Updated 2024-10-01 09:49:35

Before starting

DOWNLOAD

- **GIMP**

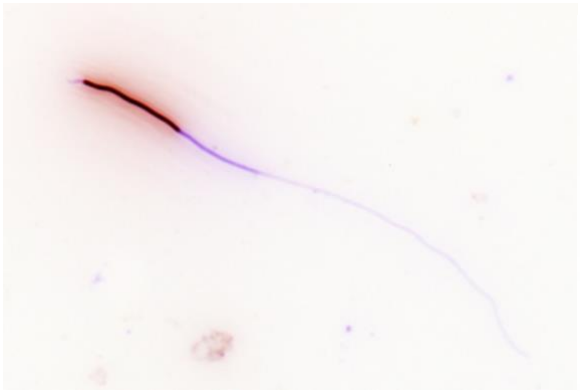
- **Photoscape** X

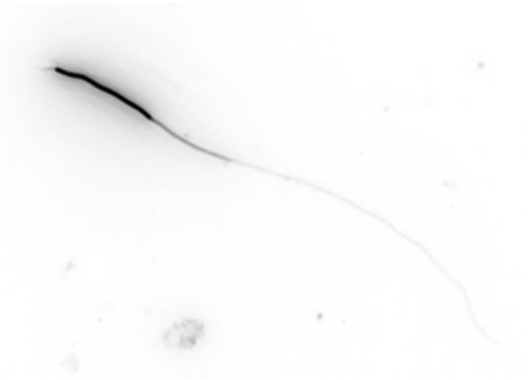
- **SpermSizer** from GitHub, read the instructions there and **before starting** for the first time, copy the modified config.ini into the folder with the program's file (exe or jar) or simply modify the config.ini file so that its variables have the following values:

```
MarkerColor=#FF0000
MarkerSize=3
LineColors=#FFFFFF
LineThickness=2
Labels=Pixels
LabelColor=#000000
LabelSize=22
LineSmoothing=4
SnapEnabled=true
TrimRadius=8
CropPadding=100
CompressCroppedImages=true
```

- file manager like freeCommander, fman helpful for copying/moving images during sperm measurements, but not necessary

CHECK whether you can see the tail of the following picture (also shown below) on your screen; if not adjust the screen brightness and contrast accordingly.





HELPFUL INFO:

SpermSizer uses the following **control keys**

Select	Pan	Zoom	Undo	Redo	Next Image	Previous Image
Left Click	Drag with Right Click	Scroll Wheel	A	D	S	W

SpermSizer displays a modified image. If you modify the original picture (that is already loaded in SpermSizer) and save it with the same name (overwrite it), the modified image will not show, but if you click on a different image in

SpermSizer and then again on the "newly" loaded one (that has the same name as the initial one), the modified picture will appear. The Measuring protocol (below) makes use of this feature when dirt or unclear background disturbs the measurements.

Shortcuts for GIMP: Save and overwrite and image – **assign this shortcut** by going to Edit -> Keyboard shortcuts (on Mac to GIMP-2 -> Keyboard shortcuts), typing overwrite in the search box, placing a cursor in the shortcut field and press ctrl+o, Close.

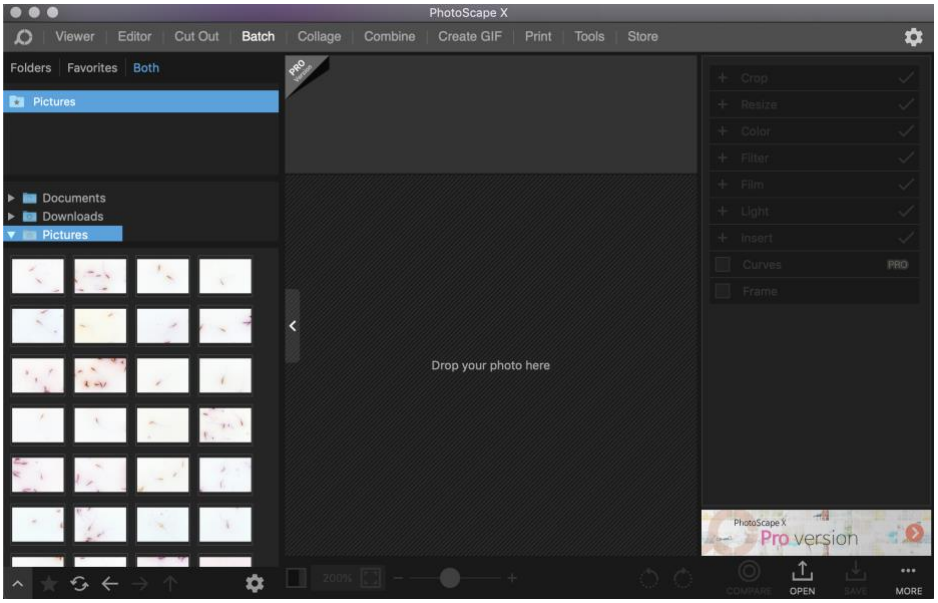
Save as – ctrl+shift+E on PC, shift+command +E on Mac

