Use type 8-bit and mode Color. Create a filled circle on each channel then open the channels tool and switch to Composite mode. Hint: To create a filled circle, make a circular selection, select white as foreground colour using the colour-picker tool and call Edit>Fill. What colour do you obtain from mixing red and

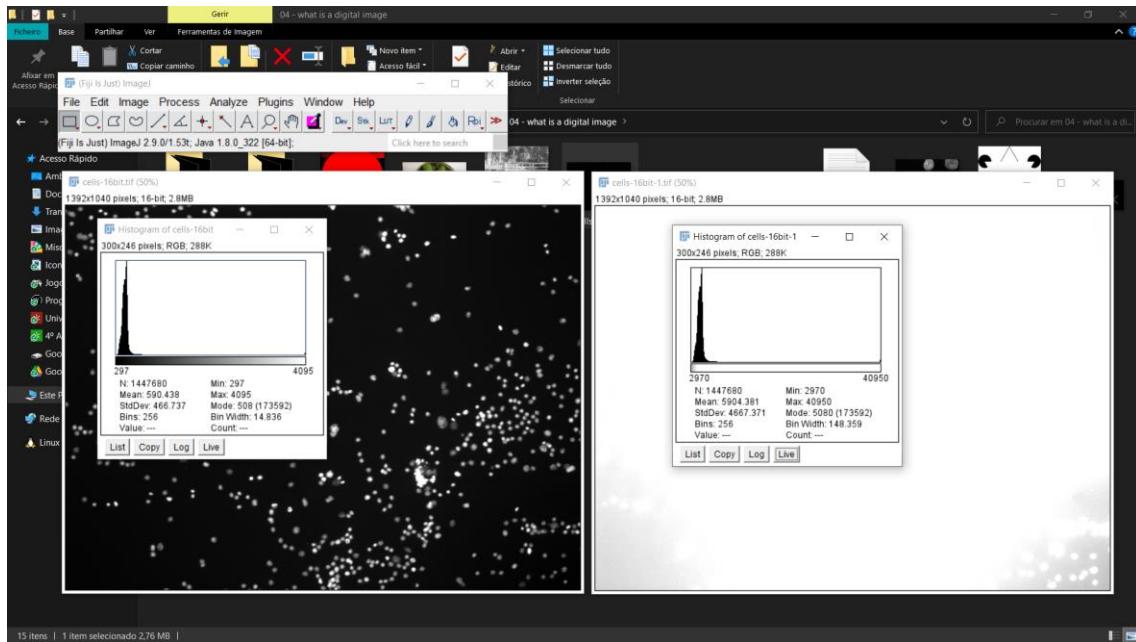
blue, blue and green, green and red? What colour do you obtain from mixing red, green and blue?

red and blue	= purple
blue and green	= cyan
green and red	= yellow
red, green and blue	= white

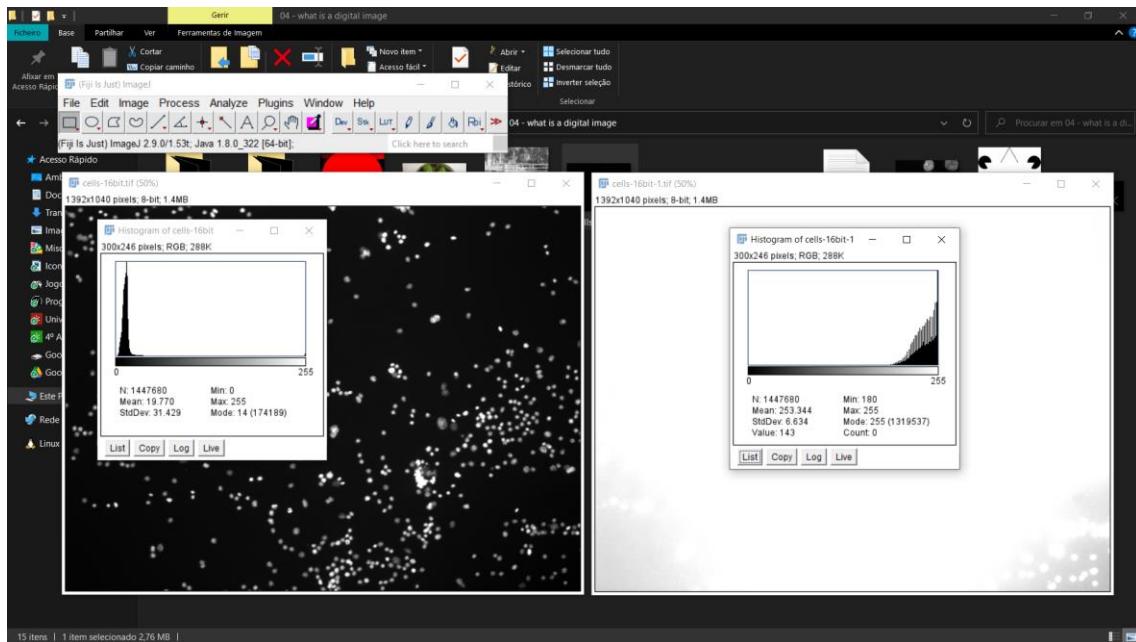
## T4.3 How Is The Image Represented By The Computer? – Conversion Traps

- a) Open the image `cells-16bit.tif`. Make a copy (`shift+d`) and multiply it by 10. Measure the mean and total intensity in the two images. Convert the images to 8bit and measure again. What is the ratio of the intensities in the two images before and after the conversion? What does a person that only knows the 8-bit versions conclude? Can you explain what happened?

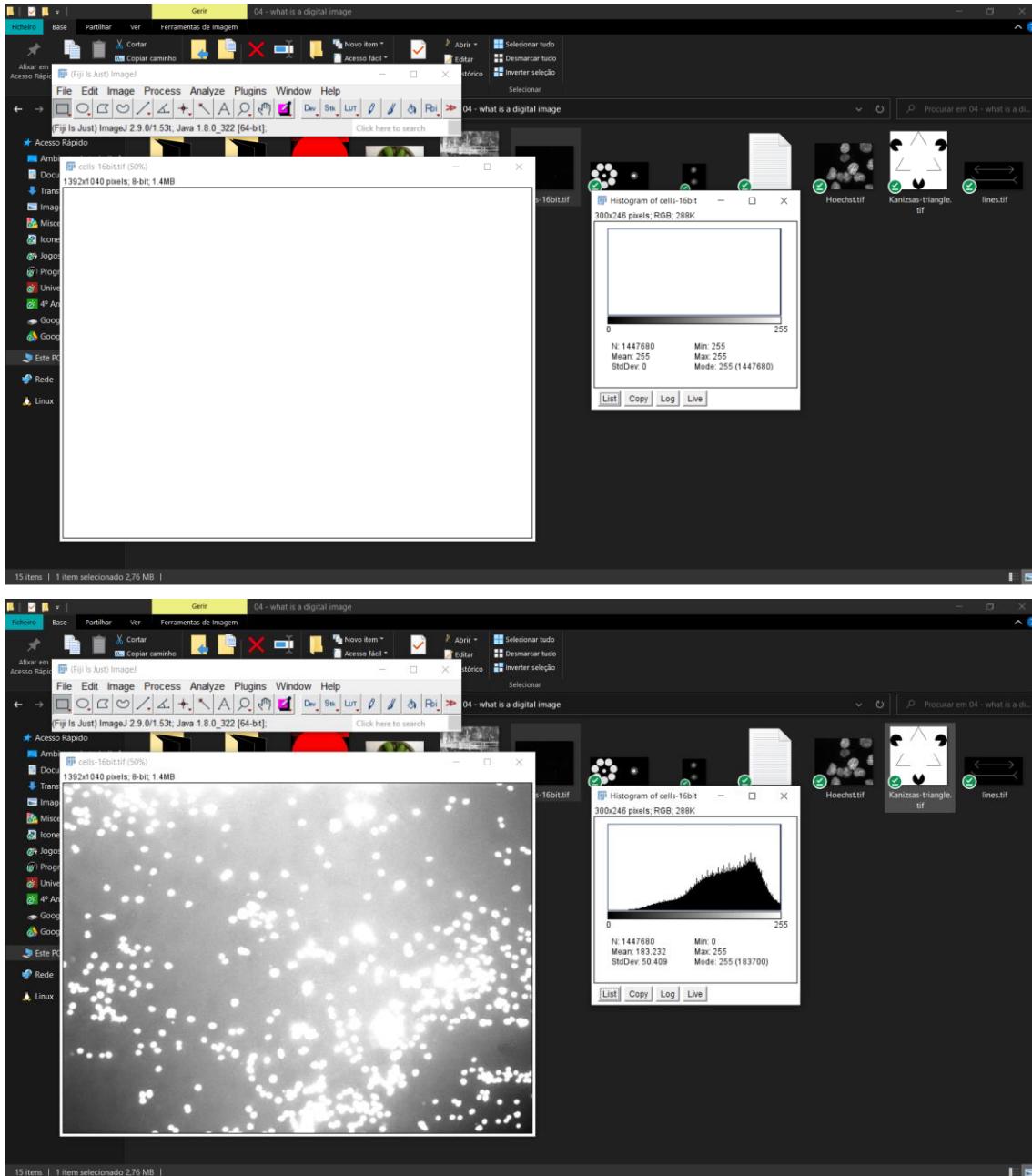
Original



Convertido em 8-bit – Ao passar de 16 bits para 8-bits, os valores superiores a  $2^8$  foram igualados a 255, pelo que se perdeu muita informação, principalmente na segunda imagem, uma vez que os valores tinham sido multiplicados por 10.



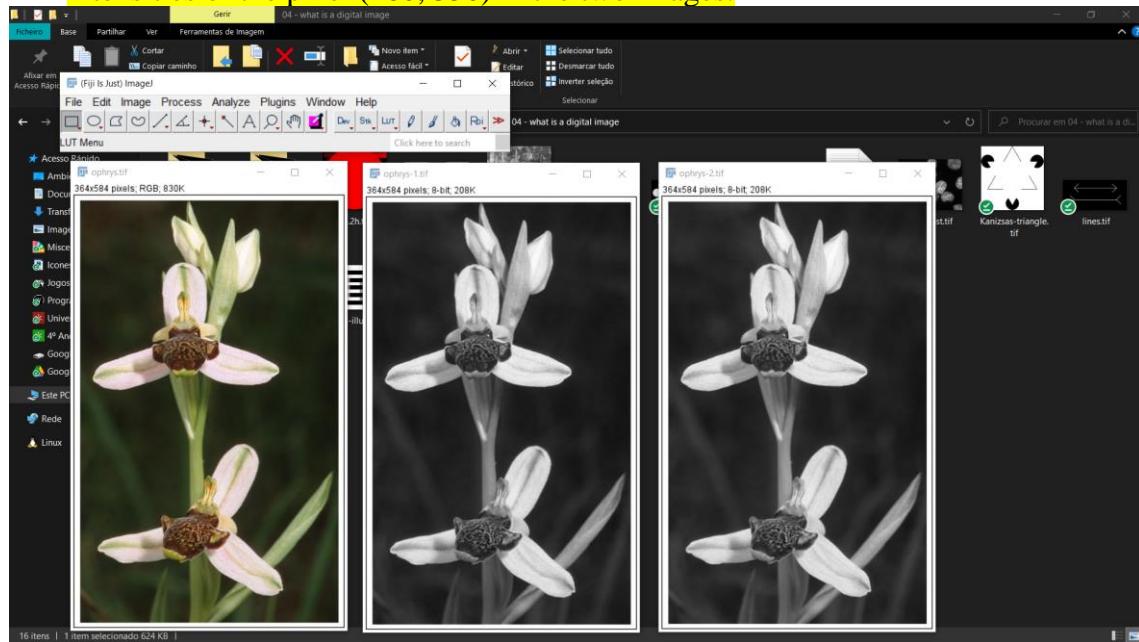
- b) Switch the scaling when doing conversions off (Edit>Options>Conversions). Convert the image again from 16 bit to 8 bit. What happens. Subtract the minimum value before doing the conversion. What happens now? Switch the scaling on again.



A remoção da opção de scaling na conversão faz com que todos os pixéis fiquem com o valor 255, visto que o pixel com menor valor na imagem original é 297. Ao subtrair o valor 297 à imagem original, a conversão retém a informação dos valores de 0 a 255, e todos os que forem superiores a 255 ficam com esse valor.

- c) Open the image ophrys.tif. Make two copies of the image ophrys-1.tif and ophrys-2.tif. Convert ophrys-1.tif to 8-bit using Image>Type>8-bit. Under Edit>Options>Conversions

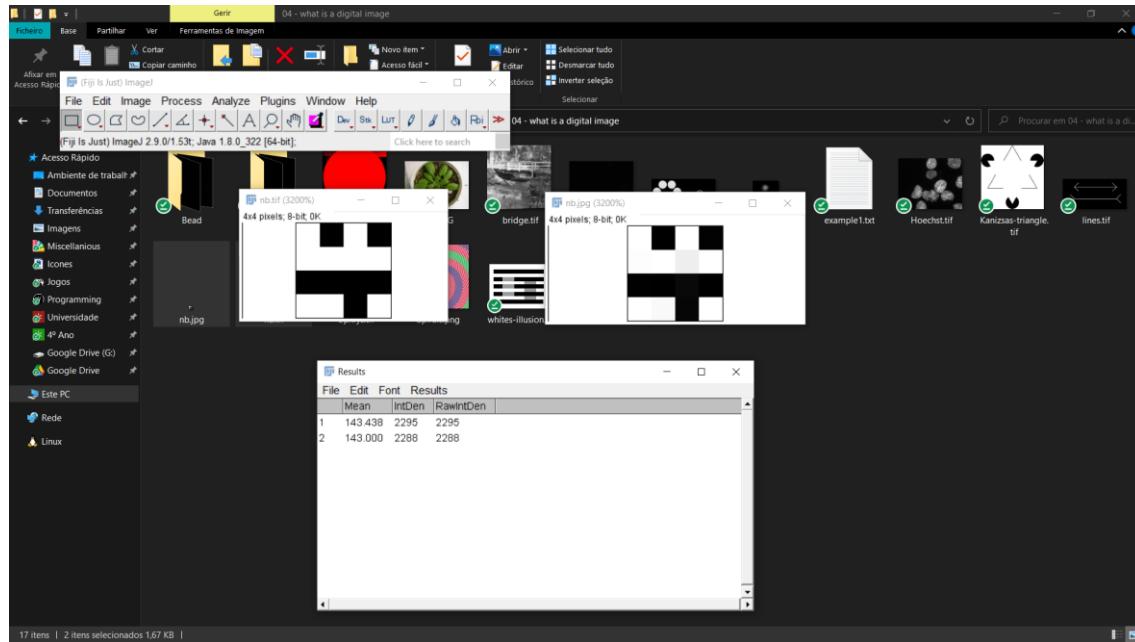
switch weighted RGB conversions on (or off if it was on before). Compare the intensities of the pixel (160, 350) in the two images.



