DL_Track_US

v0.2.1

Preface

Welcome to the DL_Track_US python package tutorial. In the next roughly 80 pages, you will learn how to automatically and manually analyse ultrasonography images and videos of human lower limb muscles. You will do so by making extensive use of the graphical user interface provided by the in the DL_Track_US package. Moreover, you will learn how to train your own neural networks using the graphical user interface as well. Have fun!

Please note that we updated the GUI from version 0.1.2 to version 0.2.1. Although it might look different, the core functionalities are the same. This is why we did not update the complete tutorial. However, we explain the new functionalities in detail.

Content

Good to know

All relevant instructions and guidelines for the installation of the DL_Track_US software package are described in our documentation, so please take a look there if anything is unclear. We have also provided information on what to do when you encounter problems during the installation process, encounter errors during the analysis process that are not caught by the GUI (no error message pop ups and advises you what to do), if you want to contribute to the DL_Track_US software package, and how you can reach us.

Before we start with this tutorial, here are some important tips:

- Test the algorithm first and train your own models if necessary, especially if you plan to analyze images taken from different muscles.
- Be cautious about the generalizability of the models, even though extensive data augmentation was used during the model training process. Different device types, muscle regions, and settings during image acquisition may impact model performance.
- Image quality is crucial. The images should have good contrast, appropriate brightness, clearly visible fascicles and aponeuroses, and clear alignment of the probe with the fascicle plane.
- If model performance is poor, visually inspect the output of the models and compare them to manual analysis results. Adjust analysis parameters or train a separate model if necessary.
- Follow the provided testing procedures in the DL_Track_US/tests folder to ensure proper functionality on your computer.

Starting the GUI

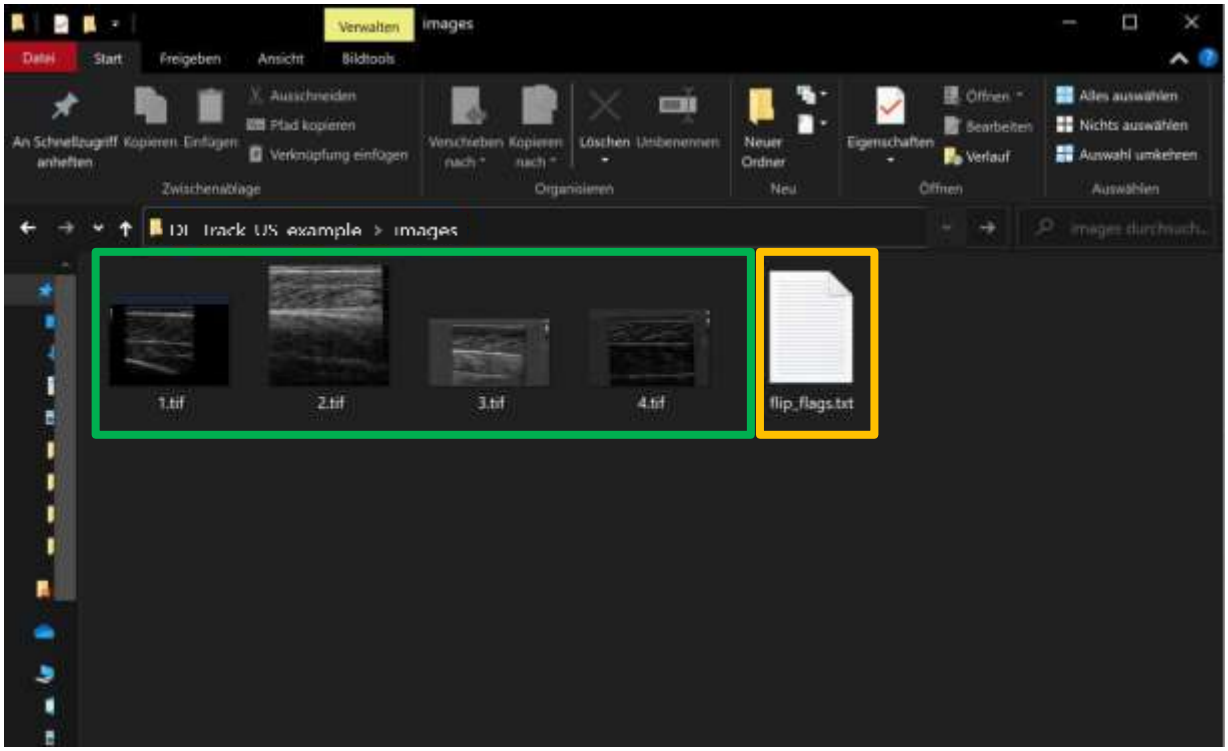
For installation of DL_Track_US and starting the graphical user interface we refer you to the online documenation. In the installation chapter, all different possibilities for installing DL_Track_US and starting the GUI are described.

Automated Image Analysis

The first analysis type this tutorial covers is the automated image analysis. The images are evaluated without user input and may be scaled. Scaling the images will ensure estimated muscle architectural parameters are converted to centimetre units. For this type of analysis, single images (not videos) are a prerequisite. These images should be contained in a single folder, like in the "DL_Track_US_example/images" folder. If you haven't downloaded this folder, please do so now (link: DL_Track_US - Examples & Models | Zenodo). Unzip the folder and put it somewhere accessible. We will make use of the included example files extensively during this tutorial. In the next few pages, we will look at every required step to successfully perform automated image analysis with DL_Track_US.

1. Creating Image Directory & FlipFlag.txt File

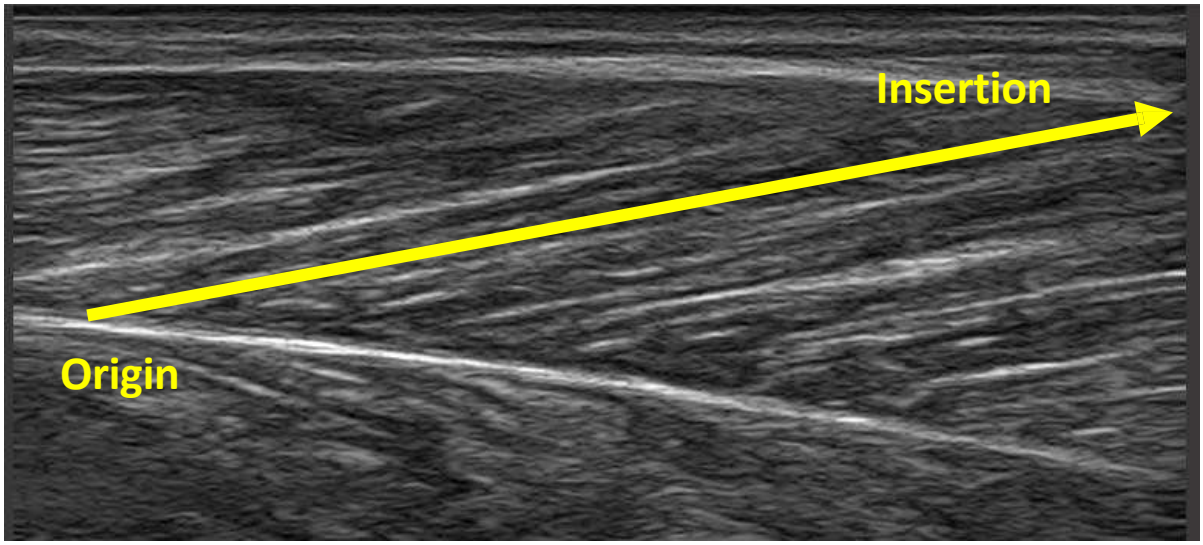
- All **images** you want to analyze shoule be placed in one folder.



- The “DL_Track_US/images“ folder contains **4 images** and a **flip_flag.txt** file. It is not required to have the **flip_flag.txt** file in the same folder as the **images** to be analysed, but it is convenient.
- Lets take a closer look at the **flip_flags.txt** file. Below you can see an the **flip_flag.txt** file in the directory.

- For every image there must be a **flip-flag**. If the number of **flip-flags** and **images** doesn't match, an error is raised.
- Another possible way to specify is displayed below. This is relevant when multiple subfolders are included, as each line then represents a subfolder.

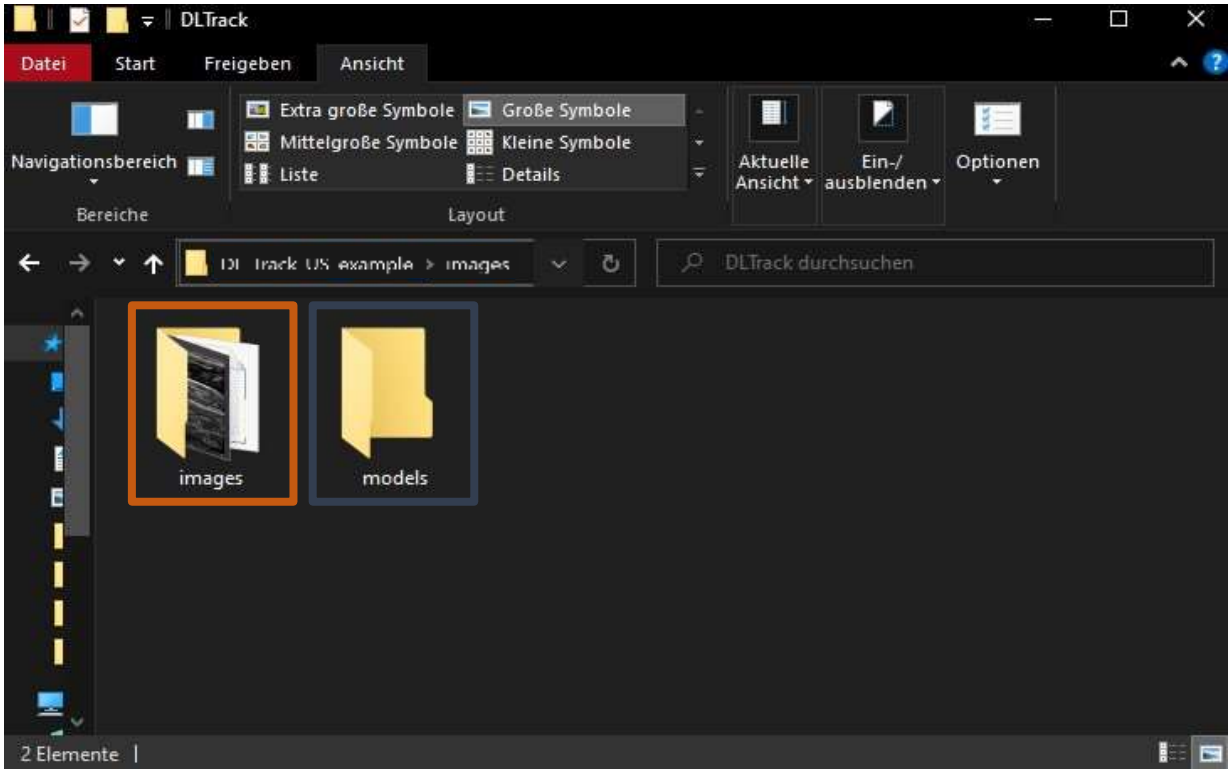
- The **flip-flag** determines if an **image** is flipped during analysis or not. A "0" stands for no flipping, whereas "1" means flip the image.
- None of the example **images** must be flipped. Their fascicle orientation is correct, with fascicles originating at the bottom left and inserting on the top right.
- Below is a visual representation of a **correct** fascicle orientation. If the fascicles in your **images** are orientated differently, please specify a "1" as a **flip-flag** for those **images**.



- For this tutorial we will use the example **images** folder “DL_Track_US_examples/images” with it’s contained images and **flip_flag.txt** file.