General procedure for measuring Ruff sperm with Sperm Sizer

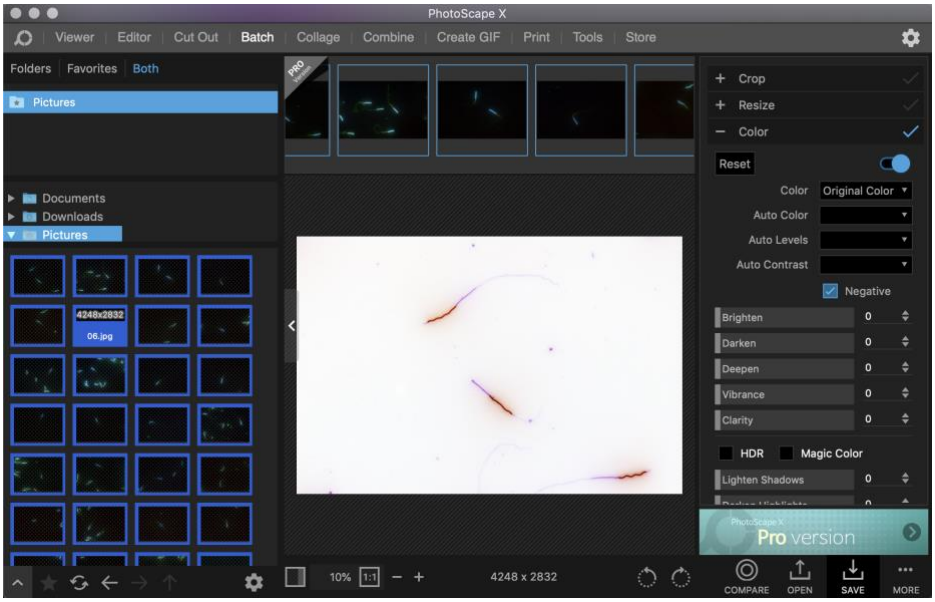
- Create 'inverted' folder and save there all inverted pictures (for how to invert see below).
- Choose the best 10 pictures to measure by deleting the rest and/or cutting images having more than one usable sperm cell and renaming those by adding a,b,c . Select the first 10 best non-aberrant sperm that meet the following criteria:
  1. sperm free of debris or contact with other sperm
  2. sperm complete – tails not broken off - recognized by a blunt end (broken) rather than a tapered tip
  3. all parts of the sperm clearly visible and easy to distinguish
- Randomize and rename the pictures into random_inv folder.
- Desaturate the pictures in the random_inv folder and save those in the random_des folder (for how to desaturate see below) – keep the image name as was.
- To measure the images with Sperm Sizer, in a 'sperm_morpho' folder create a folder with the date and your initials (like 2021-10-13_MB) and with subfolders 'measure', random_inv', and 'random_des'.
- Copy the 'to be measured batch' of 'inv' and 'des' pictures into the respective folders. Because you start measuring on the 'inv' pictures copy those also to 'measure' folder.
- You will measure acrosome, nucleus and midpiece on the 'inv' pictures for the whole batch. Then you will overwrite these pics in the 'measure' folder with those from random_des folder and measure the tail on the desaturated images.
- **In case you** need to **erase** a part of an image **to aid the measurements**, you overwrite the original image, measure on the overwritten erased image and then **save the image with the '_er' extension** (like: 0040**_inv_era**.jpeg if inverted image was used for erasing, 0040**_inv_des_er**.jpeg if inverted and desaturated image was used or if all images use same modification and such indication is redundant 0040_era.jpeg).
- **If separate erasing is needed for various parts**, e.g. acrosome and tail, you save each image separately **erA** referring to acrosome**, erN** to nucleus, **erM** to midpiece and **erT** to tail (like: 0040**_inv_erA**.jpeg if inverted image was used for acrosome specific erasing, 0040**_inv_des_erT**.jpeg if inverted desaturated image was used for tail specific erasing)
- Before exporting the measurements overwrite the greyscale or erased images with the inverted only images (those from random_inv).
- When exporting the Sperm Sizer measurements, save those also within the current folder (in this example: 2021-10-13_MB).