Na primeira imagem, as coordenadas (160, 350) (com o “Invert Y” desligado) tem o valor RGB 133, 146, 077 (Hex #85924d), na segunda tem o valor 119 e na terceira tem 134.

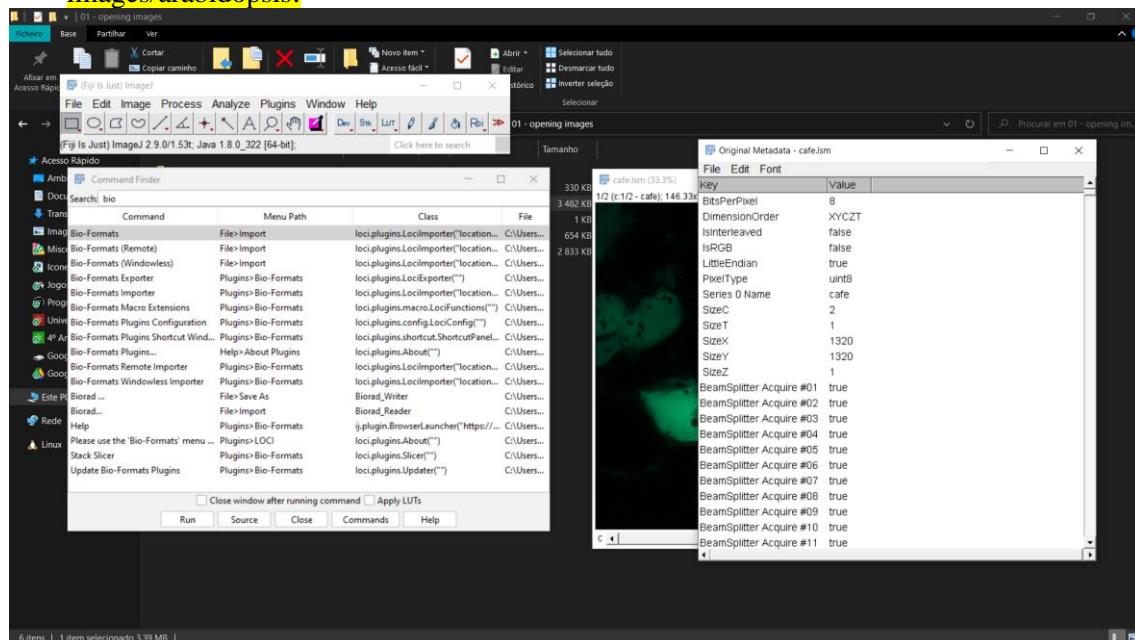
## T4.4 How Is The Image Represented By The Computer? – On Disc Iamge Formats

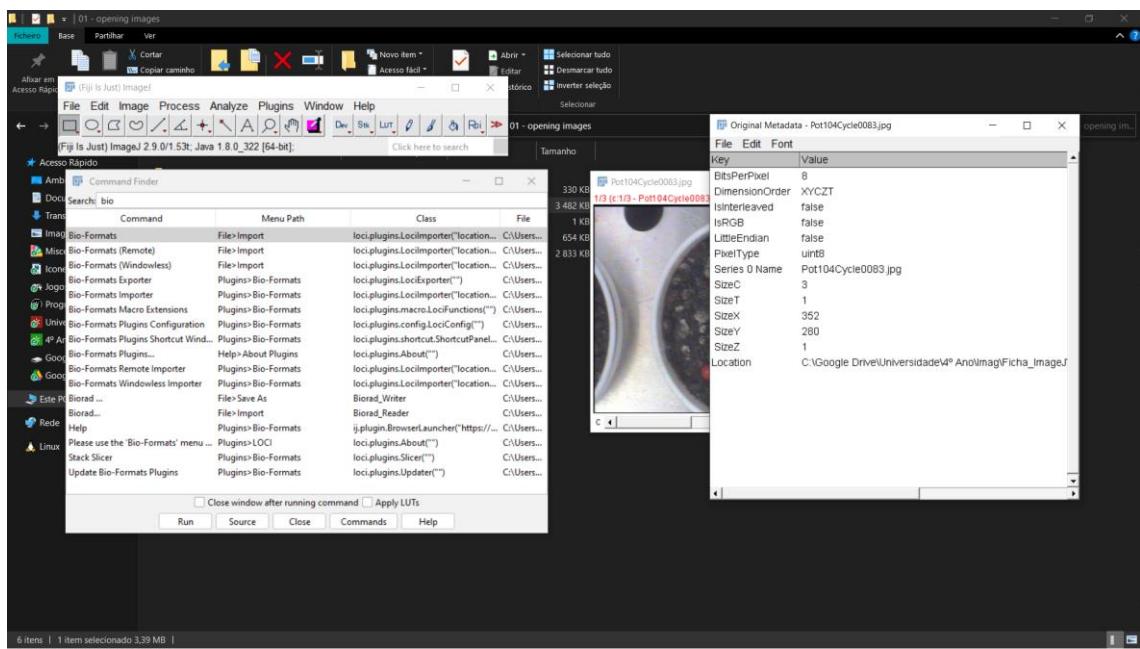
- a) Load the image nb.tif, save it as jpeg-image and load the saved jpeg image. Zoom in and compare the two images (make sure that the option Interpolate zoomed images is switched off). Measure and compare the intensity in the two images.



Verifica-se que existe alteração de informação quando se passa do formato tif para jpeg.

- b) Use the loci-bioformats importer to read the meta-data of the image cafe.lsm in folder 01 - opening images and the metadata of one of the images in the folder 01 – opening images/arabidopsis.





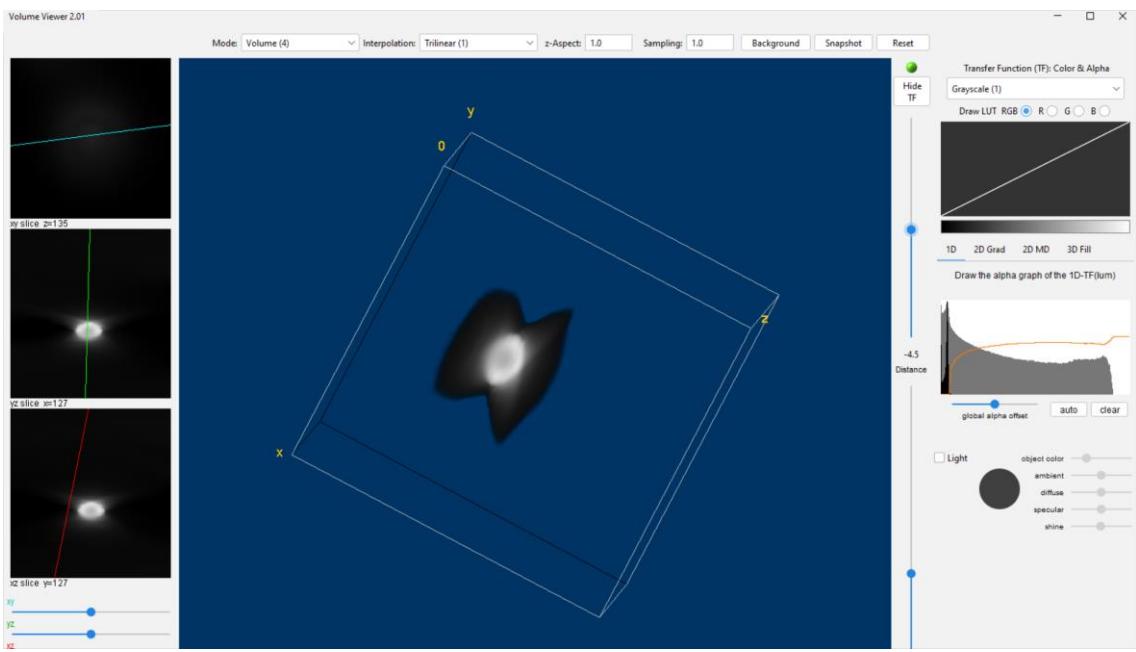
## T4.5 The Image And The Real World – Sampling And Resolution

- a) Use the loci-bioformats importer to find the excitation wavelength and numerical aperture of the system that was used to take the image cafe.lsm from the folder 01 “opening images. What is the optical resolution of the system? What is the sampling interval? Hints: It could be useful to copy the data into a text editor and to use the find-command of the text editor. The excitation wavelengths can be found under `IlluminationChannel`. The channels that are used have an entry different from 0 in the field `Aquire`. The (maximal) numerical aperture is a property of the objective. The sampling interval can be found under `VoxelSizeX` and `VoxelSizeY`. Has the image been sampled at the Nyquist-interval or is over-or under-sampled?

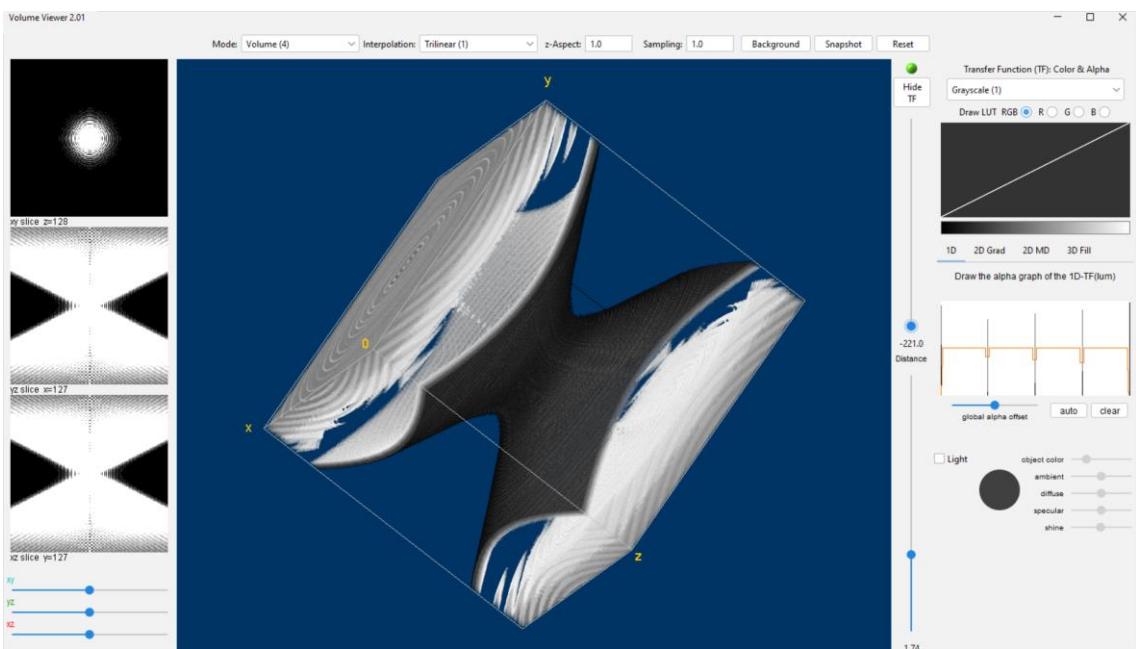
```
IlluminationChannel Acquire #01 0    IlluminationChannel Wavelength #01      458.0
IlluminationChannel Acquire #02 0    IlluminationChannel Wavelength #02      477.0
IlluminationChannel Acquire #03 0    IlluminationChannel Wavelength #03      488.0
IlluminationChannel Acquire #04 0    IlluminationChannel Wavelength #04      514.0
IlluminationChannel Acquire #05 0    IlluminationChannel Wavelength #05      543.0
IlluminationChannel Acquire #06 0    IlluminationChannel Wavelength #06      633.0
IlluminationChannel Acquire #07 0    IlluminationChannel Wavelength #07      0.0
IlluminationChannel Acquire #08 0    IlluminationChannel Wavelength #08      458.0
IlluminationChannel Acquire #09 0    IlluminationChannel Wavelength #09      477.0
IlluminationChannel Acquire #10 0    IlluminationChannel Wavelength #10      488.0
IlluminationChannel Acquire #11 0    IlluminationChannel Wavelength #11      514.0
IlluminationChannel Acquire #12 0    IlluminationChannel Wavelength #12      543.0
IlluminationChannel Acquire #13 0    IlluminationChannel Wavelength #13      633.0
IlluminationChannel Acquire #14 0    IlluminationChannel Wavelength #14      0.0
IlluminationChannel Acquire #15 0    IlluminationChannel Wavelength #15      458.0
IlluminationChannel Acquire #16 0    IlluminationChannel Wavelength #16      477.0
IlluminationChannel Acquire #17 -1   IlluminationChannel Wavelength #17      488.0
IlluminationChannel Acquire #18 0    IlluminationChannel Wavelength #18      514.0
IlluminationChannel Acquire #19 0    IlluminationChannel Wavelength #19      543.0
IlluminationChannel Acquire #20 0    IlluminationChannel Wavelength #20      633.0
IlluminationChannel Acquire #21 0    IlluminationChannel Wavelength #21      0.0
IlluminationChannel Acquire #22 0    IlluminationChannel Wavelength #22      458.0
IlluminationChannel Acquire #23 0    IlluminationChannel Wavelength #23      477.0
IlluminationChannel Acquire #24 0    IlluminationChannel Wavelength #24      488.0
IlluminationChannel Acquire #25 0    IlluminationChannel Wavelength #25      514.0
IlluminationChannel Acquire #26 -1   IlluminationChannel Wavelength #26      543.0
IlluminationChannel Acquire #27 0    IlluminationChannel Wavelength #27      633.0
IlluminationChannel Acquire #28 0    IlluminationChannel Wavelength #28      0.0

VoxelSizeX      0.11085672262346012
VoxelSizeY      0.11085672262346012
VoxelSizeZ      1.0
```