2. Creating Neural Network Directories

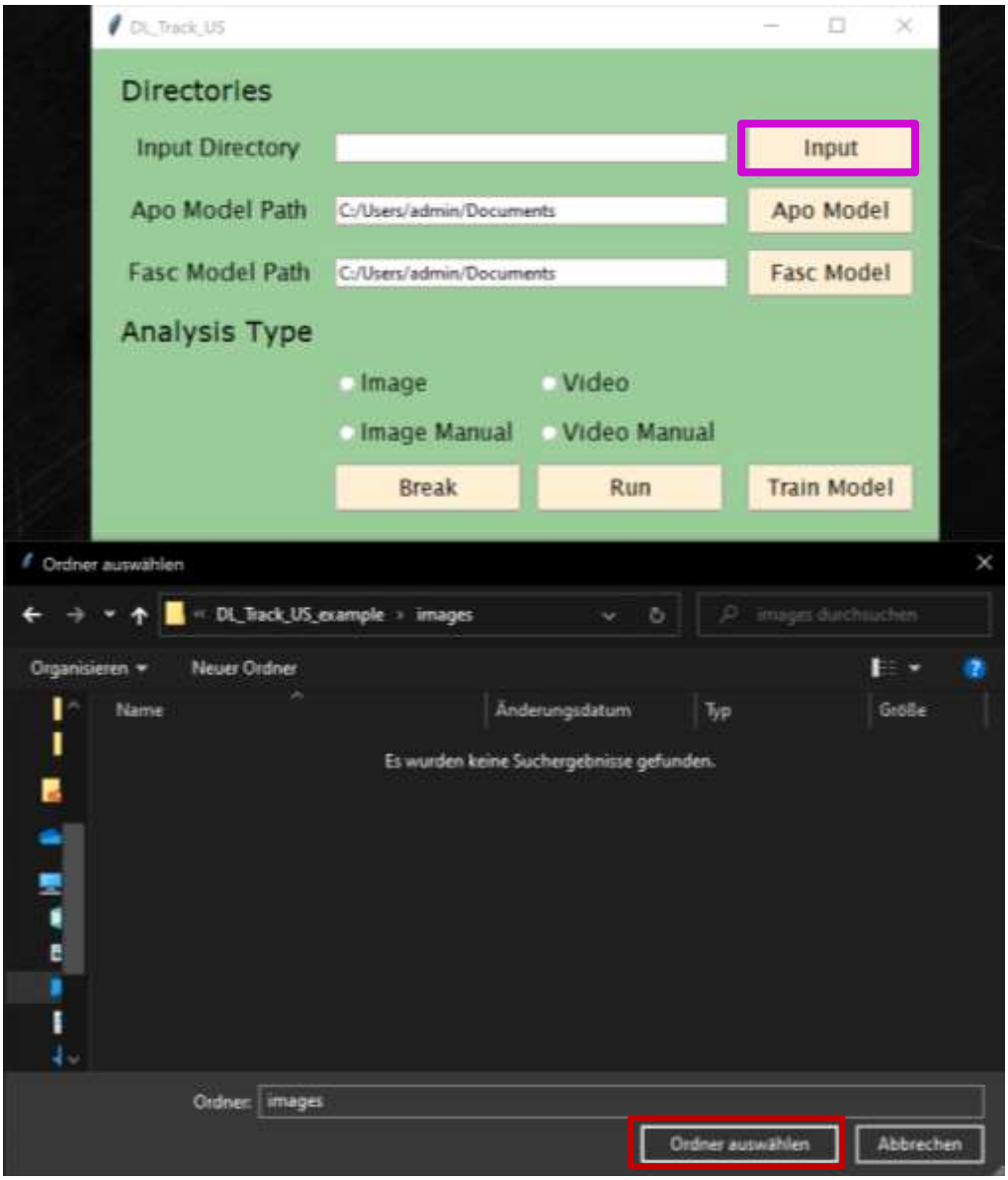
- The **folder containing the images** for this tutorial (in this case the “DL_Track_US_examples/images” folder) is already included in the “DL_Track_US_example” folder.
- The pre-trained **aponeurosis and fascicle neural networks** are located in the “DL_Track_US_example/**models**” folder. You can make use of these neural networks later as well, when you analyse your own images outside of this tutorial.



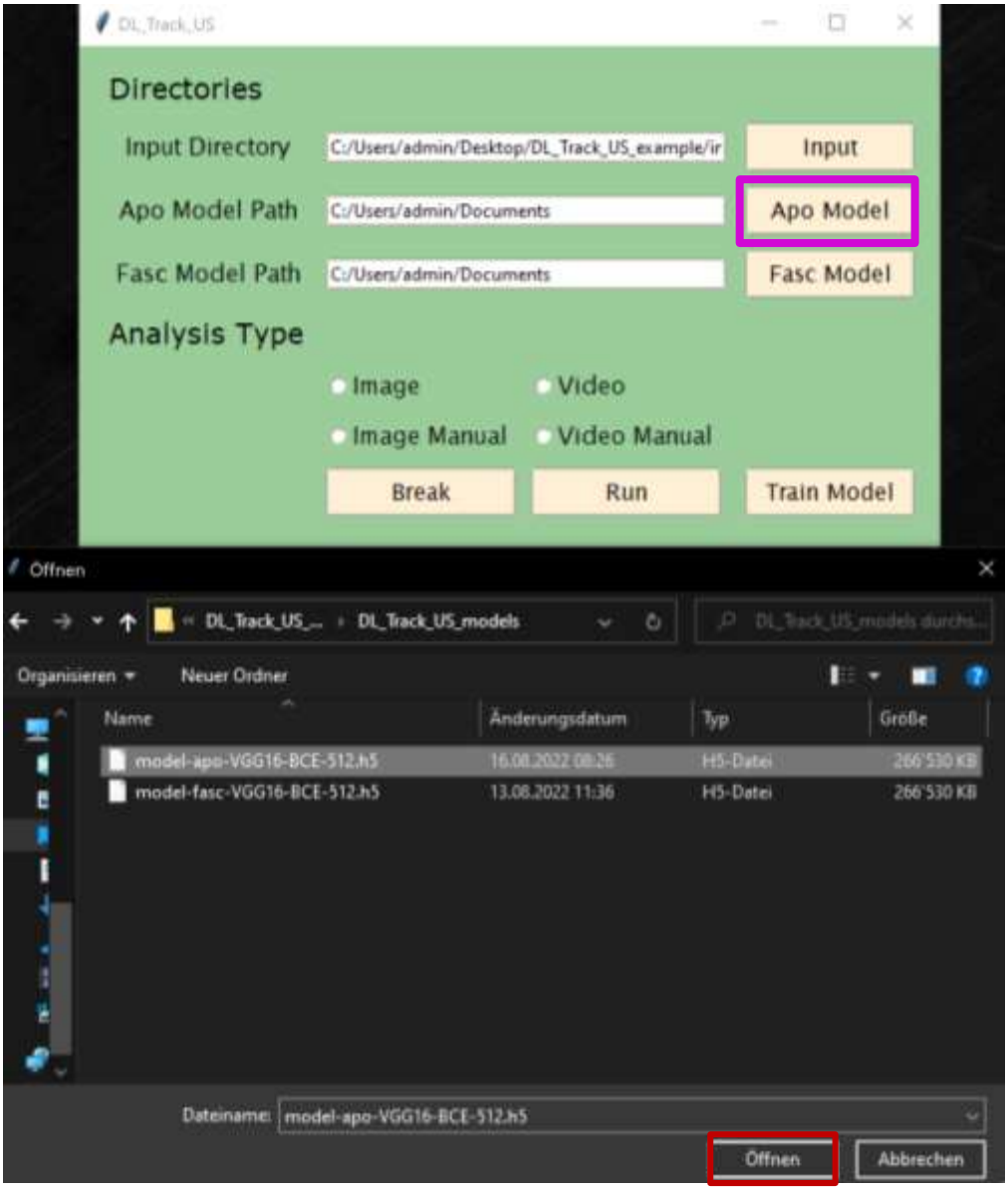
3. Specifying Input Directories in the GUI

Once the GUI is openend, the first step of every analysis type in DL_Track_US is to specify the input directories in the graphical user interface (GUI).

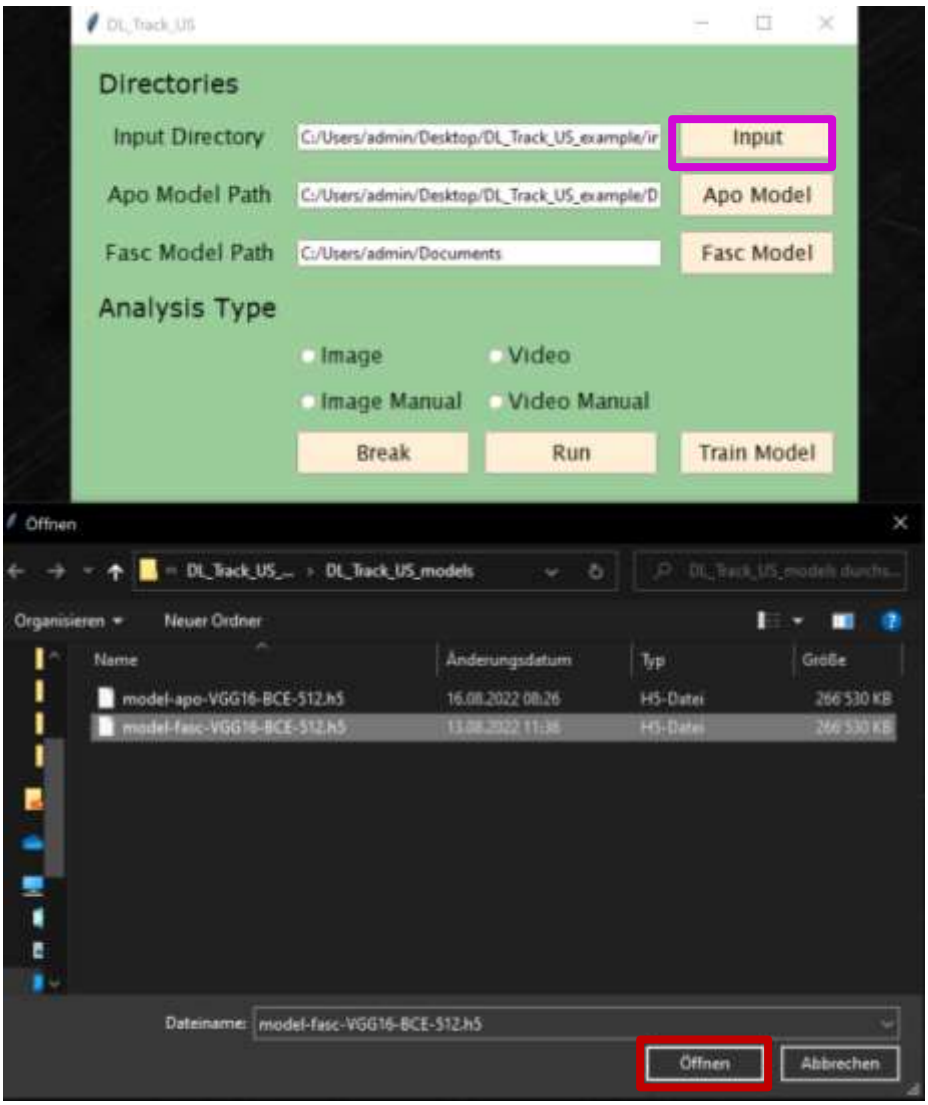
- First, specify the path to the folder containing the **images** to be analysed. Remember this was the folder “DL_Track_US_example/**images**”.
  - By clicking on the **Input** button a selection window opens were you need to select the images folder.
  - Click **select folder** to specify the path in the GUI.



- Secondly, specify the absolute path to the **aponeurosis neural network** in the "DL_Track_US_example/**models**".
  - By clicking on the **Apo Model** button, a selection window opens were you need to select the **aponeurosis neural network**.
  - Click **open** to specify the path to the **aponeurosis neural network** in the GUI.



- Thirdly, specify the absolute path to the **fascicle neural network** in the "DL_Track_US_example/**models**".
  - By clicking on the **Fasc Model** button, a selection window opens were you need to select the **fascicle neural network**.
  - Click **open** to specify the path to the **fascicle neural network** in the GUI.

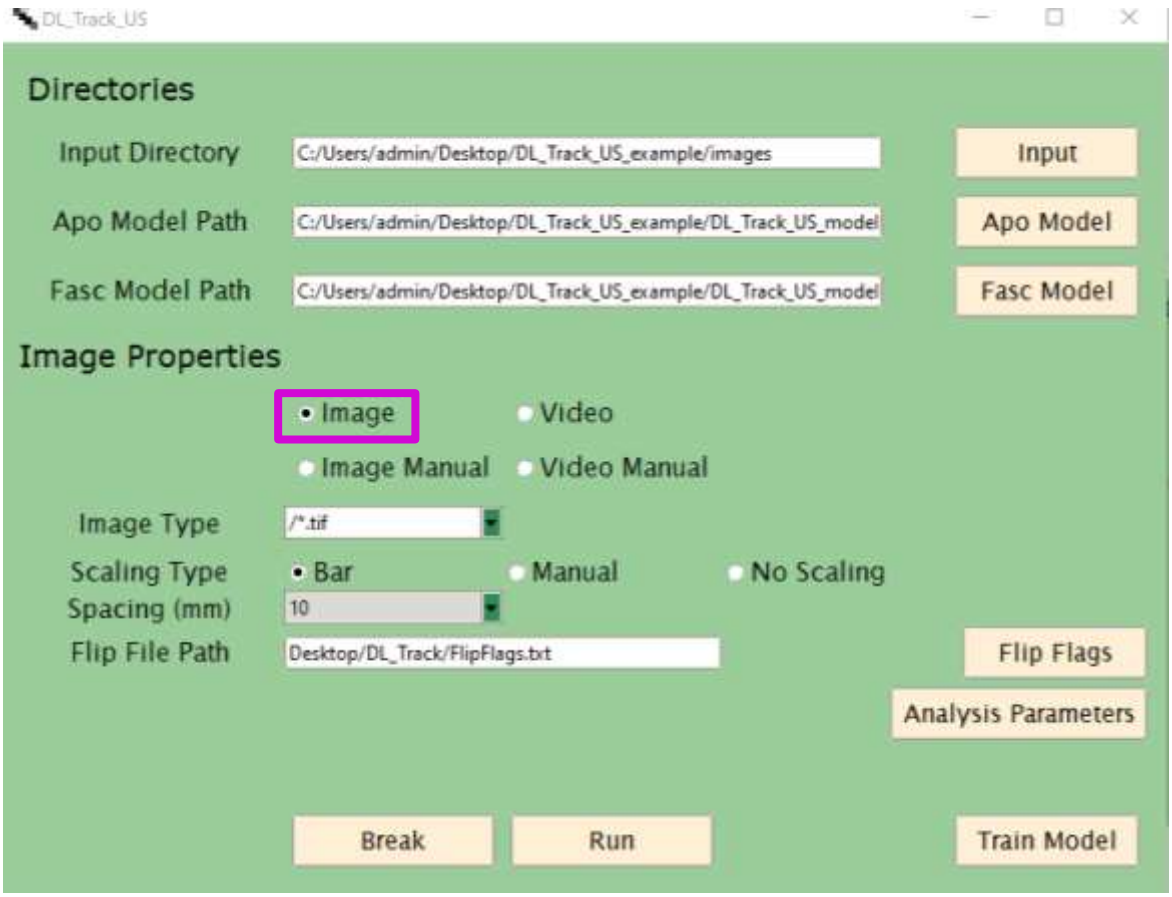


In the next section you will specify all relevant analysis parameters, including the analysis type. We will also explain what each parameter is used for.