Invert images

Open Photoscape X, go to Batch edit and drag and drop all the pictures you wish to invert.



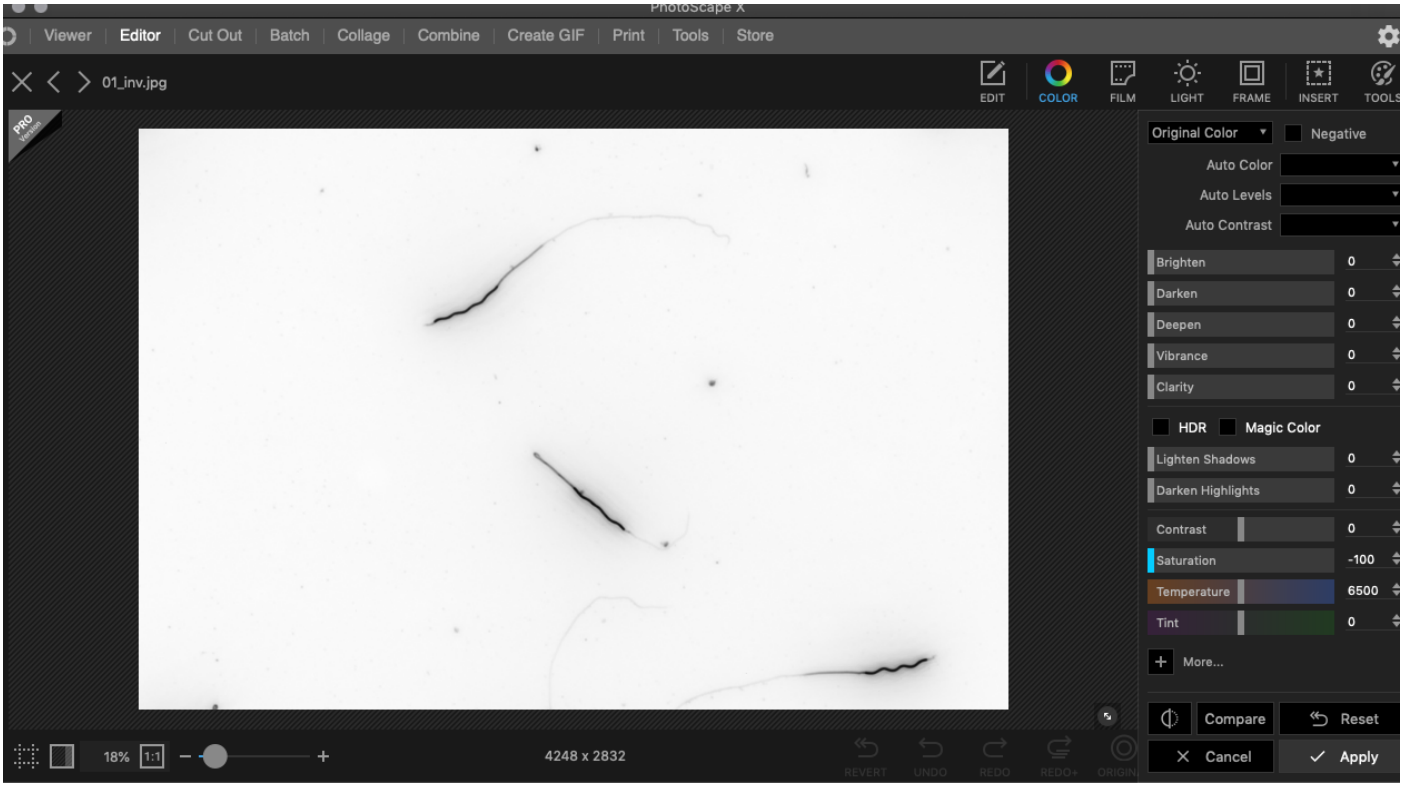
Go to the 'Color' tab on the right and tick the box next to negative.