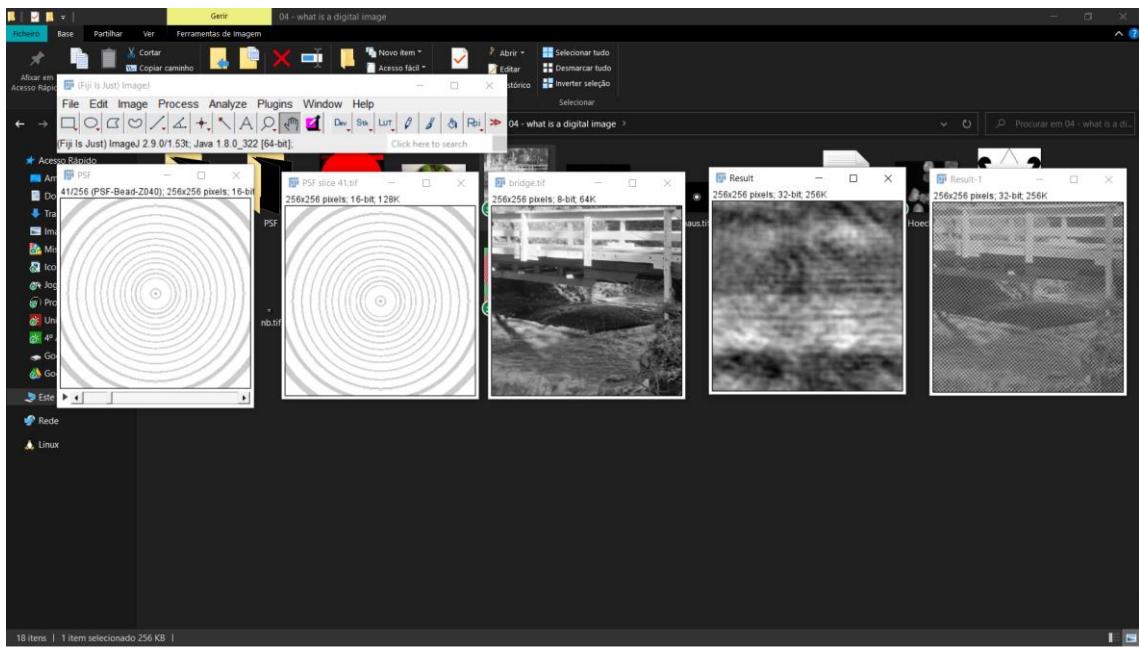
- b) Open the image 04 - what is a digital image/Bead. This is the image of a fluorescent bead taken with a widefield microscope. Display the image in 3D using the Volume Viewer or the 3D-viewer.



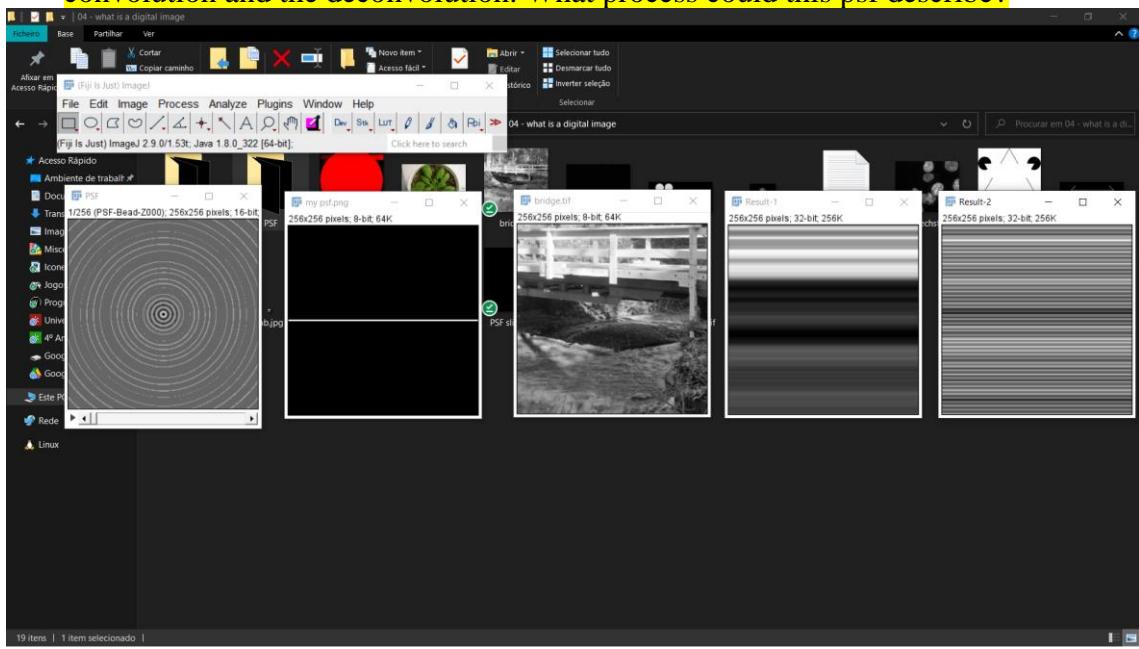
- c) Open the image 04 - what is a digital image/PSF. This is a theoretically calculated point spread function for a widefield microscope with NA=1.4 and lambda=530nm. Use Image>Properties to enter the correct sampling distances ( $dx=64.5\text{nm}$  and  $dz=160\text{nm}$ ). Display the image in 3D using the Volume Viewer or the 3D-viewer.



- d) Take one slice from the middle of the psf image and convolve the image bridge.tif with it. You can use Process>FFT>FD Math to calculate the convolution. The images must have the same size. Enlarge the canvas of the psf image using Image>Adjust>Canvas Size... Make a deconvolution of the result and the psf.

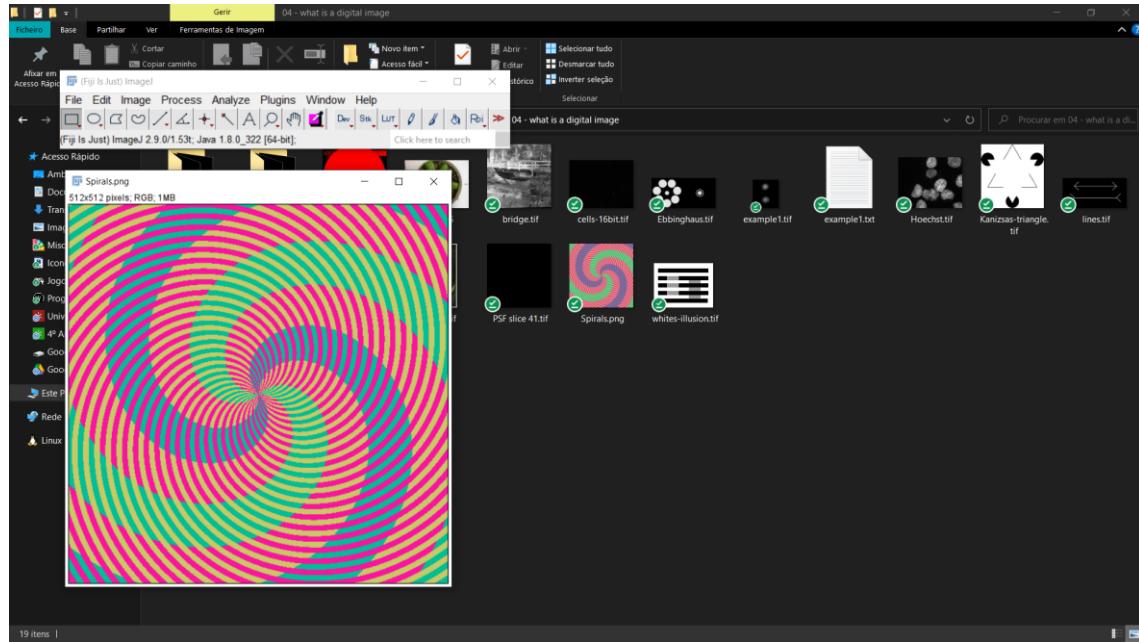


- e) Create your own 2d psf image that consists of a small horizontal white line. Do the convolution and the deconvolution. What process could this psf describe?



## T4.6 Image And Perception

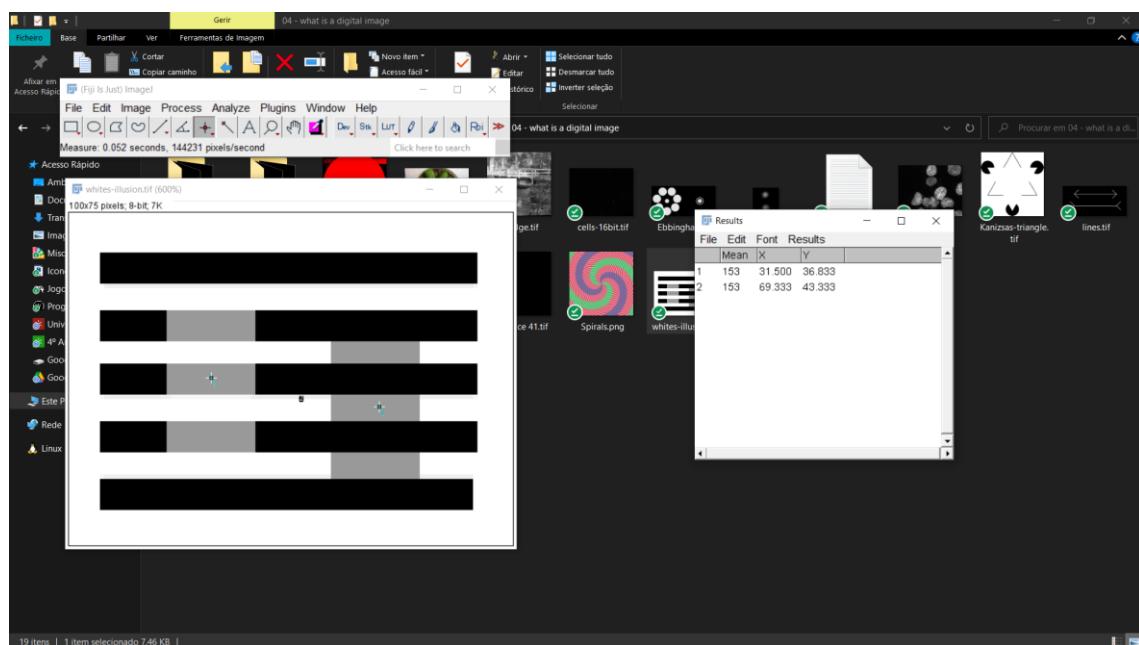
- a) Open the image Spirals.png. How many different colours are in the image? Can you list their RGB values?



Numa primeira vista, parece ter 4 cores distintas, mas na verdade só são 3, com os seus valores RGB:

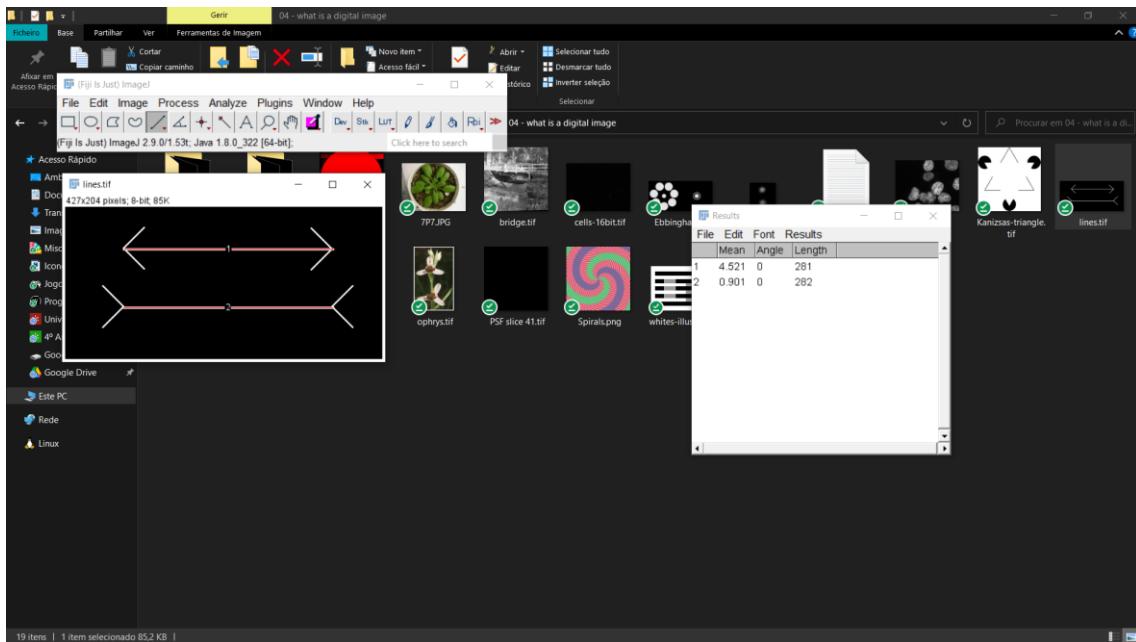
- 200, 200, 100;
- 250, 020, 160;
- 000, 190, 150.