4. Specifying Relevant Parameters

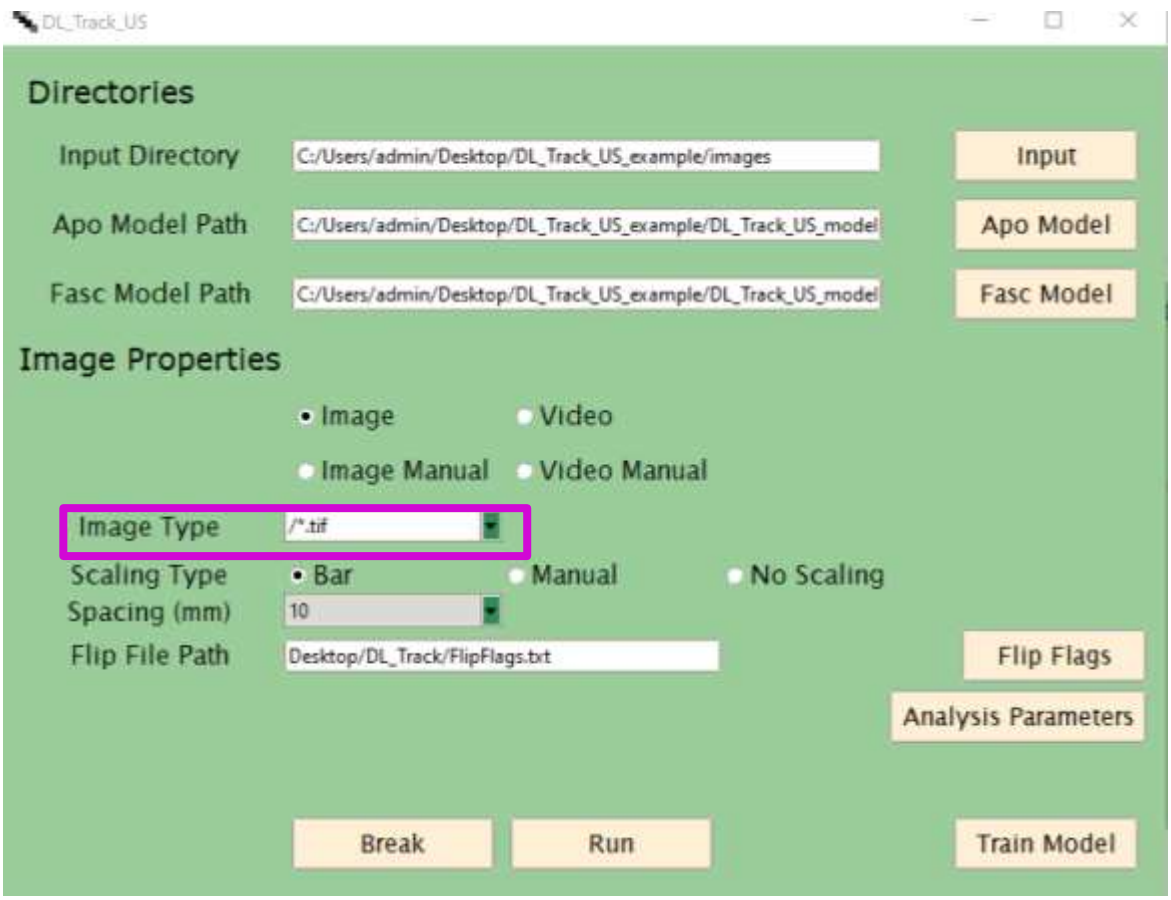
As a first step, you will select the right analysis type in the GUI.

- S elect the **Image** radiobutton.
- You can see that the GUI unfolds and several other parameters appear.
- You will set those in the next steps on the next page.



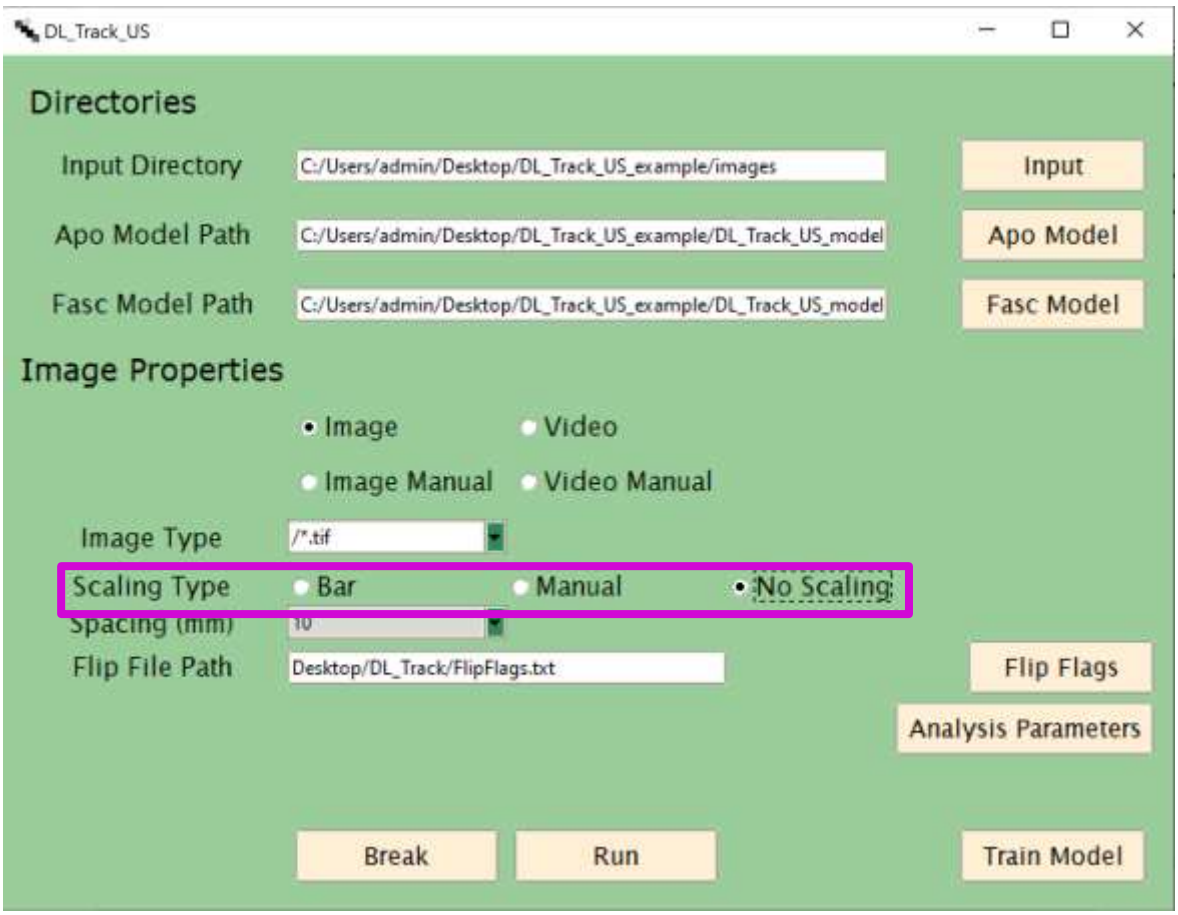
Next, you need to specify the Image Type.

- The ending of the **Image Type** must match the ending of your **images**, otherwise no files are found by DL_Track_US.
- You can either select a pre-specified ending from the dropdown list or type your own ending.
- Please keep the formatting similar to those **Image Types** provided in the dropdown list.
- All the **images** in the "DL_Track_US_example/**images**" folder are of the **Image Type** ".tif". Thus, you should select the "/*.tif" **Image Type**.

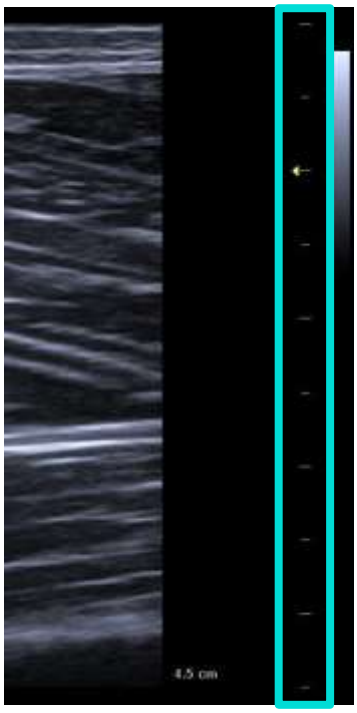


Subsequently, you need to specify the image **Scaling Type**.

- Scaling in general has the huge advantage that the resulting estimated muscle architectural features are in centimetre units rather than pixel units.
- There are three **Scaling Types** in the DL_Track_US package.
- For this tutorial however, you will select the **"No Scaling"** option as displayed below.
- We will explain the other two **Scaling Types** in the next pages.

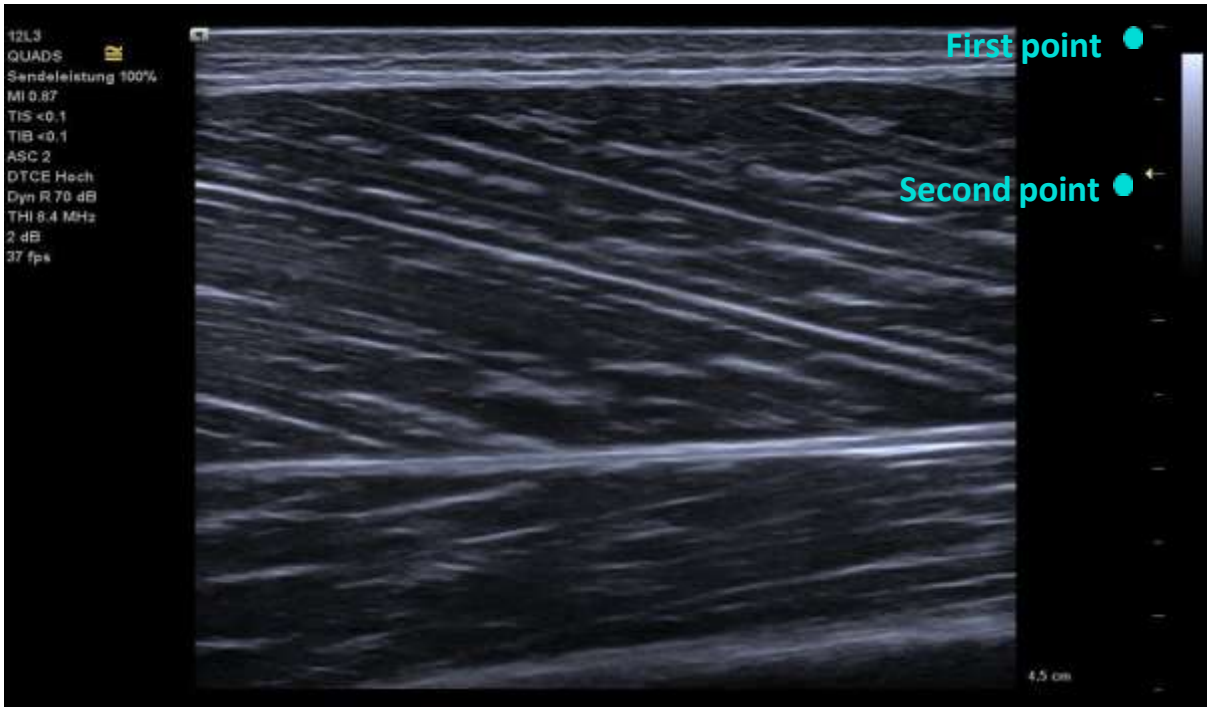


- Another **Scaling Type** except **“No Scaling”** is **“Bar”.** This **Scaling Type** is only applicable if there are **scaling bars** in the right side of the ultrasonography image:



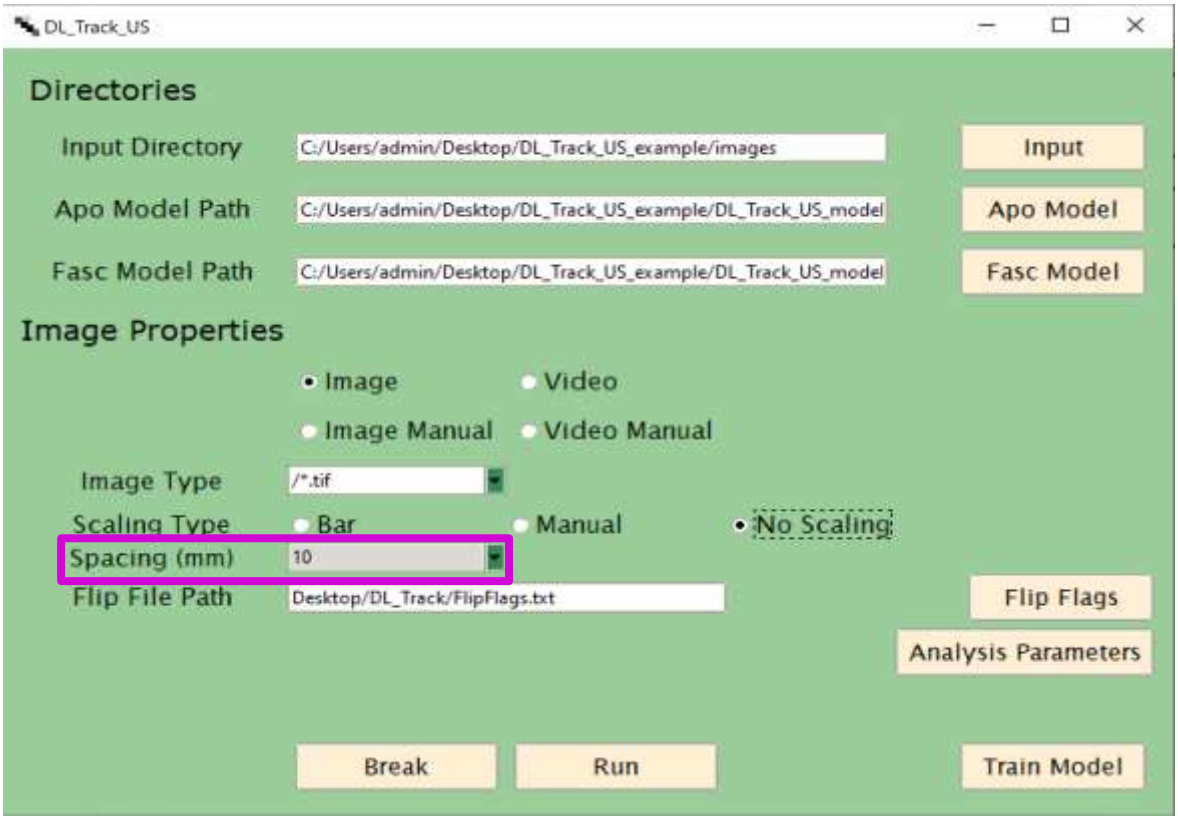
  - The **scaling bars** do not need to look exactly like the ones in the above image. They just need to be **next to the image** and **clearly separated** from each other.
  - We advise you to try this **Scaling type** on a few of your images and find out for yourself if it works.
  - Files that cannot be analysed with this **Scaling type** will be recorded in an failed_images.txt file in the image input folder.

- The last of the three **Scaling Types** is “Manual”.
  - This **Scaling Type** requires input from the user.
  - When you choose “Manual” as your Scaling type, you need to manually place **two points** on the image using the left mouse button

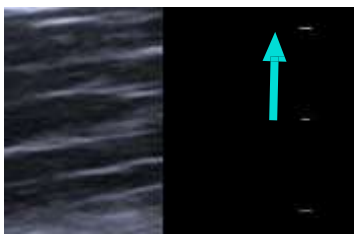


  - Just click one time with your left mouse button to record the **first point** (nothing will be displayed on the images during actual analysis).
  - Place the **second point** at a known distance of either 5, 10, 15 or 20 millimetre.
  - The distance you chose must be represented in the Spacing (see next page) parameter in the GUI.

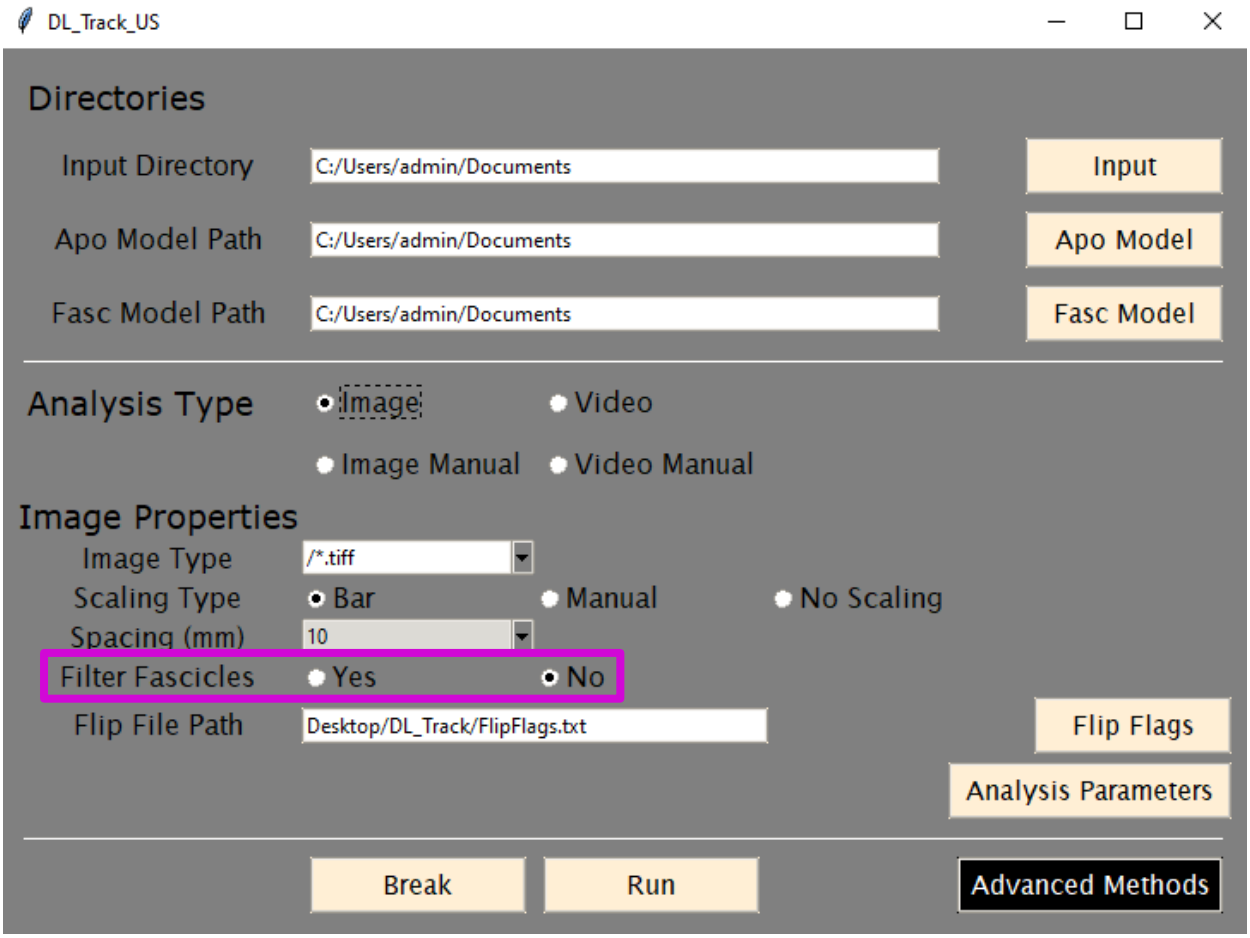
- Whenever you use **“Bar”** or **“Manual”** as your Scaling Type, make sure that the minimal distance between the scaling bars or the known distance between the manually specified points is represented in the **Spacing** parameter.



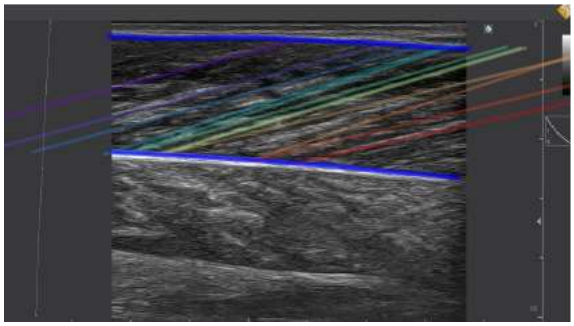
- Select the **Spacing** parameter from the dropdown list as 5, 10, 15 or 20 millimetre. For this tutorial it is not necessary to select anything, as the Spacing parameter is not used during an analysis with Scaling Type **“No Scaling”**.
- The minimal **distance** between the scaling bars in an image. This is simply the **distance** in millimeter between the two nearest scaling bars in the image. If you do not know this **distance**, please use **“Manual”** or **“No Scaling”** Scaling Type. For example in the image from before, the **distance** between the nearest bars is 5 millimetre.

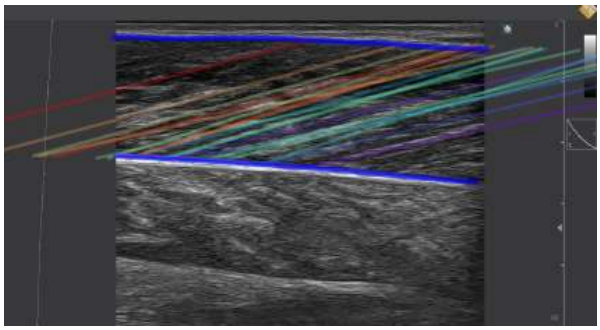


- In version 0.2.1 we introduced a new feature to DL_Track_US, called the **Filter Fascicle** option.
- Here, you have two options, **“YES”** or **“NO”**.
- Using **“YES”** all fascicles that overlap will be removed.

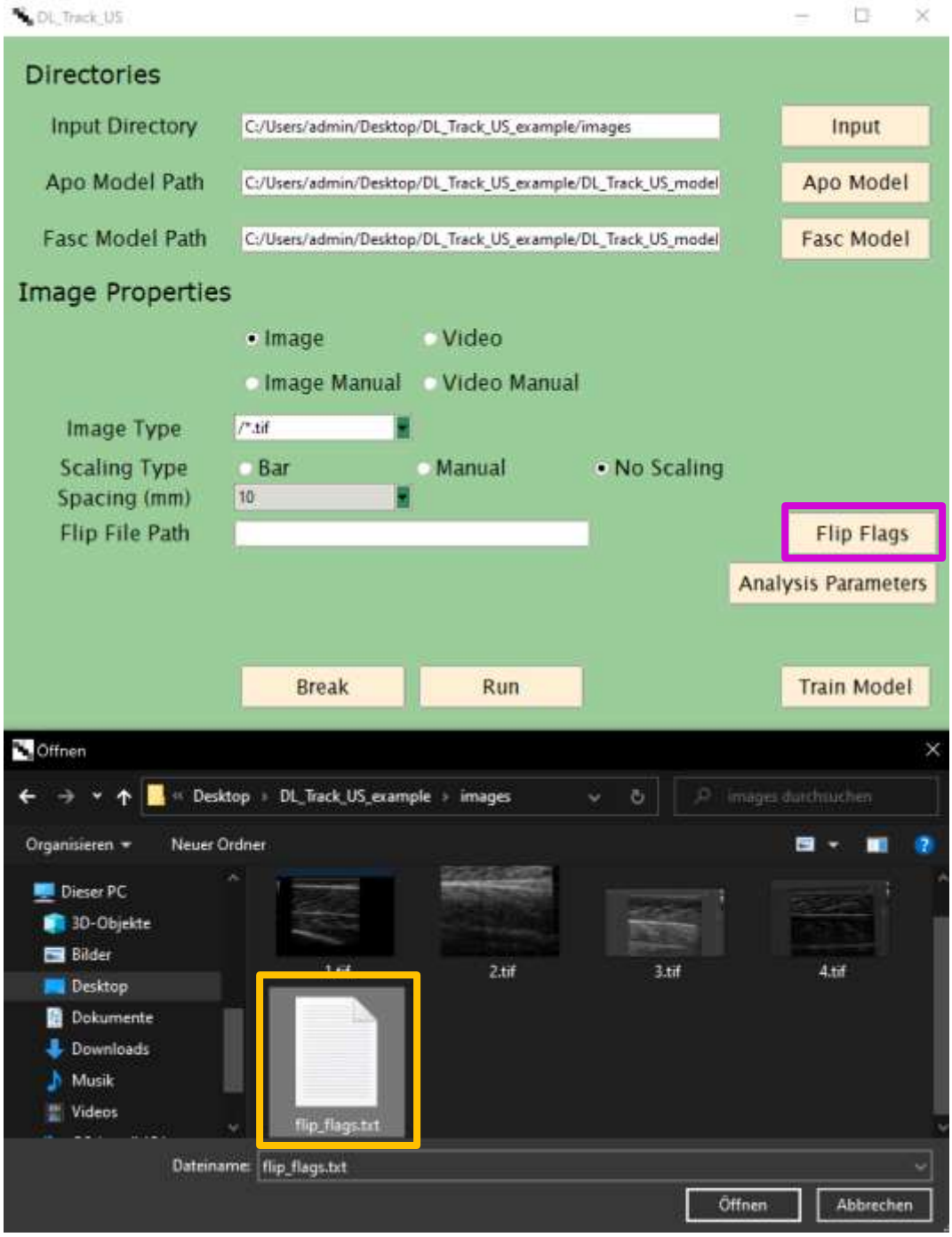


Here are some results demonstrating the difference:





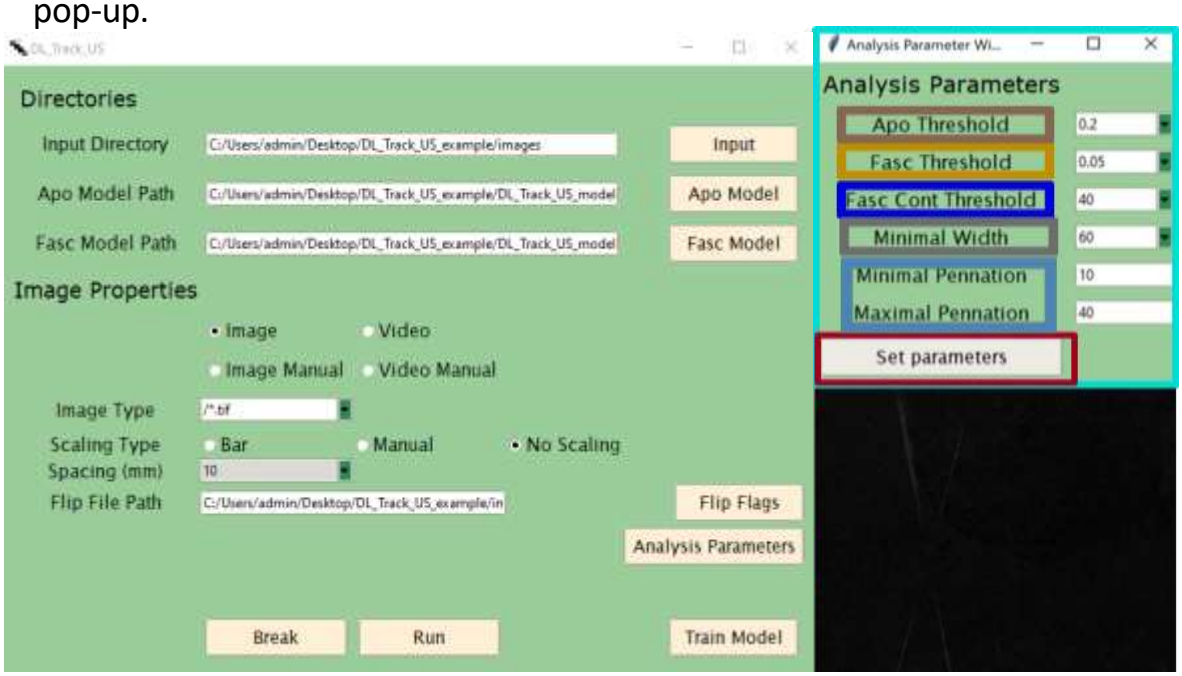
- Next, specify the absolute path to the **flip_flag.txt** file.
- By clicking the **Flip Flags** button, a dialogue will pop up and you can select the **flip_flag.txt** file.
- In this example, the **flip_flag.txt** file is located at "DL_Track_US_example/images".
- Remember, the amount of **flip-flags** in the flip_flag.txt file must equal the amount of **images** in the **images** folder.



5. Specifying Analysis Parameters

As a last step, you need to specify the analysis parameters for the **aponeurosis and fascicle neural networks.**

- When you press the **Analysis Parameters** button, a separate window will pop-up.

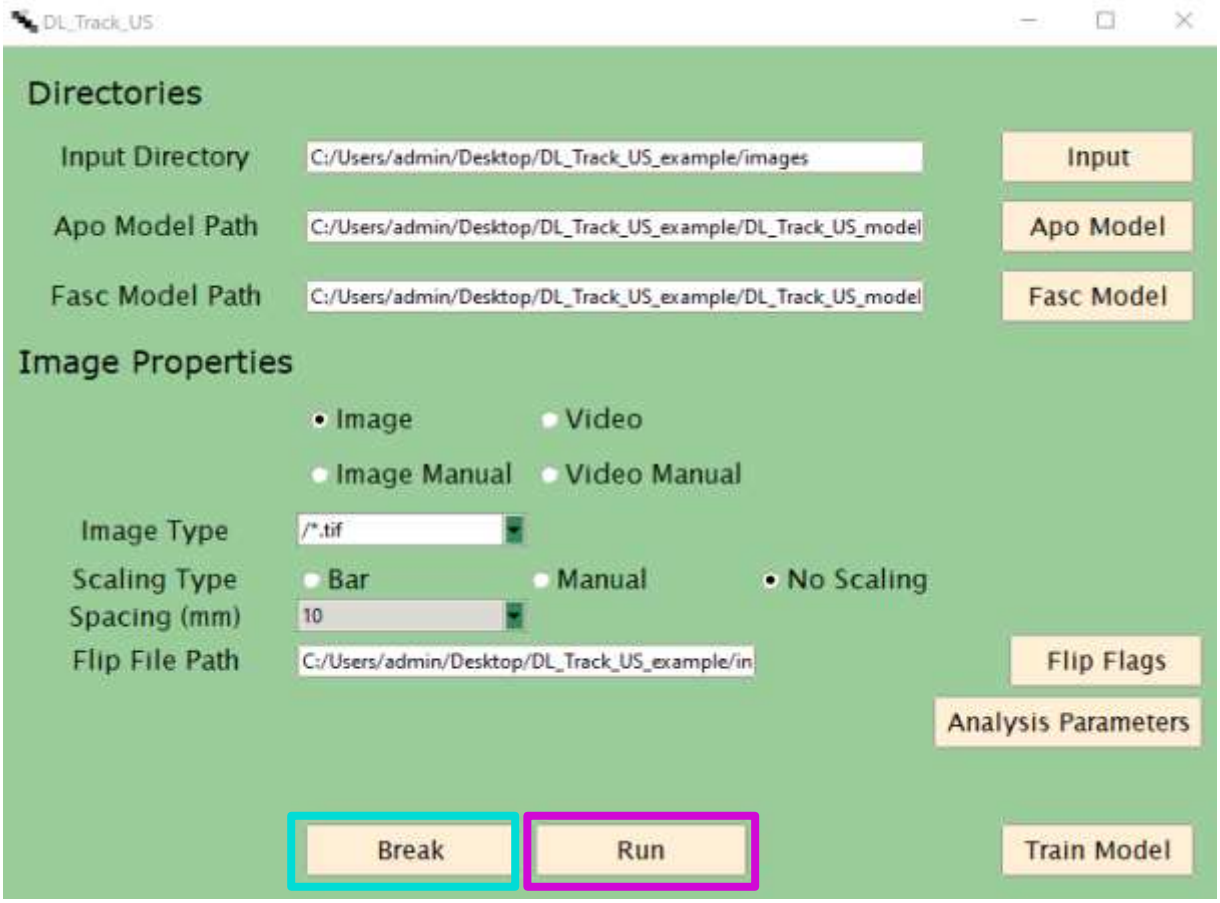


  - In the **Analysis Parameter window**, all parameters used by the **aponeurosis and fascicles neural networks** during inference are specified.
  - The **Apo Threshold** parameters determines the threshold of the minimal acceptable probability by which a pixel is predicted as aponeurosis. The lower, the more pixels will be classified as aponeurosis.
  - The **Fasc Threshold** is the same thing just for fascicle segments.
  - The lower the **Fasc Cont Threshold**, the shorted the minimal acceptable length of detected fascicle segments to be included in the results.
  - The **Minimal Width** determined the minimal acceptable distance between superficial and deep aponeurosis.
  - **Minimal and Maximal Pennation** describe the respective minimal and maximal pennation angle that is physiologically possible in the analysed image/muscle.
  - **In v0.2.1 of the GUI, we added the parameter „Apo Length Thresh“. This is set to 600 px as default. Changing this value will result in longer or shorter structures detected as aponeurosis.**

- For this tutorial, you can leave all parameters the way they are.
- You can set the parameters by clicking the **Set parameters** button. Adapt these parameters according to your images in analyses.
- For future analyses, it’s best you test the ideal parameter configuration in a small sample of your images prior to the actual analysis.

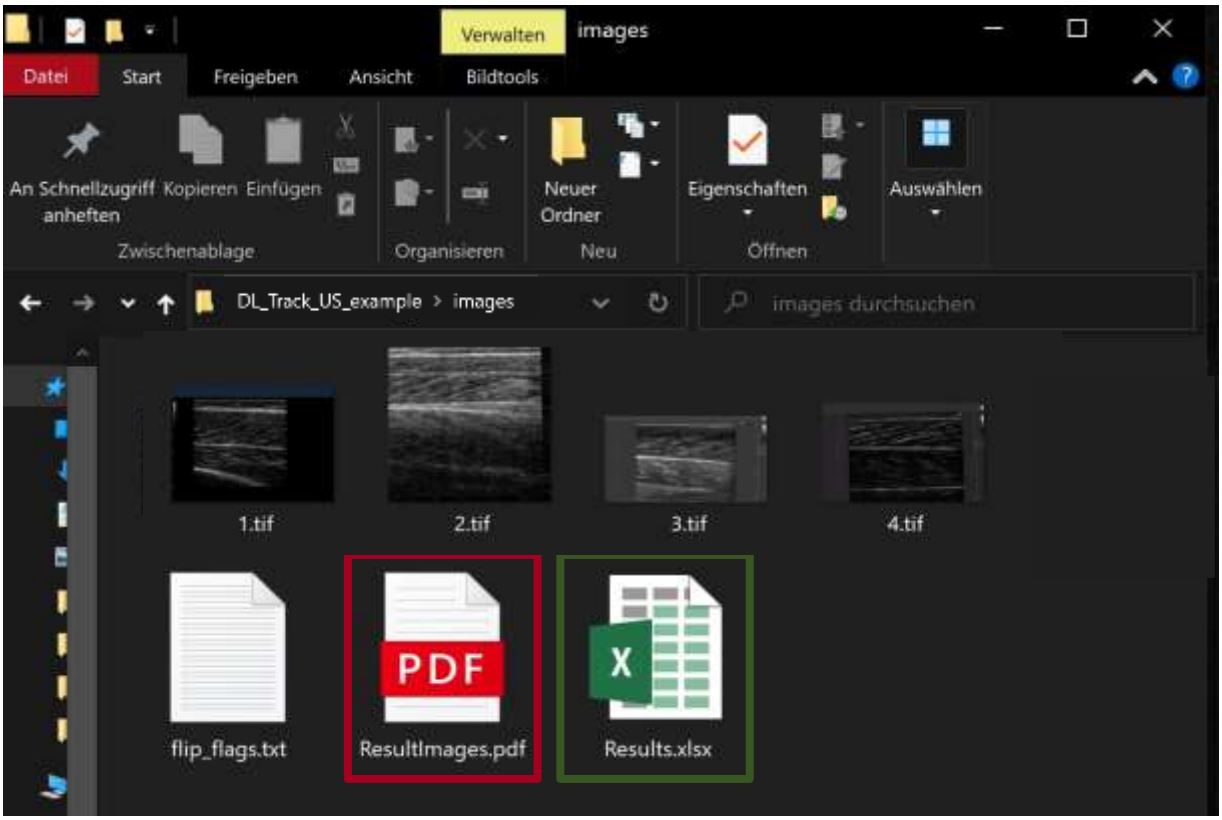
6. Running / Breaking DL_Track_US

- By clicking the **Run** button in the main GUI window, you can start the analysis.



- Moreover, you can see that there is a **Break** button placed in the GUI as well.
- Clicking the Break button allows you to stop the analysis at any point. The currently evaluated image will be processed and then the analysis is terminated.