Press 'SAVE'. Choose JPEG Quality 100, folder 'inverted', Suffix '_inv' and press OK. The inverted folder is used to randomize and anonymize the images for measurement. Such images are in random_inv folder.

Desaturate images

Open Photoscape X, go to Batch edit and drag and drop all the inverted images from random_inv folder you wish to desaturate. Go to 'Color' tab on the right, if ticked, untick the box next to the 'negative', and slide the Saturation bar (turquoise on the below picture) to the left (-100).

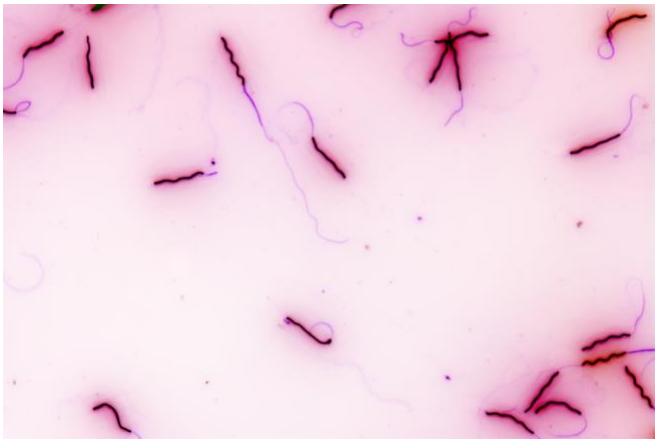


Press 'SAVE', use folder ranomd_des, and press OK.

Choose images for SpermSizer measurements

(a) It is best to use images with a clean background, with a single sperm (or other sperm far away), and a sperm cell that is +/- straight and not glowing much (see test image 38 - ruff 1386 2021-07-12 Snap-1509.jpg - below). It is helpful to choose the images from the inverted ones.

Example of poor images (glowing, with colorful background and those where sperm cells are close to each other):



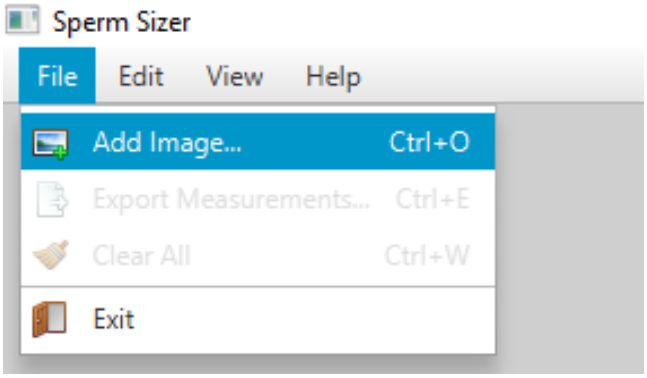
(b) **If plenty of sperm is available for a given male**, use the best (see a-b) and ideally where tail and midpiece are distinguishable.

(c) **If more sperm cells are on one image**, cut single sperm cells out so that you can measure those on separate images because that is the only way to attribute each part of the sperm to a specific sperm cell. The cutting out of the sperm can be done in GIMP using the Rectangle select tool, selecting the cell and cropping to selection by right-clicking, choosing Image and Crop to selection, pressing shift + command + E on mac (ctrl + shift + E) or going to Export and export the image while adding 'a' to the end of ist name. Do the same for the other sperm cells on the picture naming those b, c, d, etc.

Measure the sperm with Sperm Sizer

LOADING IMAGES

Once the program is on, **add** as many **images** as you want to measure to the Sperm Sizer.