- b) Open the image whites-illusion.tif. Which grey bars are brighter, the ones on the left or the ones on the right? Measure the intensity values. Can you explain this?



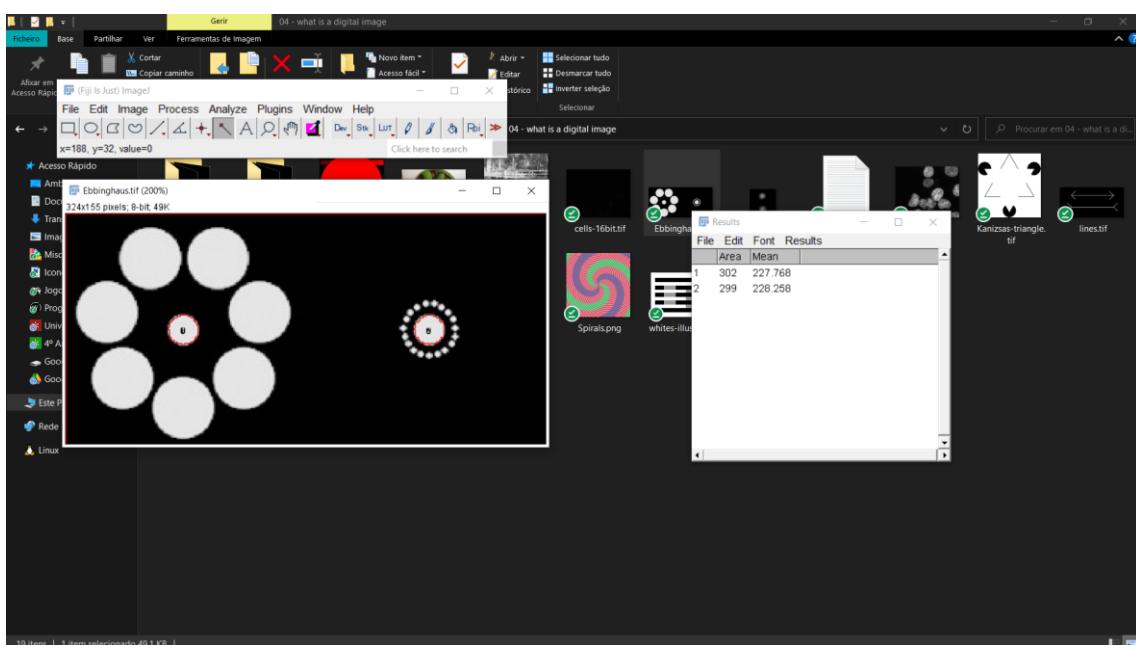
Parece que a barra da esquerda é mais clara do que a da direita, porém é uma ilusão de ótica e ambas as barras têm a mesma cor. Isto é explicado pois a barra da direita tem preto a envolve-la e a da direita tem branco, o que faz parecer, por contraste, que a da direita é mais clara do que a da esquerda.

- c) Open the image lines.tif. Which line is longer, the upper line or the lower line? Measure the lengths.



Parece que as duas linhas têm o mesmo tamanho, porém, novamente, trata-se de uma ilusão de ótica, e têm ambas o mesmo tamanho.

- d) Open the image Ebbinghaus.tif. Which central dot is larger, the left one or the right one? Use thresholding and the wand-tool to measure the areas of the two dots.



Trata-se de outra ilusão de ótica. O ponto central da esquerda parece maior, mas não o é.

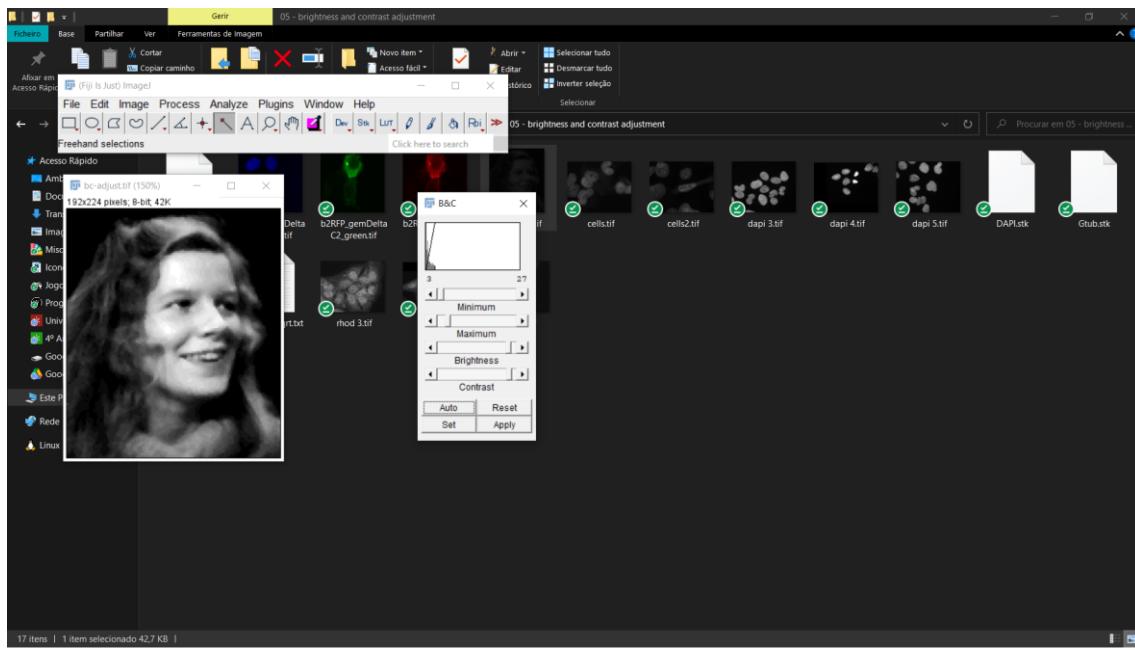
- e) Open the image Kanizsas-triangle.tif. Do you see the white triangle that is standing on its head? Use thresholding and the wand-tool to measure its area.

Visto que o triângulo referido é resultante de uma ilusão de ótica, não consegui calcular a sua área com uso exclusivo do thresholding e da wand-tool, visto que estas ferramentas agrupam pixéis semelhantes, e o triângulo não tem as suas fronteiras definidas.

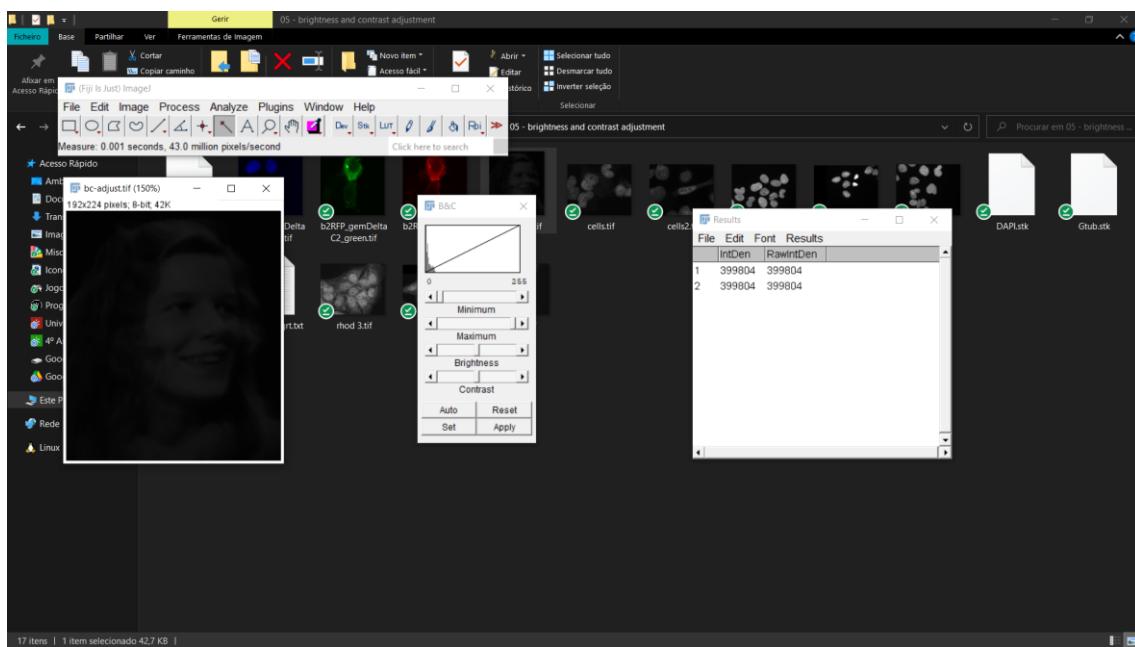
# T5

## T5.1 Linear Display-Adjustments

- a) Open the image bc-adjust.tif from folder 05 - brightness and contrast adjustment. Use the B&C-Adjustment tool (Image>Adjust>Brightness/Contrast) to optimise the display of the image.

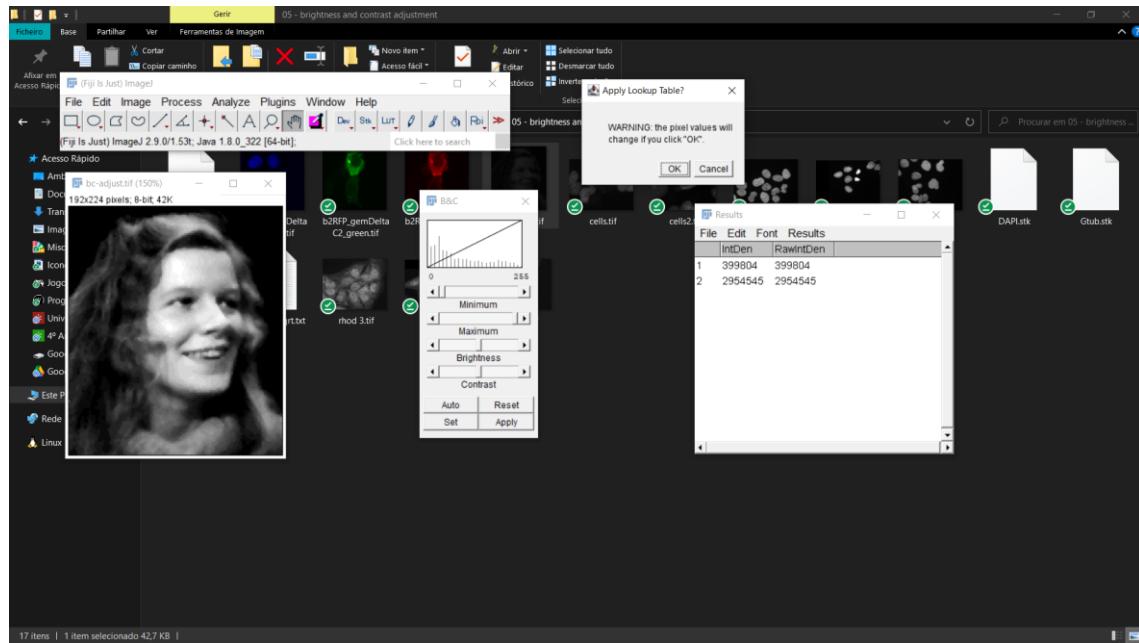


- b) Measure the total intensity (IntDen) in the image (make sure Integrated density is selected under Analyse>Set Measurements). Change the brightness and measure again. Does the measured intensity value change?



A mudança do brilho e do contraste é apenas visual, pelo que o valor da intensidade não é alterada.

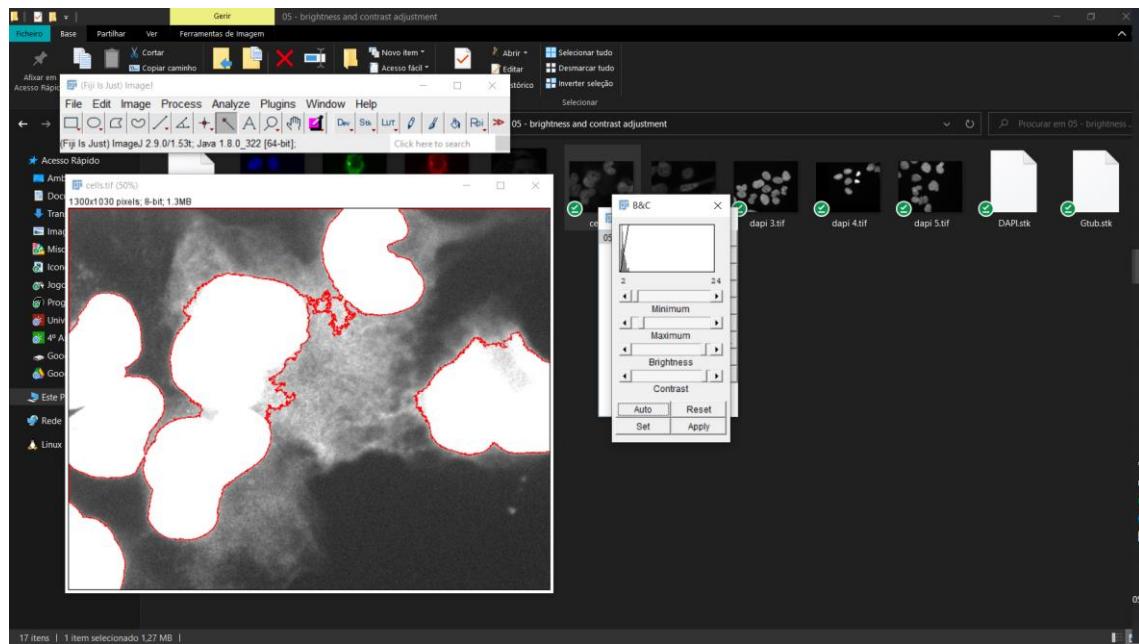
- c) Measure the total intensity (IntDen) in the image. Change the brightness, press the Apply-button and measure again. Does the measured intensity value change?