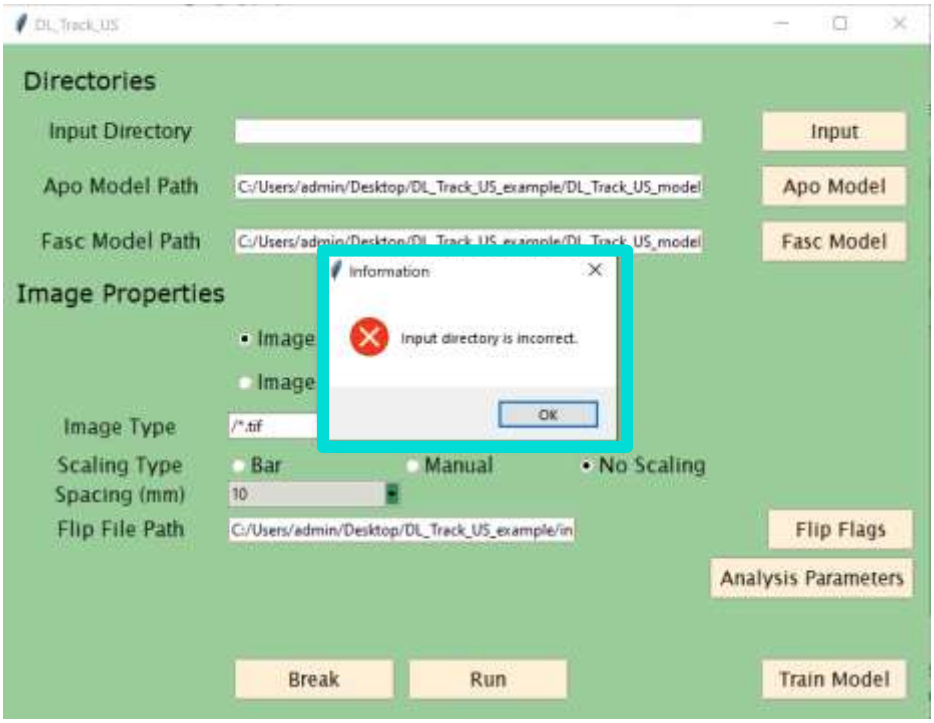
- In the “DL_Track_US_example/images” folder, you will see that two files will be / have been created, **ResultImages.pdf** and **Results.xlsx**.
- The **ResultImages.pdf** file contains each original input image and concomitant prediction results with fascicles and aponeurosis displayed.
- The **Results.xlsx** file contains the actual architectural parameter estimates for each input image. There, the median value of all detected muscle fascicle length and pennation angles as well a the calculated muscle thickness will be displayed. Each input image is displayed in a separate row.
- Note that the **ResultImages.pdf** file can be opened only after the **Results.xlsx** was created.



You have now completed the DL_Track_US tutorial for automated image analysis! There is one more thing though, error handling. Take a look at the next section to get more information.

7. Error Handling

Whenever an error occurs during the analysis process, the DL_Track_US GUI will open a **messagebox**. This looks always similar to this:



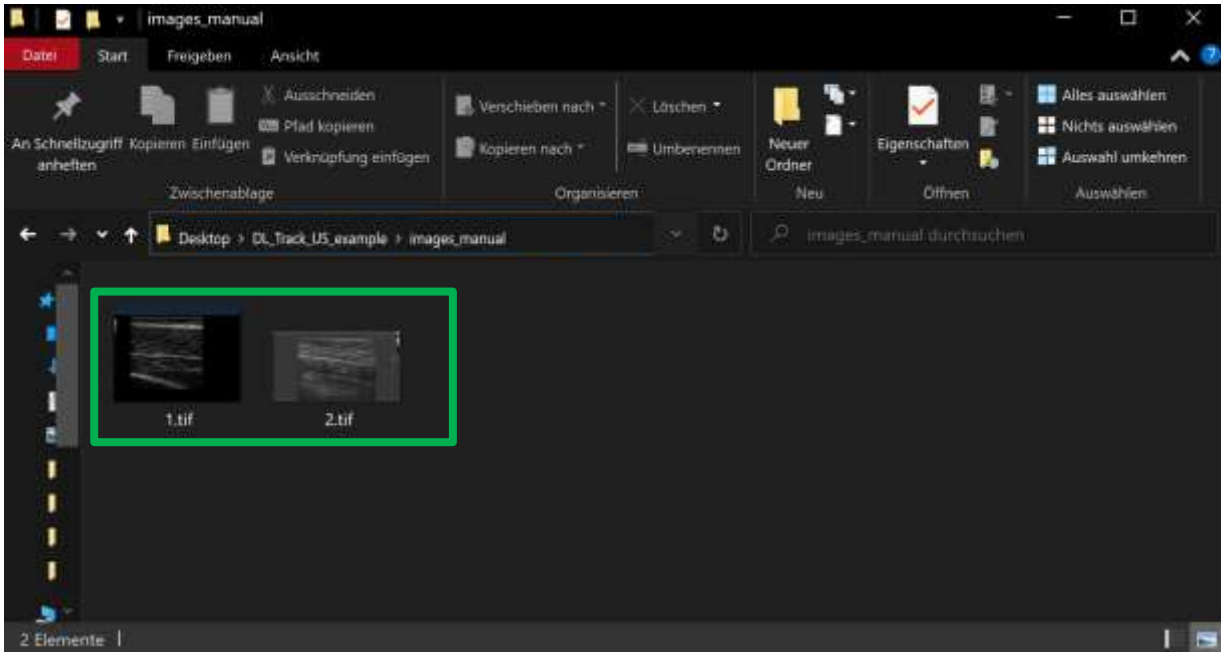
We tried to formulate these **messageboxes** as concise as possible. Just follow their instructions to fix the error and run the analysis anew. In case an error occurs that is not caught by an error **messagebox,** don't hesitate to report this in the Q&A section in the DL_Track_US discussion forum. Please take a look here how do best do this.

Manual Image Analysis

The next analysis type this tutorial covers is the manual image analysis. The images are evaluated manually by drawing the muscle thickness, fascicle length and pennation angles directly on the Image. For this type of analysis, single images (not videos) are a prerequisite. These images should be contained in a single folder, like in the "DL_Track_US_example/images_manual" folder. If you haven't downloaded this folder, please do so now (link: DL Track US - Examples & Models | Zenodo). Unzip the folder and put it somewhere accessible. We will make use of the included example file included in the DL_Track_US_examples folder extensively during this tutorial. In the next few pages, we will look at every required step to successfully perform manual image analysis with DL_Track_US.

1. Creating Image Directory

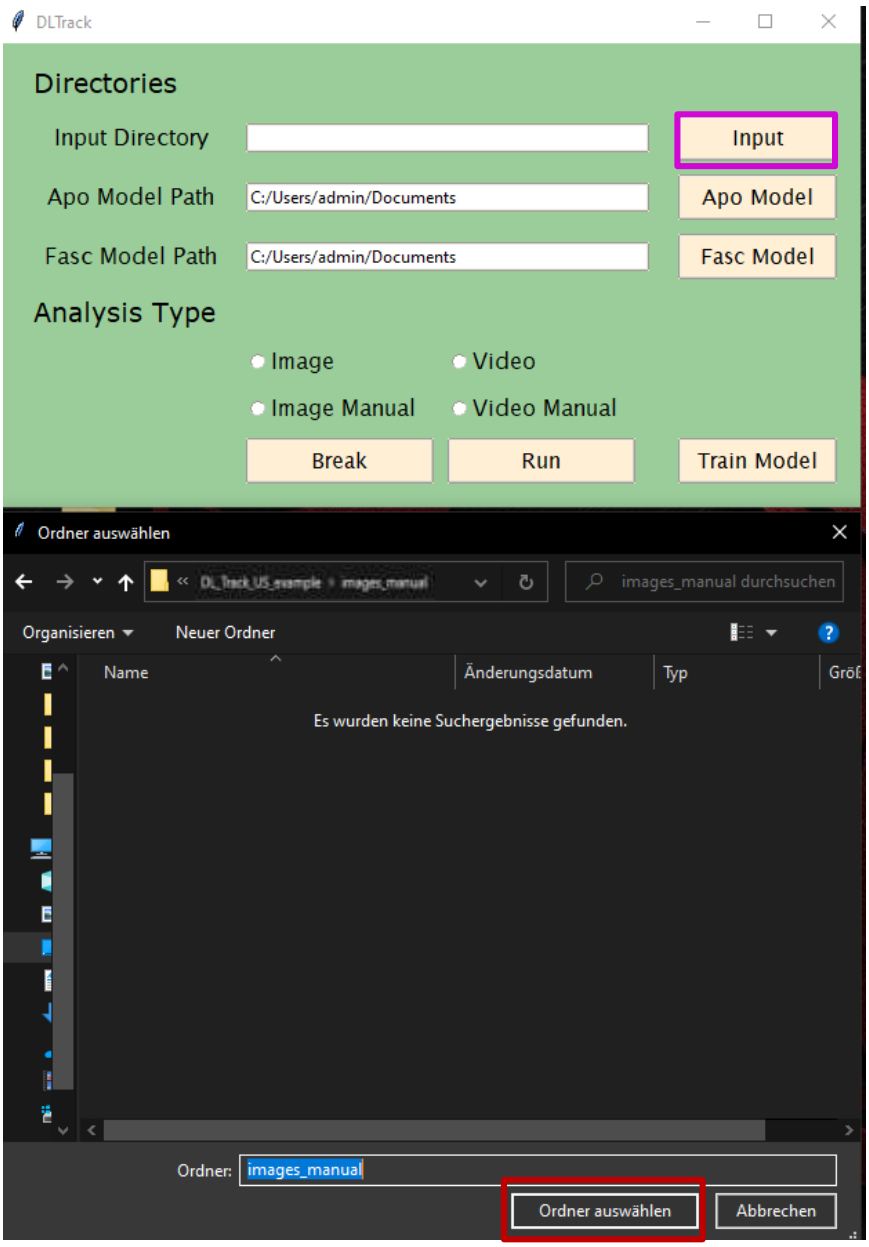
- All **images** to be analyzed should be in a single folder.



- The “DL_Track_US/image_manual“ folder contains **2 images.**
- In contrast to automated image analysis, you do not need a flip_flag.txt file nor do you need neural networks that do predictions.
- In manual image analysis, you are the neural network.
- The next step is to specify the input directory in the GUI.

2. Specifying Input Directories in the GUI

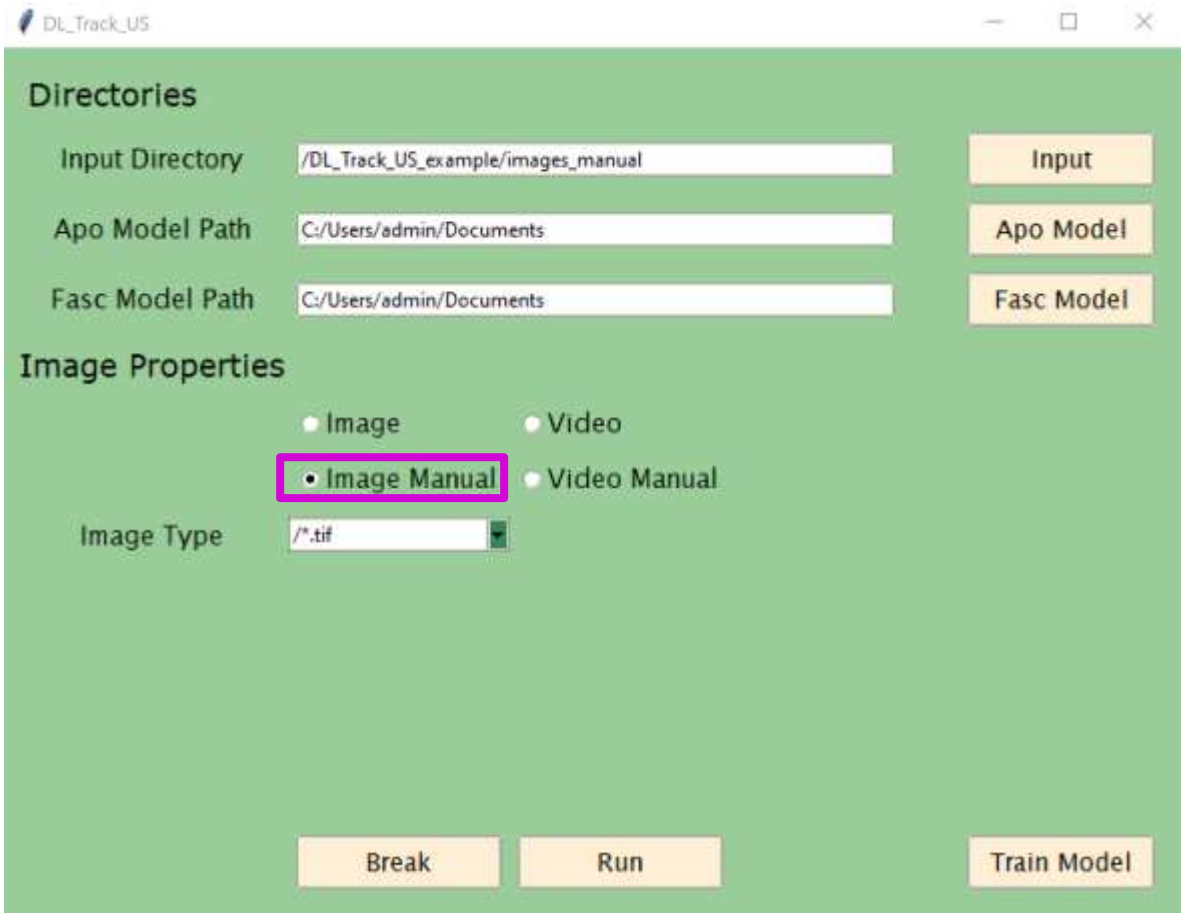
- You will begin with specifying the path to the folder containing the **images** to be analysed, the "DL_Track_US_example/images_manual" folder.
- By clicking on the **Input** button in the GUI a selection window opens were you need to select the images folder.
- Click **select folder** to specify the path in the GUI.