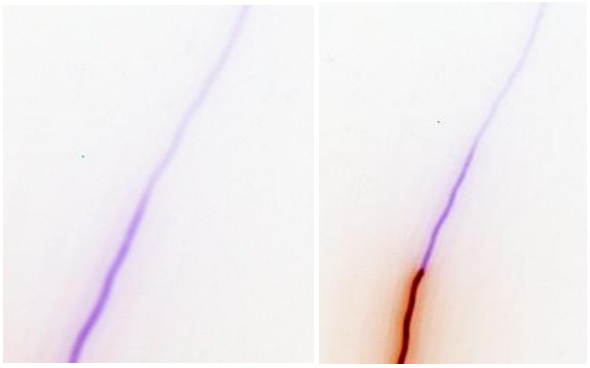


MEASUREMENTS

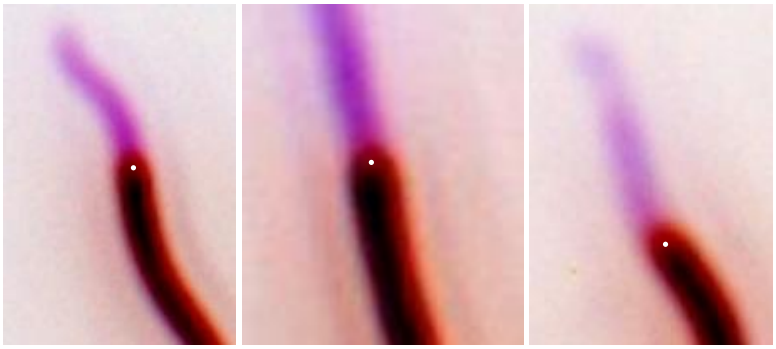
Each part of the sperm is measured separately and is enclosed by the two dots you place on the sperm. The program calculates the distance in pixels as soon as you place the second dot. If the acrosome line is faded at the start, you can help SpermSizer by dragging a line from the faded start until the acrosome is easier to see and then making the dot where the acrosome ends (see GitHub example of this straight line extension). The same can be done with the faded tail, but in reverse order, i.e. you place a dot where the tail starts and then drag the line from where the tail is clear until the end where it fades.

It is essential to keep the order of the measurements: Acrosome, Nucleus, Midpiece, Tail, albeit Acrosome, Nucleus, Midpiece can be measured in one batch of inverted images and Tail in the desaturated images, with which the initial images are overwritten.

If the divide between the midpiece and tail is unclear, it helps to zoom out.

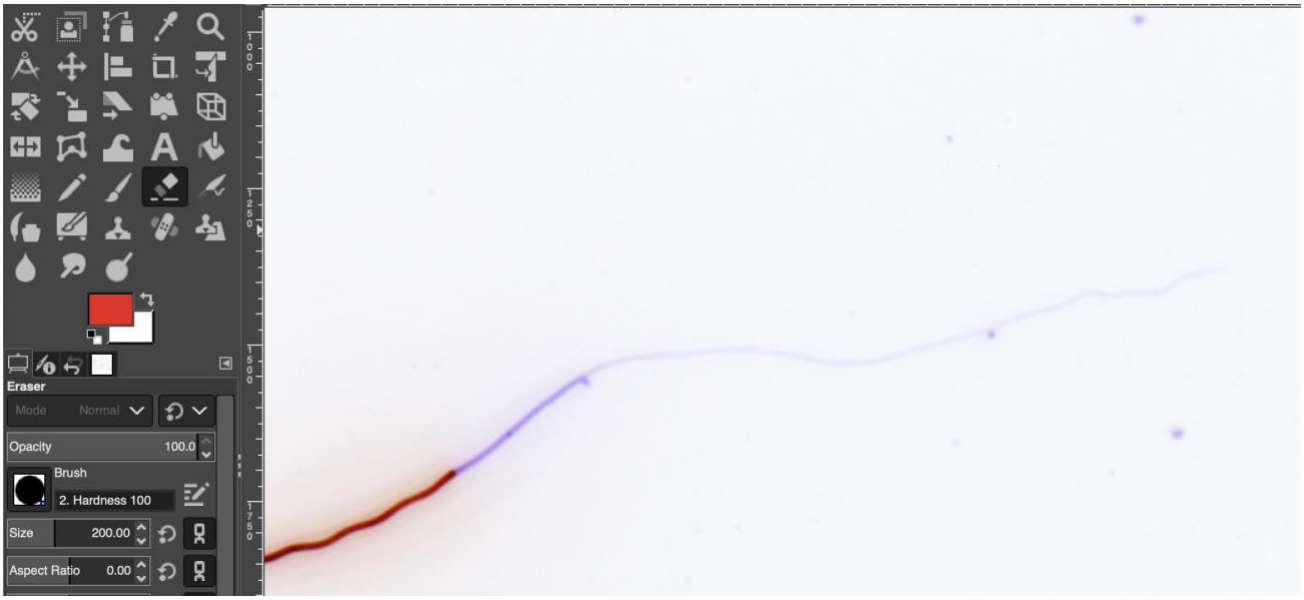


If sperm parts are glowing (see pics 2,3, 5, 10, 14, 16, 35 here), **go for the midpoint between the glow and the dark part.**