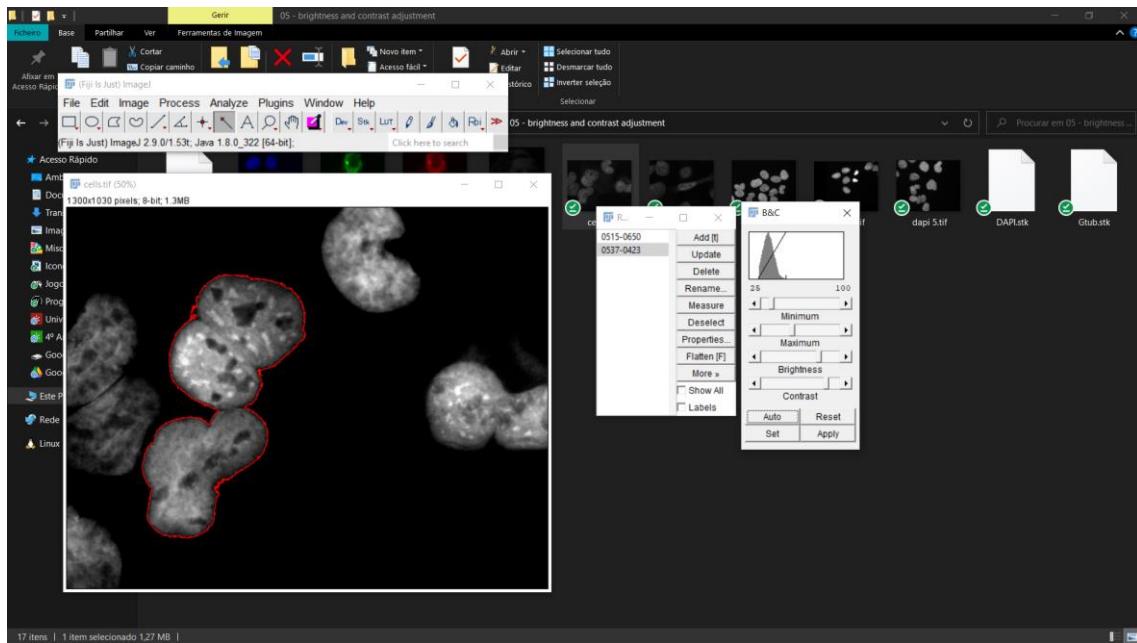
Quando se aplica a alteração do brilho e contraste, os pixéis são alterados, pelo que muda a intensidade.

- d) On the image cells.tif, press the auto-button multiple times. Press the reset button. Make a selection on the background and press the auto-button. Make a selection in the foreground and press the auto-button. What do you observe?

Ao selecionar diversas vezes a opção auto, a imagem fica progressivamente mais clara, até chegar a um ponto que volta ao estado inicial.



Ao selecionar o fundo, o auto foca-se apenas no fundo, realçando os seus detalhes, mesmo que isso implique a perda de detalhes no resto da imagem.

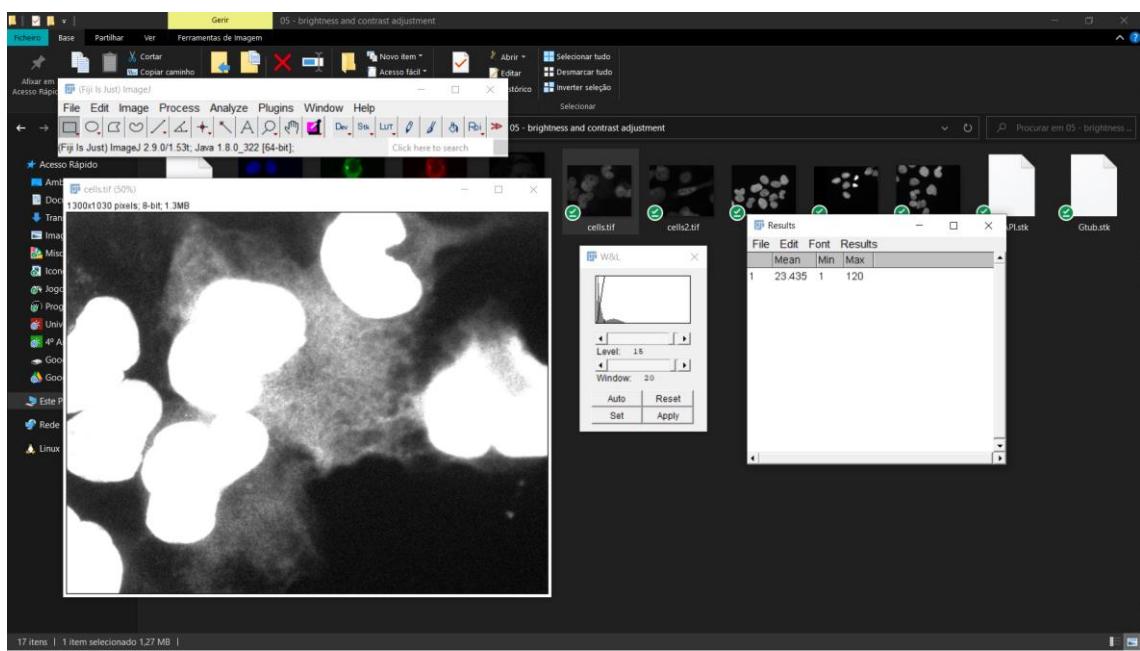


Quando está selecionado uma célula, acontece o inverso, os detalhes da célula são realçados em deterioramento dos detalhes do plano de fundo.

- e) Can you describe what happens to the line that maps the intensity-values in the image to the displayed intensity values, when you move the Brightness-slider and when you move the Contrast-slider?

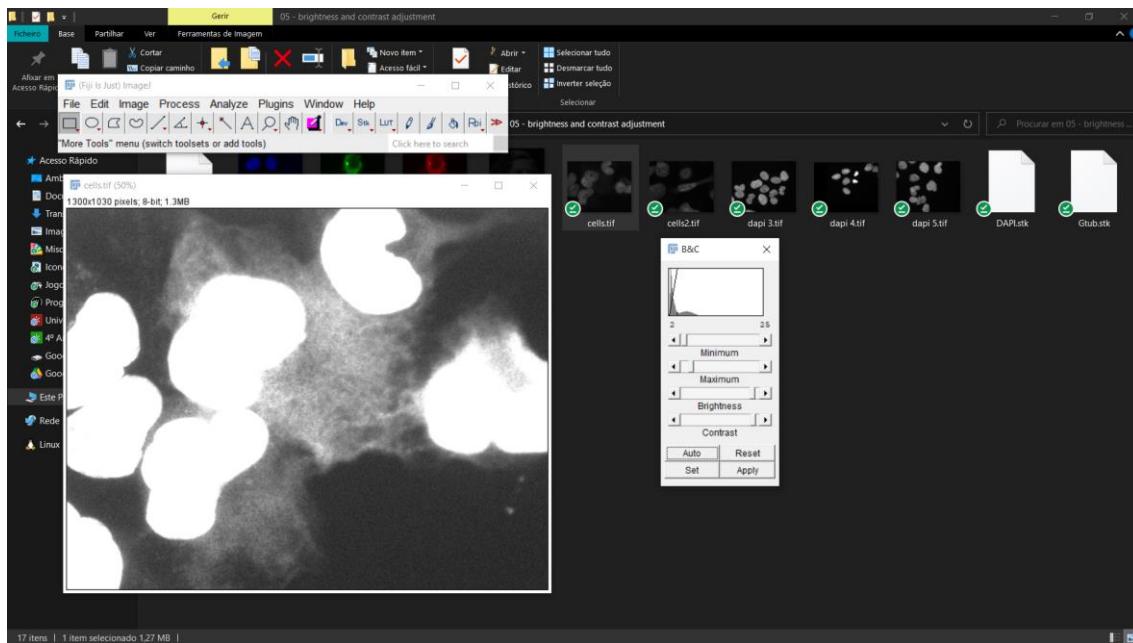
O slider do brilho faz uma translação da linha segundo o eixo do x, ou seja, quando movemos o slider para a direita, a linha move-se para a esquerda. Já o slider do contraste faz com que o declive da reta aumente, de forma diretamente proporcionar (ou aumentam ou diminuem os dois simultaneamente).

- f) Another tool to change the contrast and brightness is the Windows/Level-Adjustment tool. Here you set the middle value and the size of the window around it. If you set the level to 15 and the window to 20, what are the corresponding min. and max. values?

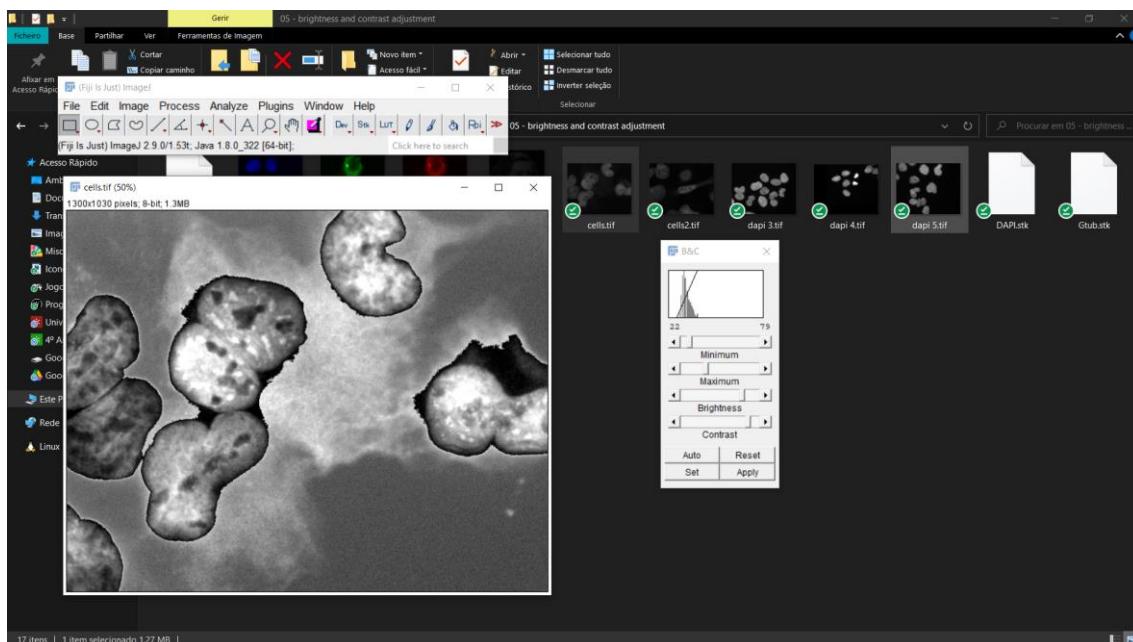


## T5.2 Non-Linear Display-Adjustments

- a) Open the image cells.tif from folder 05 - brightness and contrast adjustment. Try to use the B&C-Adjustment tool to make the intensities in the background visible. What happens to the bright values in the nuclei when you do this? Use Process>Math>Gamma to make a non-linear adjustment. Can you make the background-intensities visible, without saturating the bright spots on the nuclei?

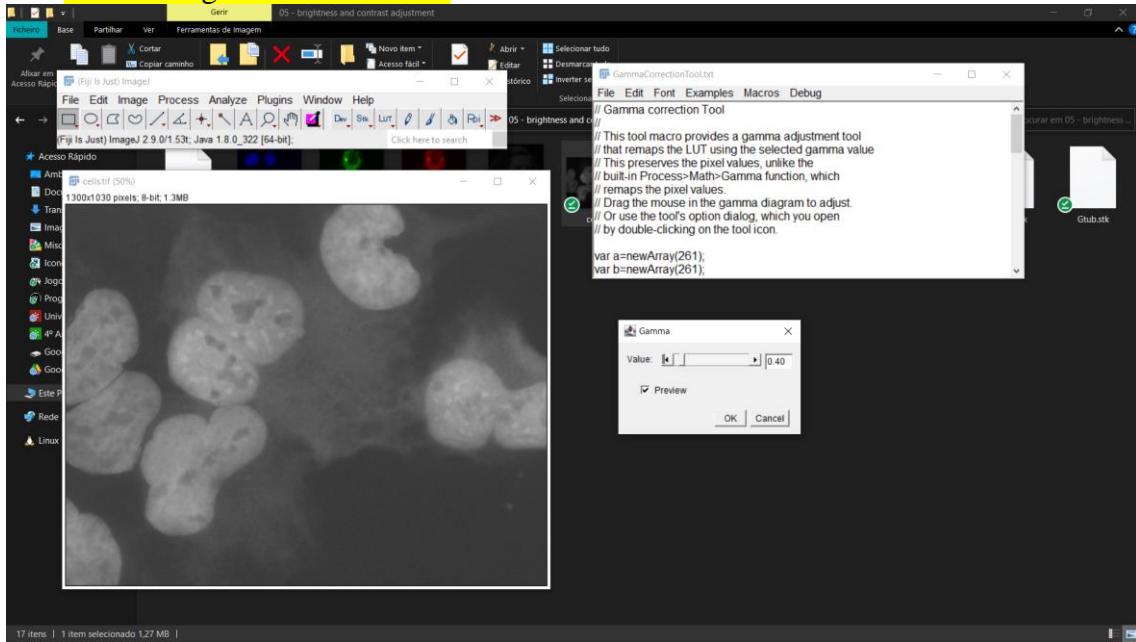


Como se pode ver, ao ajustar-se apenas o brilho e contraste com o B&C, perde-se muitos detalhes nas células. Aplicando primeiro o gamma ao fundo da imagem e depois o B&C à imagem toda, é possível realçar a imagem sem perder muita informação.