- Once that is done, the path will be displayed in the entry filed and you can start to specify the relevant parameters for the analysis.

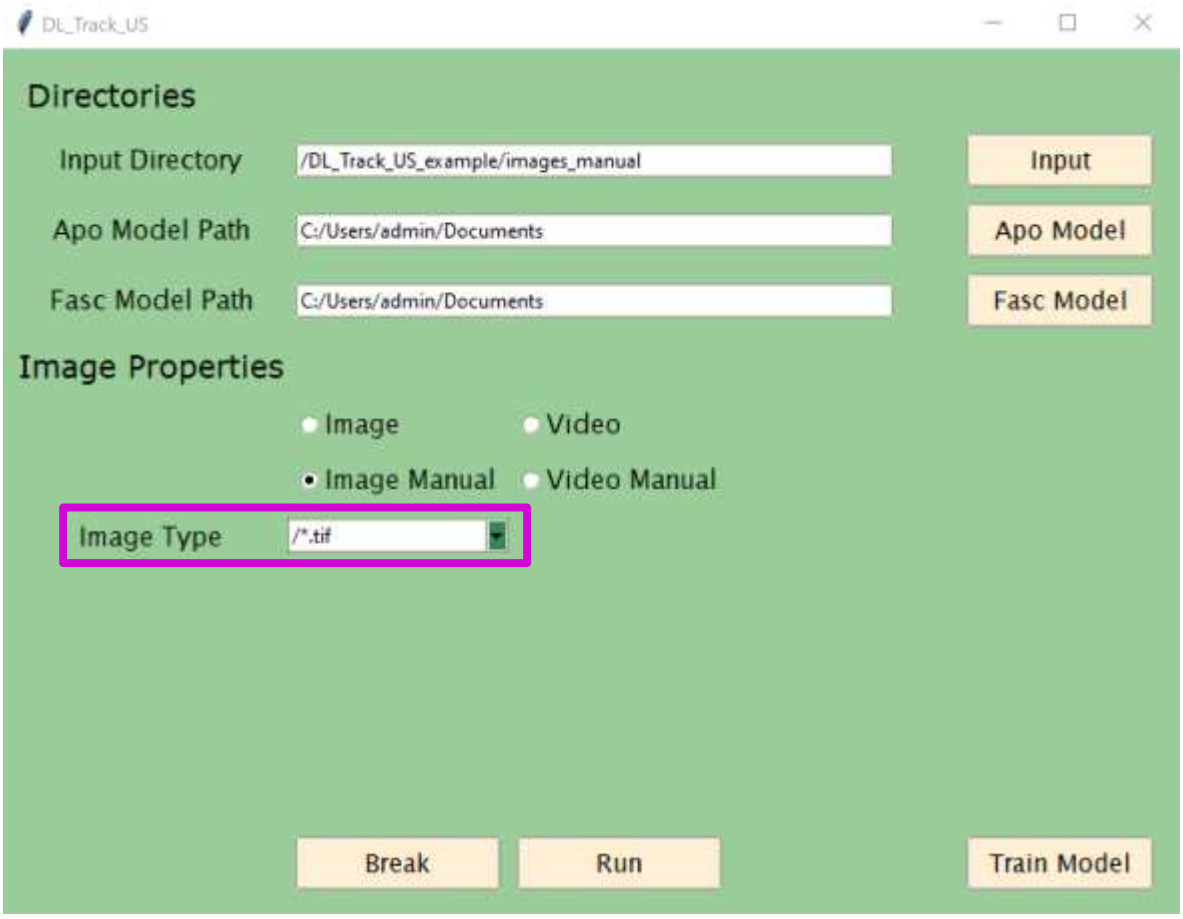
3. Specifying Relevant Parameters

- Please select the **Image Manual** radiobutton.
- You can see that the GUI unfolds and another parameter appear.
- You will set this one in the next step on the next page.



Next, you need to specify the Image Type.

- The ending of the **Image Type** must match the ending of your **images**, otherwise no files are found by DL_Track_US.
- You can either select a pre-specified ending from the dropdown list or type your own ending.
- Please keep the formatting similar to those **Image Types** provided n the dropdown list.
- All the **images** in the "DL_Track_US_example/images_manual" folder are of the **Image Type** ".tif".
- Thus, you should select the "/*.tif" **Image Type**.



- Once you have specified the Image Type, you can start with the analysis of the images contained in the "DL_Track_US_example/images_manual" folder.
- You can start the analysis by clicking the **Run** button in the main GUI.

- Take a look at the next page to see how to continue in the "Manual Analysis window" that pops up.

4. Manual Analysis of Images

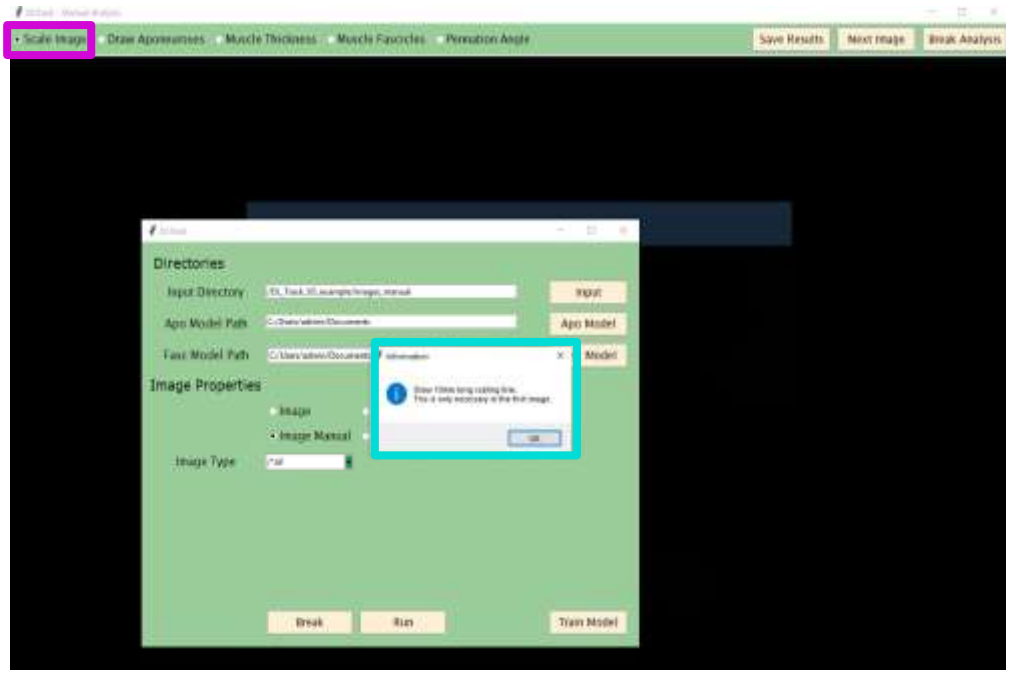
Subsequent to clicking the Run button in the main GUI, the “Manual Analysis window” opens. Here is how it looks like:

Important to note:

- The actual lines you draw are not used during the computation of the architectural parameters.
- The start- and endpoints of each line are relevant.
- The start point is defined as the point where you clicked the left mouse button to start drawing the line.
- The endpoint is defined as the point where you released the left mouse button to stop drawing the line.
- The line follows the cursor as long as the left mouse button is pressed.
- The calculations of the scaling line length, muscle thickness, fascicle length and pennation angle are dependent on the number of specified lines/segments.
- **Do NOT click somewhere random** on the image during the analysis of a parameter and exactly follow the instructions. If additional clicks happened, start the analysis anew by selecting the radiobutton representing the parameter again.
- If you do not follow the instructions presented in this tutorial, we cannot guarantee the correctness of the analysis results.

First of all, you will scale the images manually so that the calculated architectural parameters are returned in centimetre rather than pixel units.

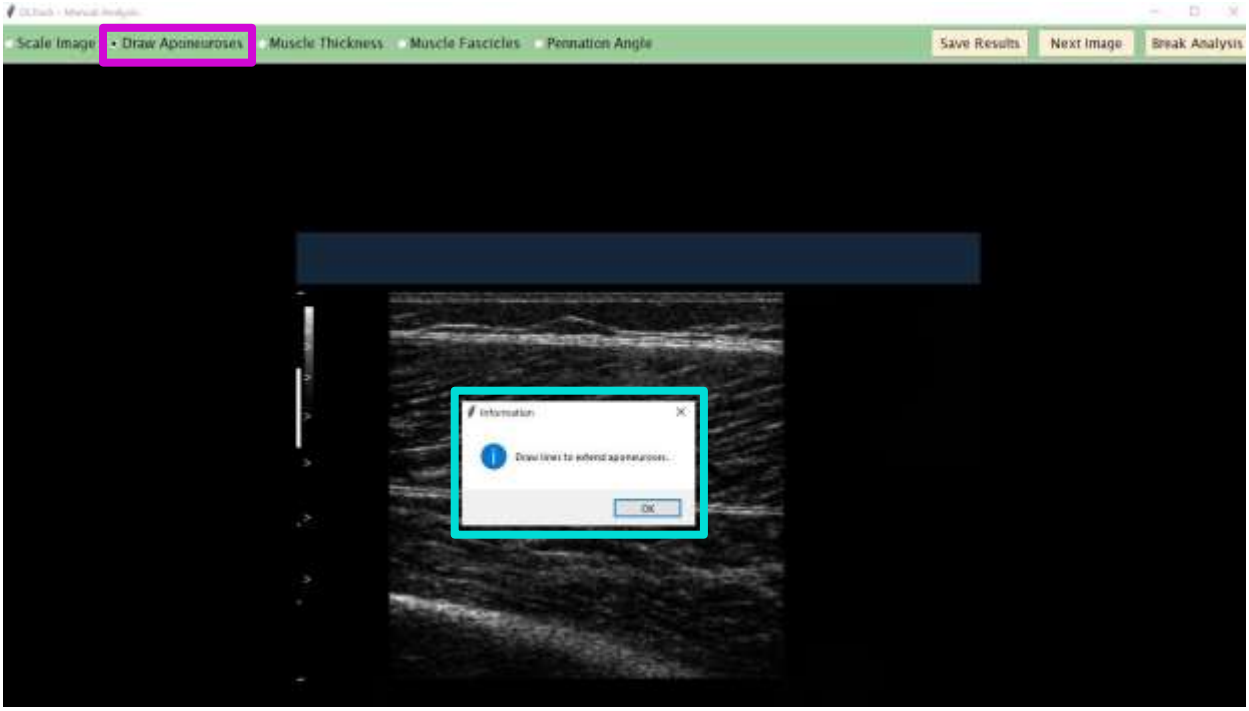
- Draw a **one centimetre long straight line in the image**.
- The distance of one centimetre is usually recognizable in the scaling bars in the image.
- You can initiate the scaling process by selecting the **Scale Image** radiobutton in the "Manual Analysis window".
- A **messagebox** will appear advising you what to do.



- The **drawn line** should look like this.