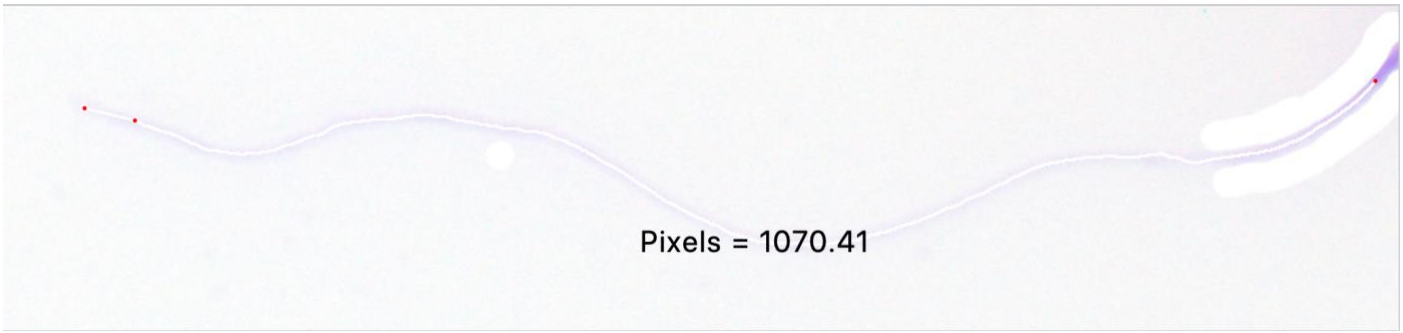
IN CASE A MEASUREMENT (usually of tail) FAILS or PROGRAM TRACKS A PART WRONGLY

The tail may fail to be picked up by the Sperm Sizer program, or big object adjacent to the sperm (another sperm or some background dirt) induces the program to create a wrong outline of the shape of the sperm. In those cases, undo the last point defining the end of the Tail and try again (even several times or with a longer final line). If the tries fail, click on a previous image in SpermSizer and then open the image of interest in GIMP. Choose Eraser Toolset the Size to 200 and erase the area around the tail (for fine details – if needed – use eraser Size 20-50). You may need to zoom in with + (or mouse; on mac hold Command and use trackpad).



Once done, press ctrl+o, you will be prompted to overwrite the image which you confirm. Go back to SpermSizer click on the image of interest and the erased image will be loaded while your measurements of previous parts remain intact. Place the dot where the Tail ends and the Tail will be measured. Press on a previous image, then return to GIMP and press export (or use the shortcut – ctr+shift_E on PC, shift+command+E on Mac) to export the modified image. Save it with the '_era' suffix (or 'gre_era' if erasing was done on a greyscale image, 'des_era' if done on desaturated image), which will allow us to backtrack, which images were measured on modified images and if needed to identify the actual modified image. Then copy the same image from the 'randomized_inv' folder to the 'measuring' folder. This will overwrite the erased image with the original one so that the SpermSizer exported pictures show the actual sperm and not the erased parts. Go to SpermSizer, click on the image you have just measured and the original picture will appear. Continue with the next image.

Albeit in the previous example the whole area around the tail is erased, erasing only most glowing/dirty parts is often enough.



The aim is to remove unnecessary glow, which can be caused even by other cells (see the SpermSizer tracking below, before and after the glow is removed).

You can use this method to improve the measurements of all other parts as well (e.g. if part of the midpiece is not being picked up because of a dirt nearby).

EXPORTING THE MEASUREMENTS

Once you finished measuring, press “Export measurements”, which will save the measurements and pictures of measurements into a folder containing the date in its name. Rename the folder by adding your initial (e.g. _MB for Martin Bulla) at the end.