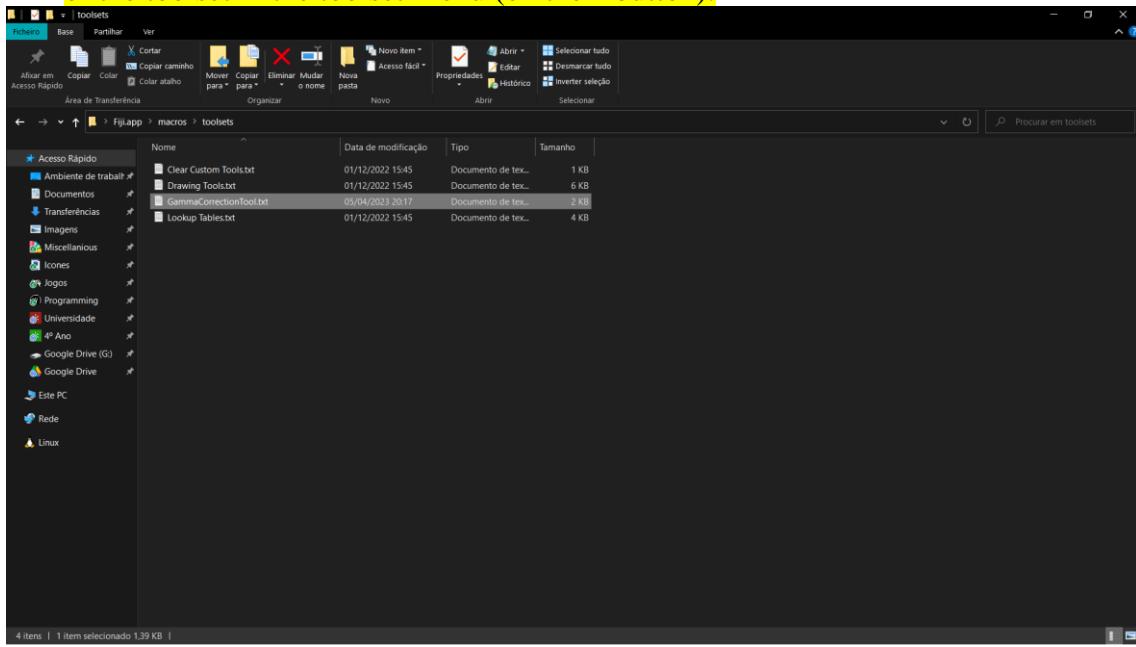


- b) The command Process>Math>Gamma changes the pixel values in the image. You can use the microtool GammaCorrectionTool to modify the display using a gamma-function, without modifying the pixel values. Drag the link from the ImageJ macro-

tools page onto the Image-launcher window. Press the gamma-button and click on the image. Drag to change the gamma value. The function is displayed on the image. Which gamma value gives a good display? Hint: Double-clicking on the gamma-button image with **ctrl+shift+a**.

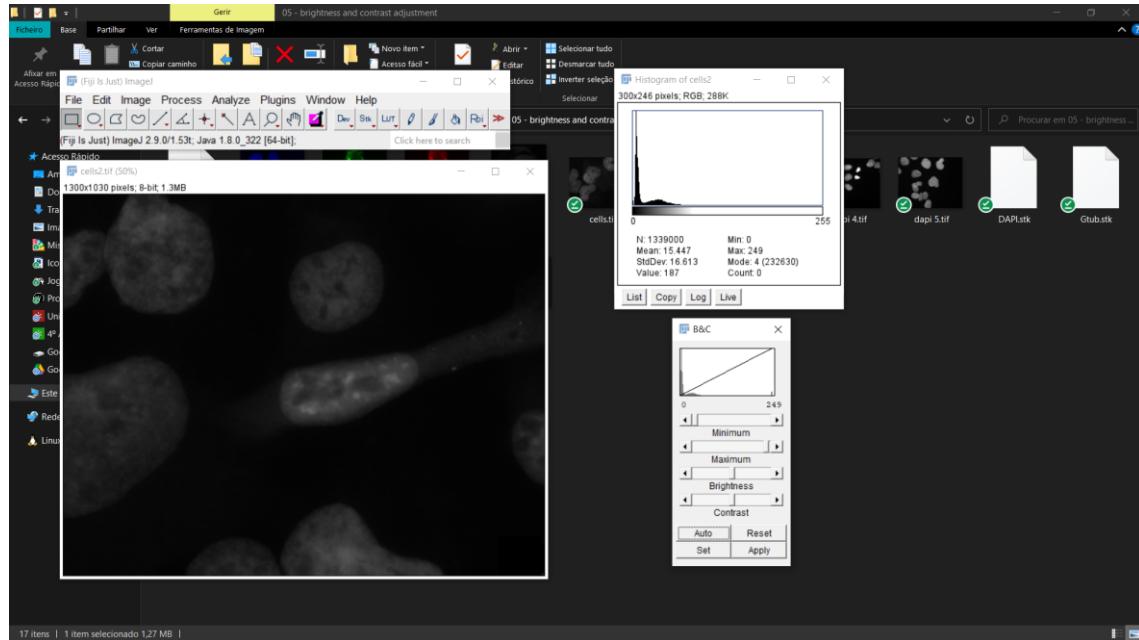


- c) Add the GammaCorrectionTool-macro to your toolsets. To do this you need to save the file into the ImageJ/macros/toolsets folder. The name of the file will be the name of the toolset in the toolset-menu (on the » button).



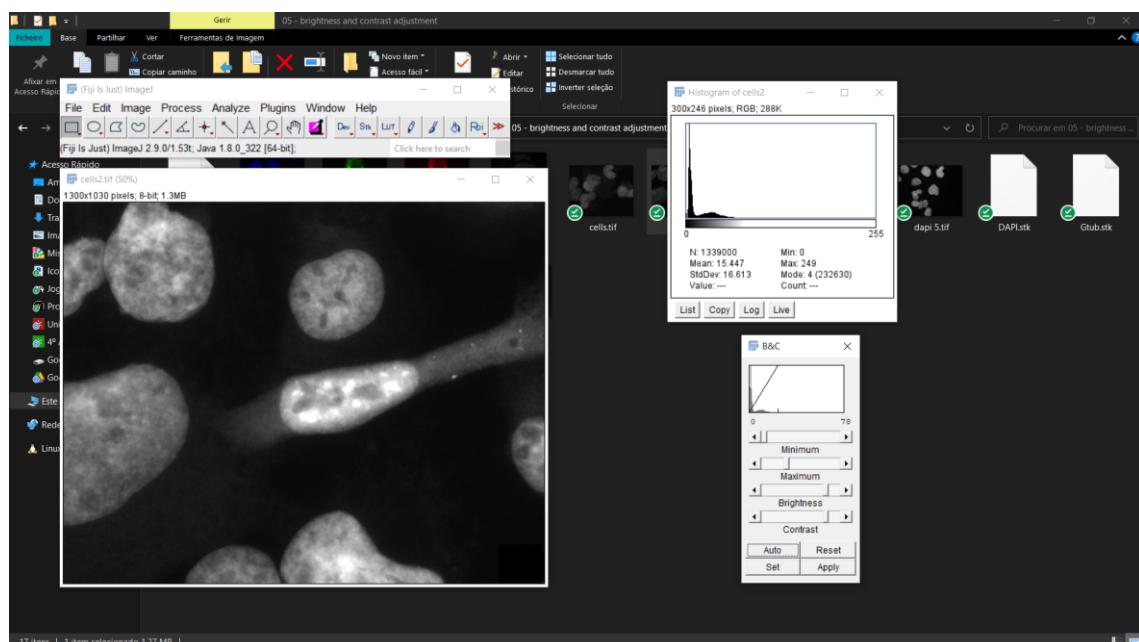
## T5.3 Enhance Contrast

- a) Open the image cells2.tif from folder 05 - brightness and contrast adjustment. Use the histogram to find out the min. and max. intensities in the image. Set the min. and max. values for the display-adjustment to these values (you can use the set button on the B&C adjuster). Is the display appropriate?

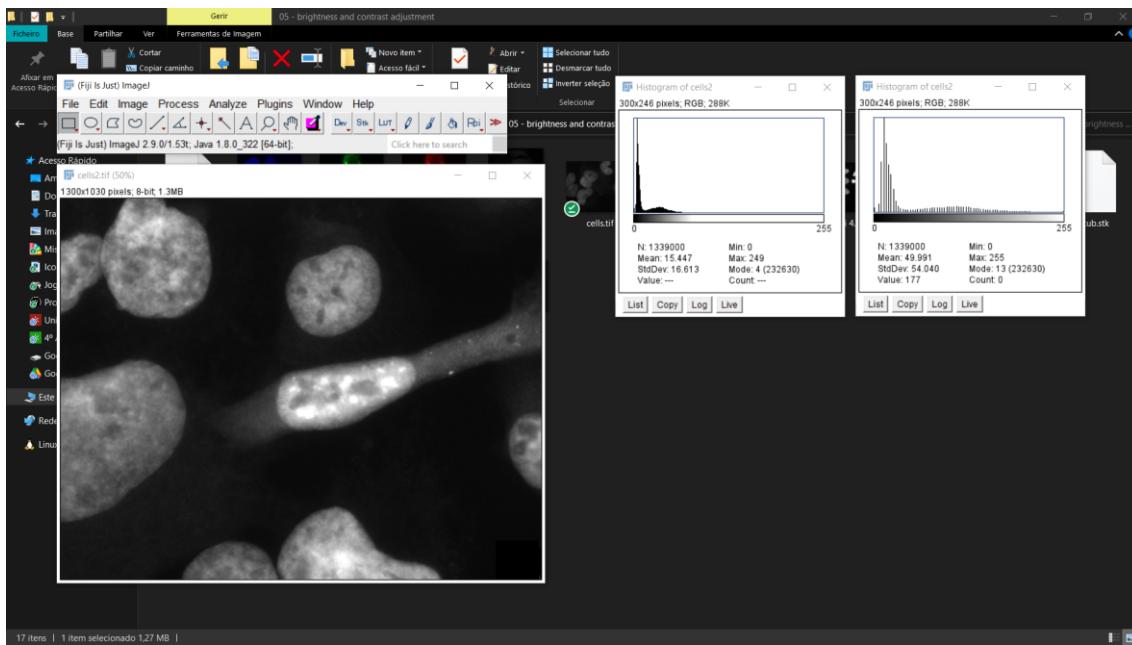


A imagem fica muito escura, de difícil análise.

- b) Run Process>Enhance Contrast and make sure that no option is selected. Enter 0.5% in the Saturated Pixel-field and press ok. What are the min. and max. values that will be set by the tool?

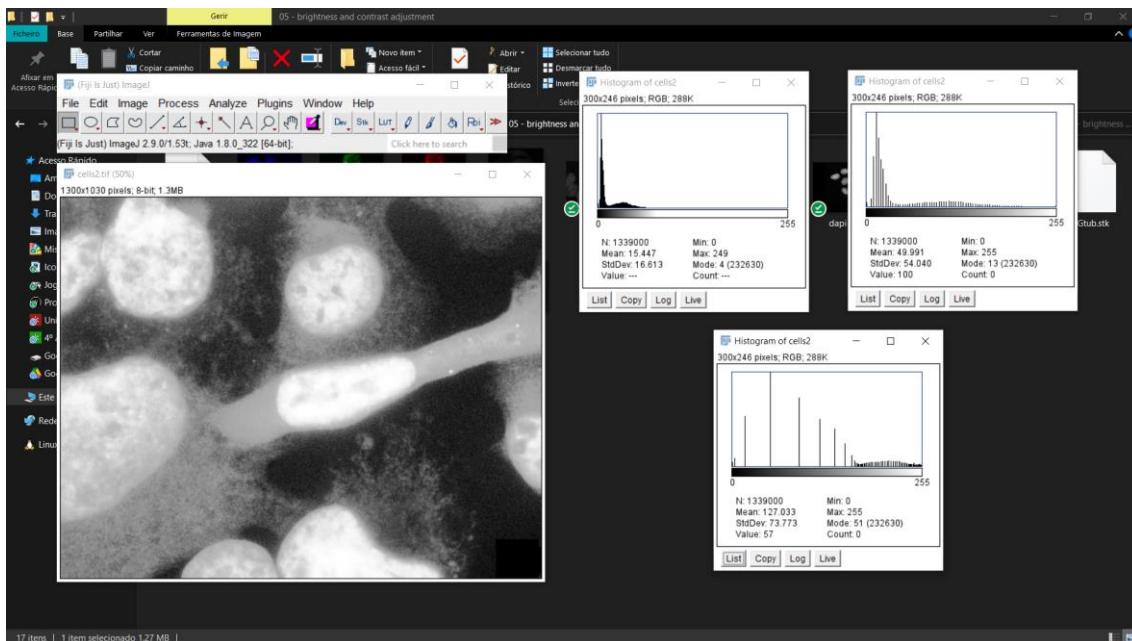


- c) Display the histogram of the image. Run Process>Enhance Contrast again, but this time select the normalize option. Compare the histogram of the resulting image with the histogram of the original image.



Comparando o histograma da esquerda (imagem original) e o da direita (imagem normalizada) verifica-se que a normalização agrupa alguns dos valores originais em certos valores.

- d) Run Process>Enhance Contrast again. This time select Equalize Histogram. Do it again, but hold down the alt key while pressing the ok button. Compare the histograms of the original image, the image after histogram-equalization and the image after histogram-equalization with the alt key pressed.



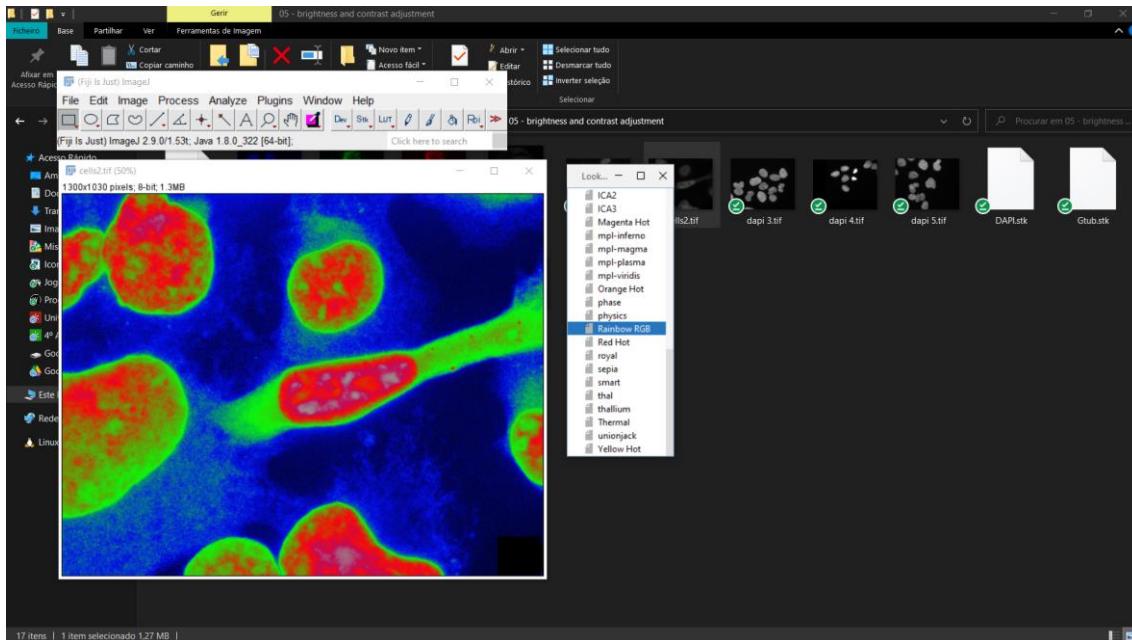
O histograma superior esquerdo é o original, o superior direito o segundo, com a equalização, e o de baixo o que tem o alt pressionado. Verifica-se que os dois primeiros

são semelhantes aos analisados na alínea anterior, mas o último te maior espaçamento nas colunas maiores.

- e) (OPTIONAL) Histogram-equalization calculates a normalized, cumulative histogram, i.e. each histogram value is replaced by the sum of the values up to this value and the values are normalized to the available range of grey values. Each grey value in the image is replaced by its value in the normalized, cumulative histogram.

## T5.4 Lookup-Tables

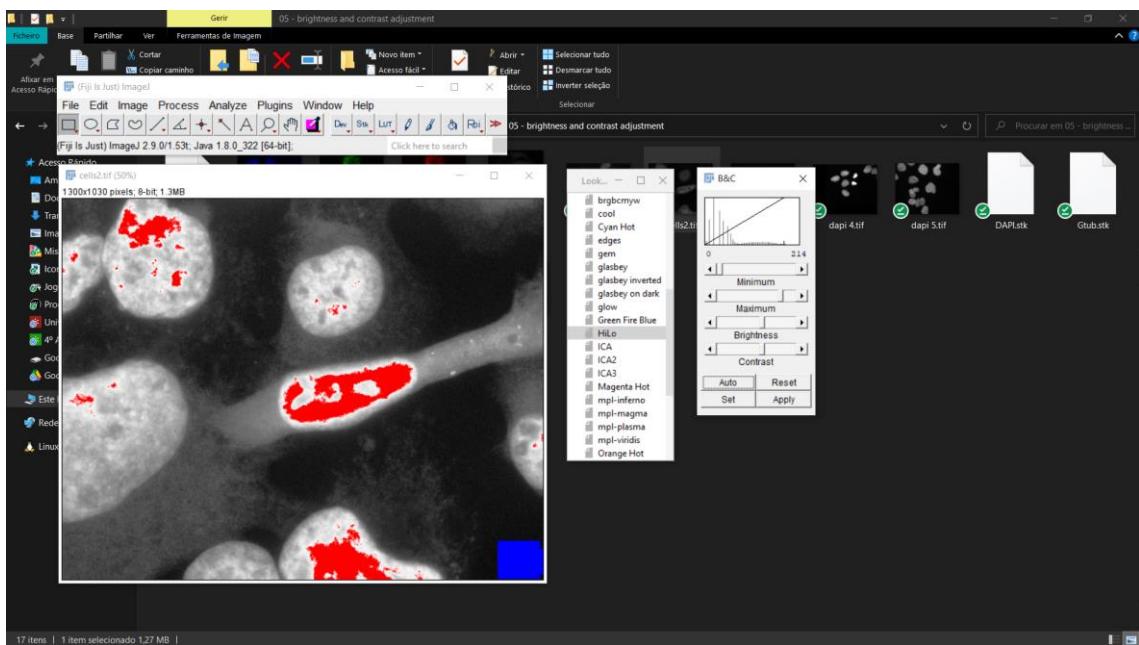
- a) Open the Control Panel from Plugins>Utilities>Control Panel (ctrl+shift+u). In the control panel go to Image>Lookup Tables and tear this menu off. Close the parent menu. Make sure that no image is opened. Click on the different lookup-tables. An image showing the colours for the 255 intensity values is displayed. Now open the image cells2.tif, do a histogram-equalization and apply the lookup-tables to it, by clicking on the lookup-tables again.



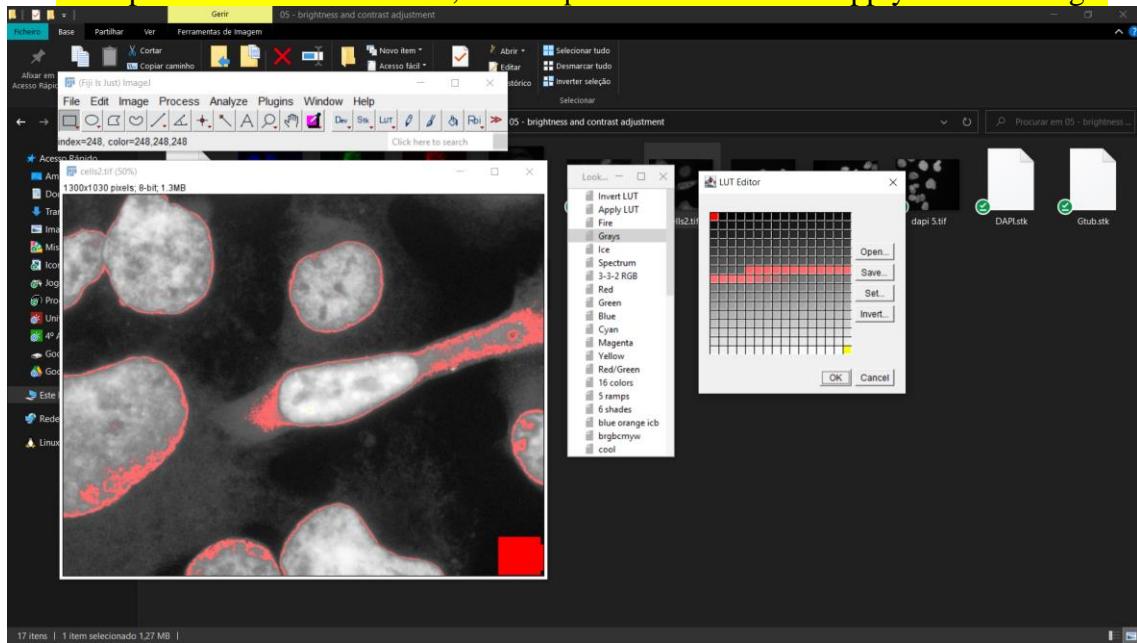
- b) Open the Jet lut. Can you tell in which colour the intensity value 200 is displayed? What are the RGB components of this colour (use the list button on Image>Color>Show Lut)?

Não tem a Jet LUT.

- c) The lookup table HiLo is useful to adjust the display of our image. 0 will be displayed in blue, 255 in red and values in between will be displayed in grey. Have a look at the HiLo lookup-table. Use it in combination with the b&c-adjustment tool to optimize the display of the image cells2.tif.

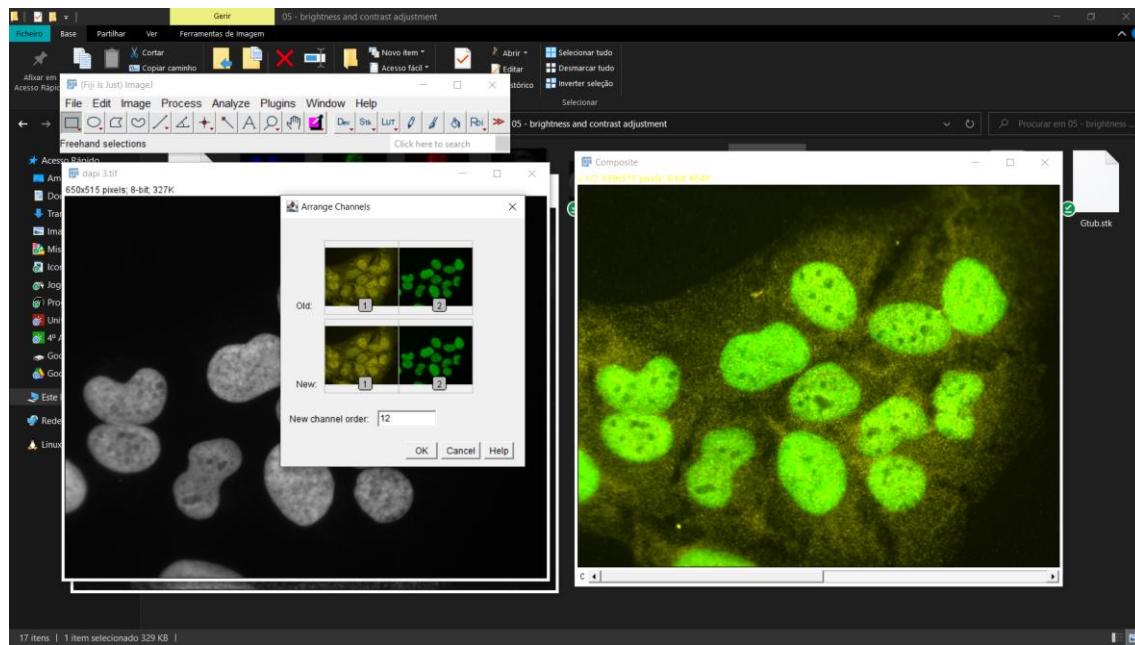
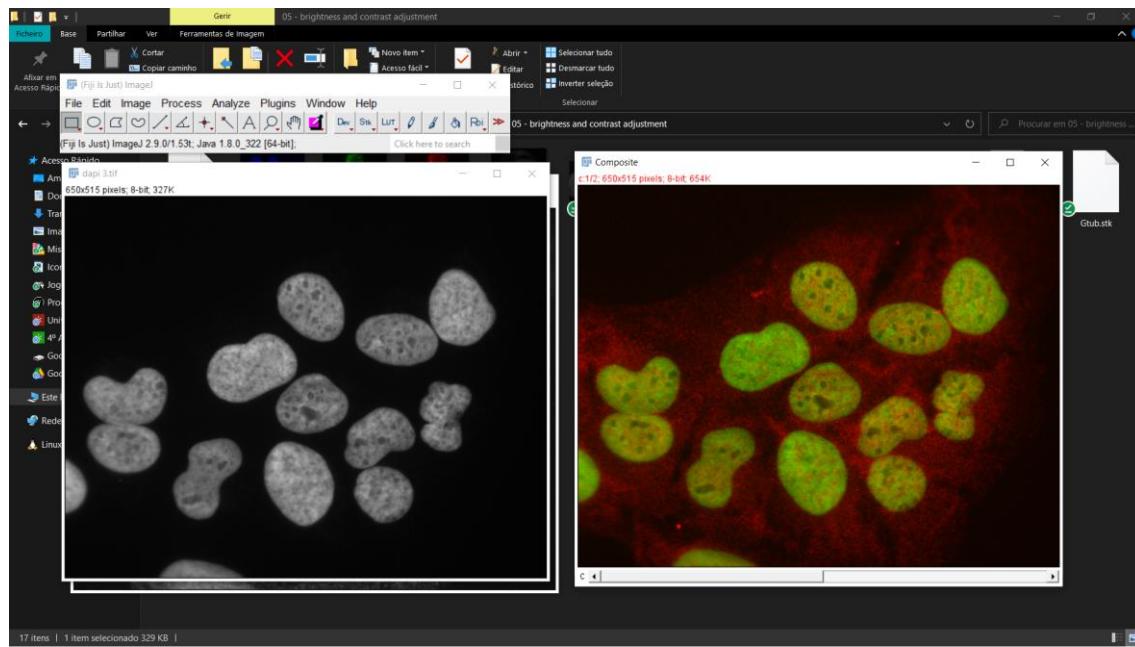


- d) Select the grey-lookup table. Use the lut-editor from Image>Color>Edit>Lut to create a lookup table that displays 0 in green, 255 in yellow and the values between 100 and 120 in different shades of red, becoming lighter with higher intensity. Save the lookup-table into the lut-folder, call Help>Refresh Menus and apply it to the image.

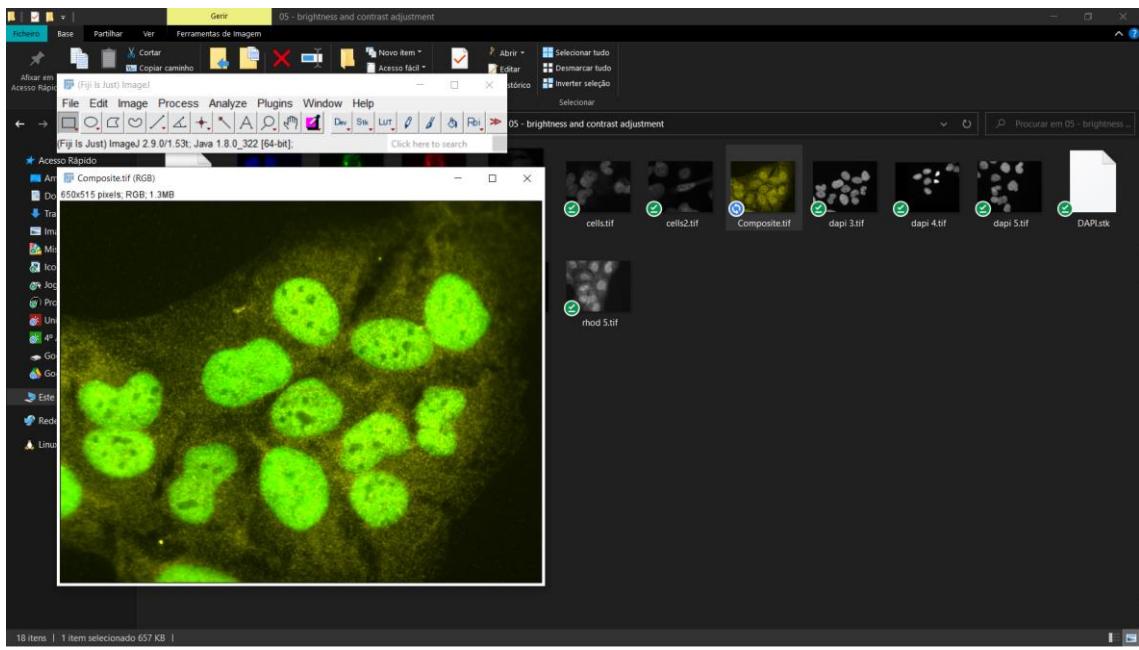


## T5.5 Overlay Of Multiple Channels

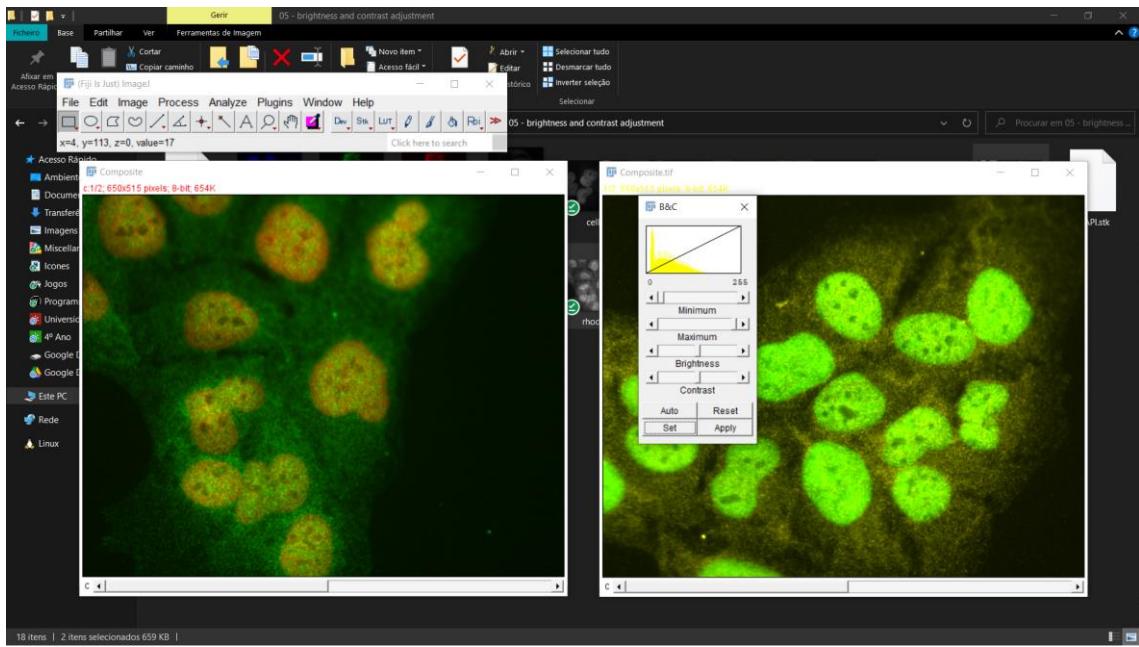
- a) Open the images dapi 3.tif and rhod 3.tif. Run the Merge Channels... command from the menu Image>Color. Try different lookup tables to change the colours of the two channels. Use the Channels tool from Image>Color>Channel Tool... to switch channels on and off. Use the B&C-Adjuster to optimize the display of the two channels independently.



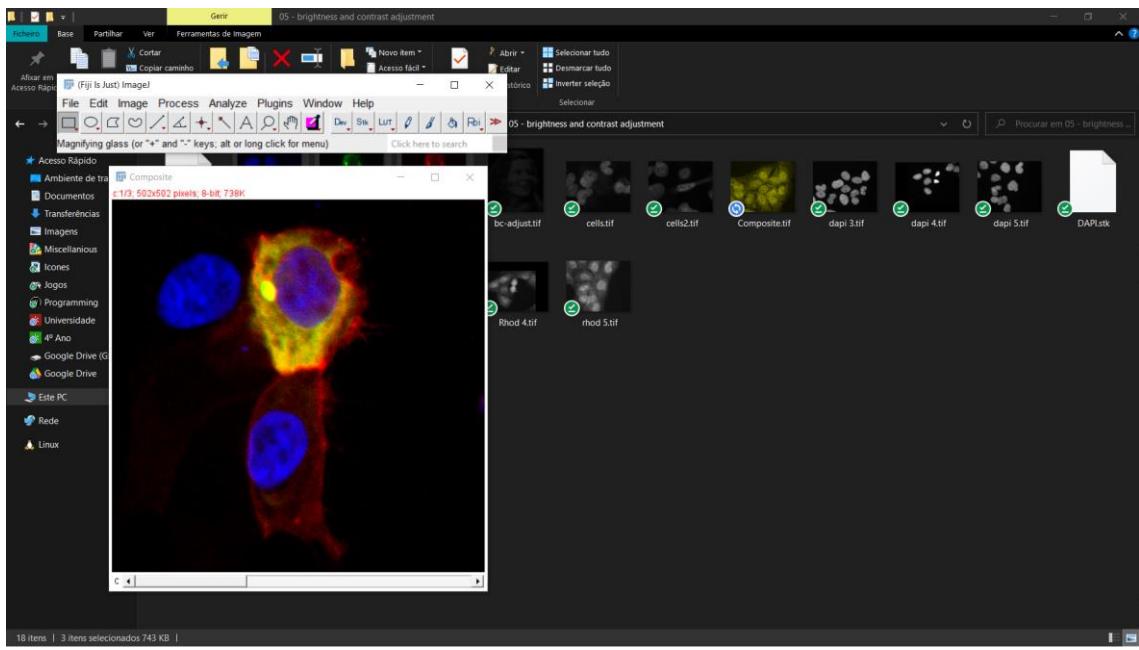
- b) Save the hyperstack as tif-image. Close it and load it again. Create an RGB-snapshot, using the command Convert to RGB from the more-button of the Channels tool.



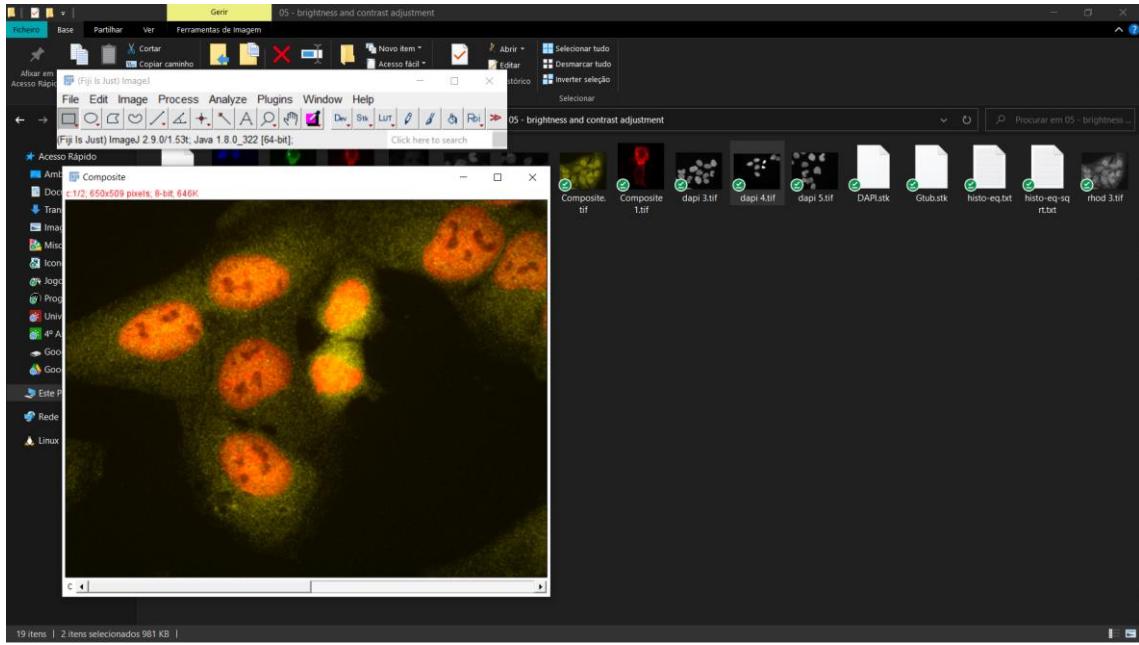
- c) Create a second hyperstack from the images dapi 5 and rhod 5. Transfer the display settings from the first hyperstack (dapi and rhod 3) to the second by using the propagate functionality of the set command on the b&c adjuster.



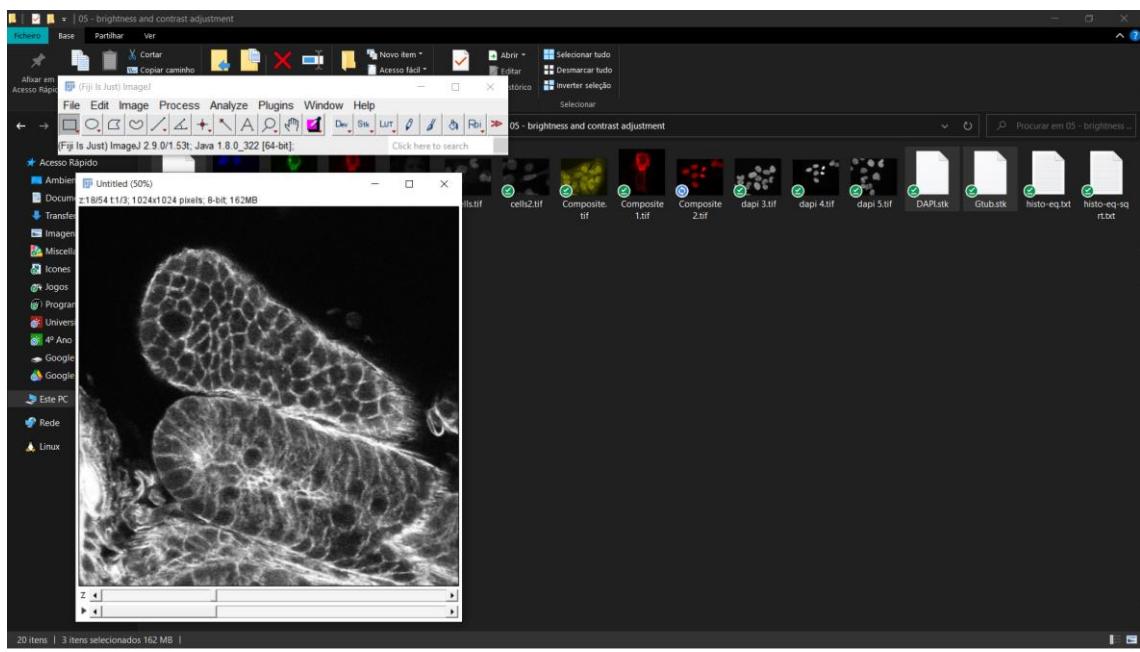
- d) Create an overlay from the three images b2RFP\_gemDeltaC2\_blue.tif, b2RFP\_gemDeltaC2\_green.tif and b2RFP\_gemDeltaC2\_red.tif and adjust the display.



- e) Create an overlay of the images dapi 4.tif and Rhod 4.tif. Correct the alignment by using select all (ctrl+a), cut (ctrl+x) and paste (ctrl+v). If the rectangular-selection tool is selected you can now move the pasted channel. After the correction, crop the image to get rid of the empty area.



- f) Open the three images Actine.stk, DAPI.stk and Gtub.stk. Concatenate the three stacks with Image>Stacks>Tools>Concatenate. Use Image>Hyperstacks>Stack to Hyperstack... to create a composite image. Be careful to select the right order of dimensions (xyzct).



g) Make a z-projection of the result of step