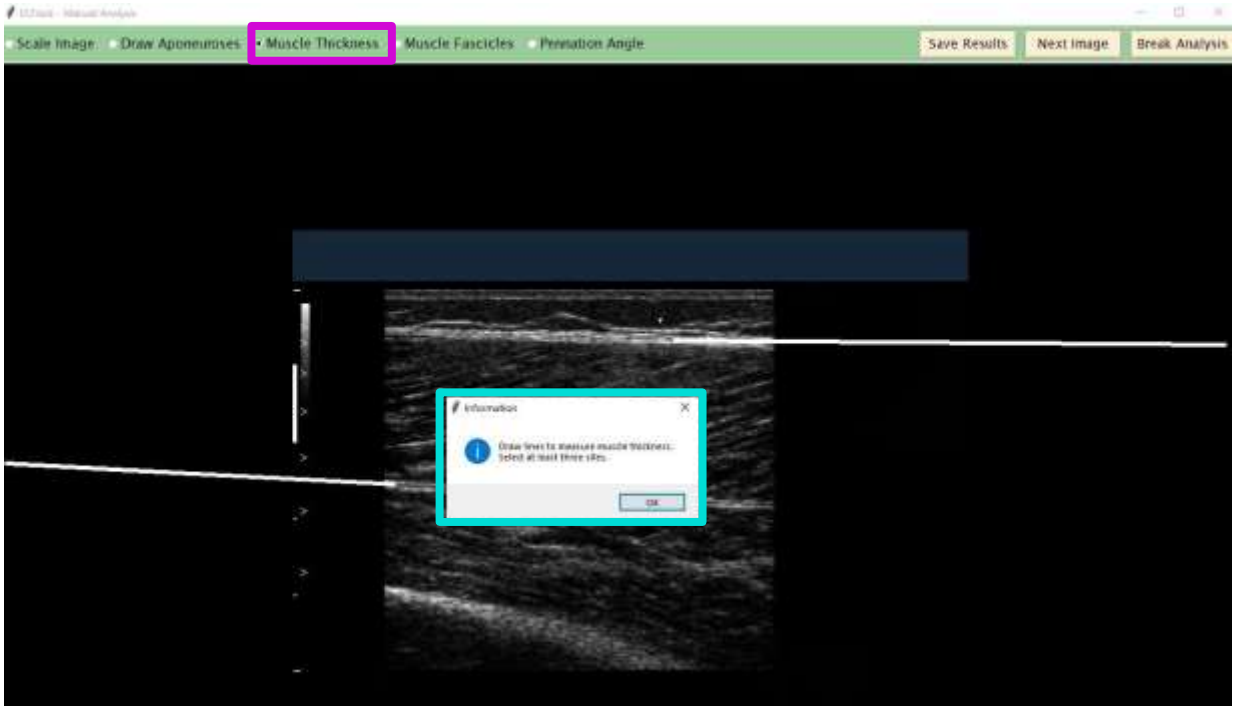
As a next step you have the option to extend the muscle aponeuroses to ease the extrapolation of fascicles extending outside of the image.

- Select the **Draw Aponeurosis** button in the "Manual Analysis window" and draw the **aponeurosis lines** on the image as shown below.
- A **messagebox** will appear advising you what to do.



Now you can start with the muscle thickness assessment.

- Select the **Muscle Thickness** radiobutton in the “Manual Analysis window”.
- A **messagebox** will appear advising you what to do.
- Draw **three straight lines** reaching from the superficial to the deep aponeurosis in the middle right and left portion of the muscle image.

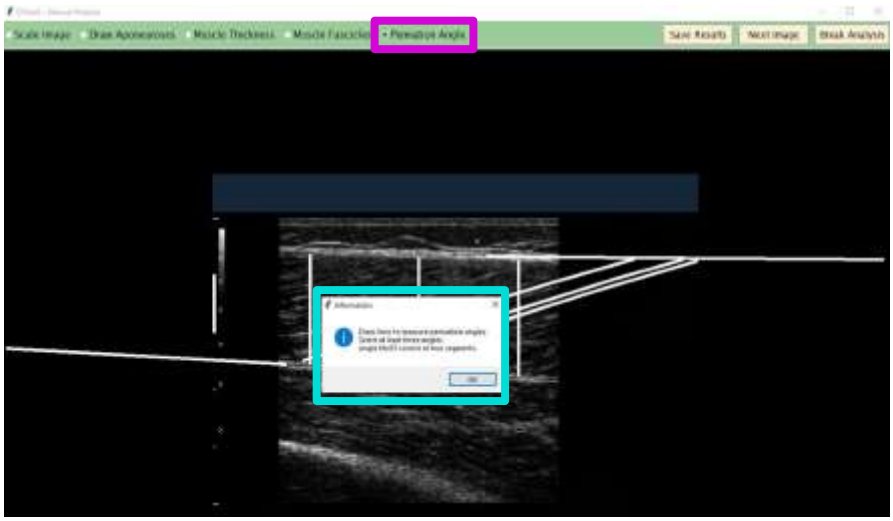


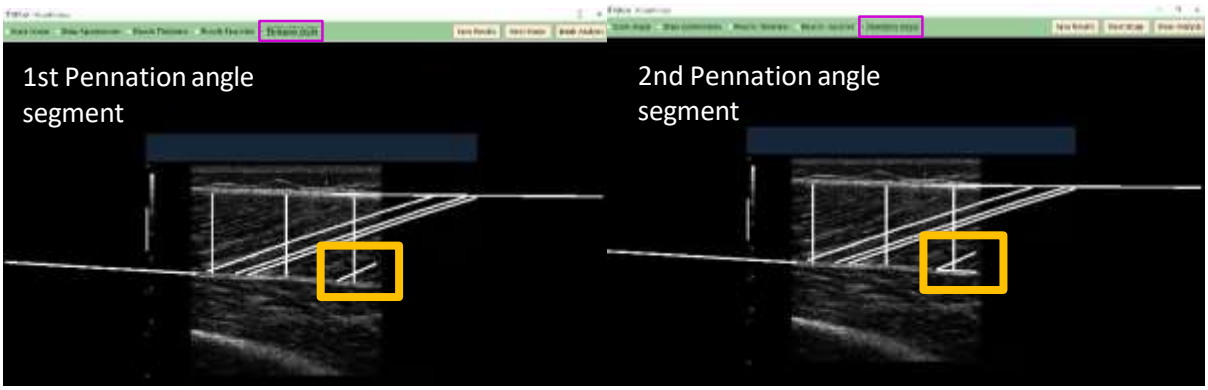
Next you can mark single fascicles on the image.

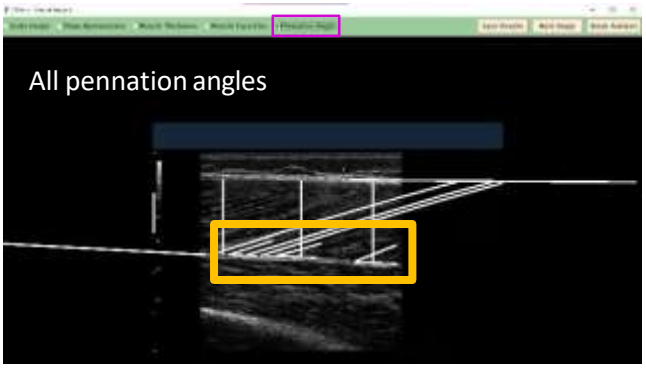
- Select the **Muscle Fascicles** radiobutton in the "Manual Analysis window".
- A **messagebox** will appear advising you what to do.
- Draw at least three **fascicles** per image in different regions of the image.
- It is possible to extrapolate the **fascicles** outside of the image region.
- Each **fascicle** MUST consist of three segments.
- Do not draw more or less segments per **fascicle** and pay attention to avoid any extra unwanted mouse clicks.
- One segment **MUST** start where the previous segment ended.
- Take a look at the image sequence below to see how it is done:

Next you can manually analyse the pennation angle.

- Select the radiobutton **Pennation Angle**.
- A **messagebox** will appear advising you what to do.
- Draw at least three **pennation angles** per image at different regions of the image.
- Each drawn **pennation angle** MUST consist of two segments. The first segment should follow the orientation of the fascicle, the second segment should follow the orientation of the deep aponeurosis. The segments should both originate at the insertion of the fascicle in the deep aponeurosis.
- Please pay attention to avoid unwanted clicks on the image.



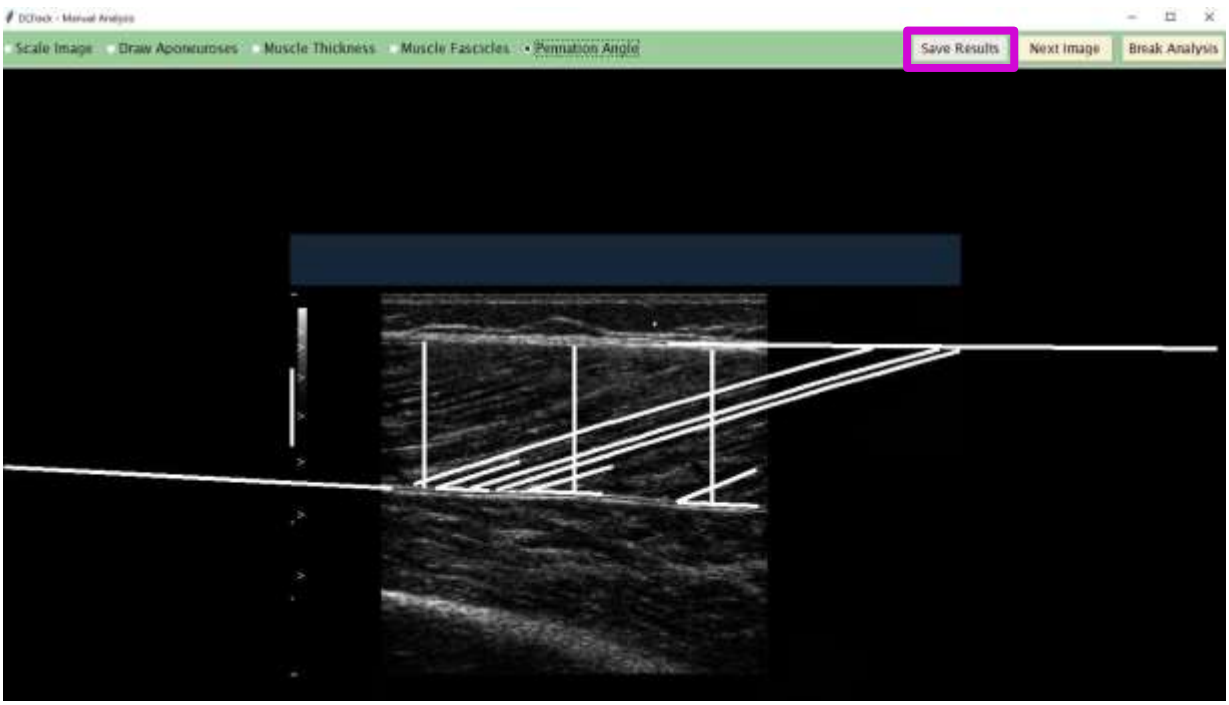




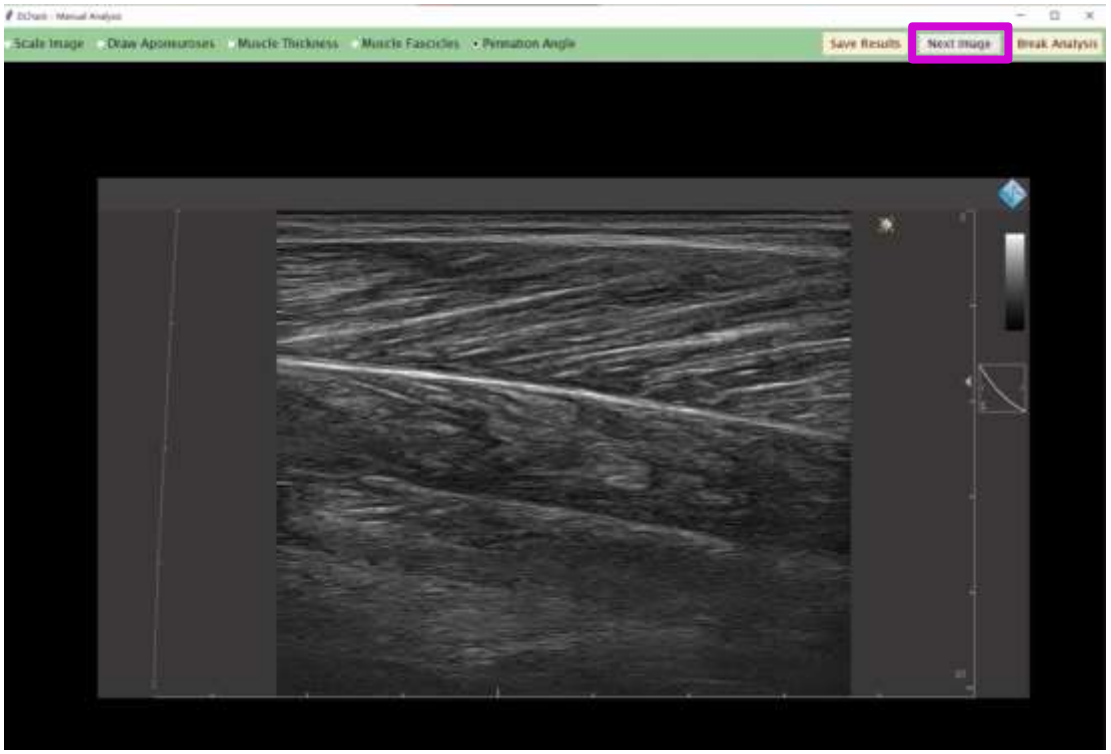
5. Saving / Breaking / Next Image

There are three buttons in the “Manual Analysis window” left to explain.

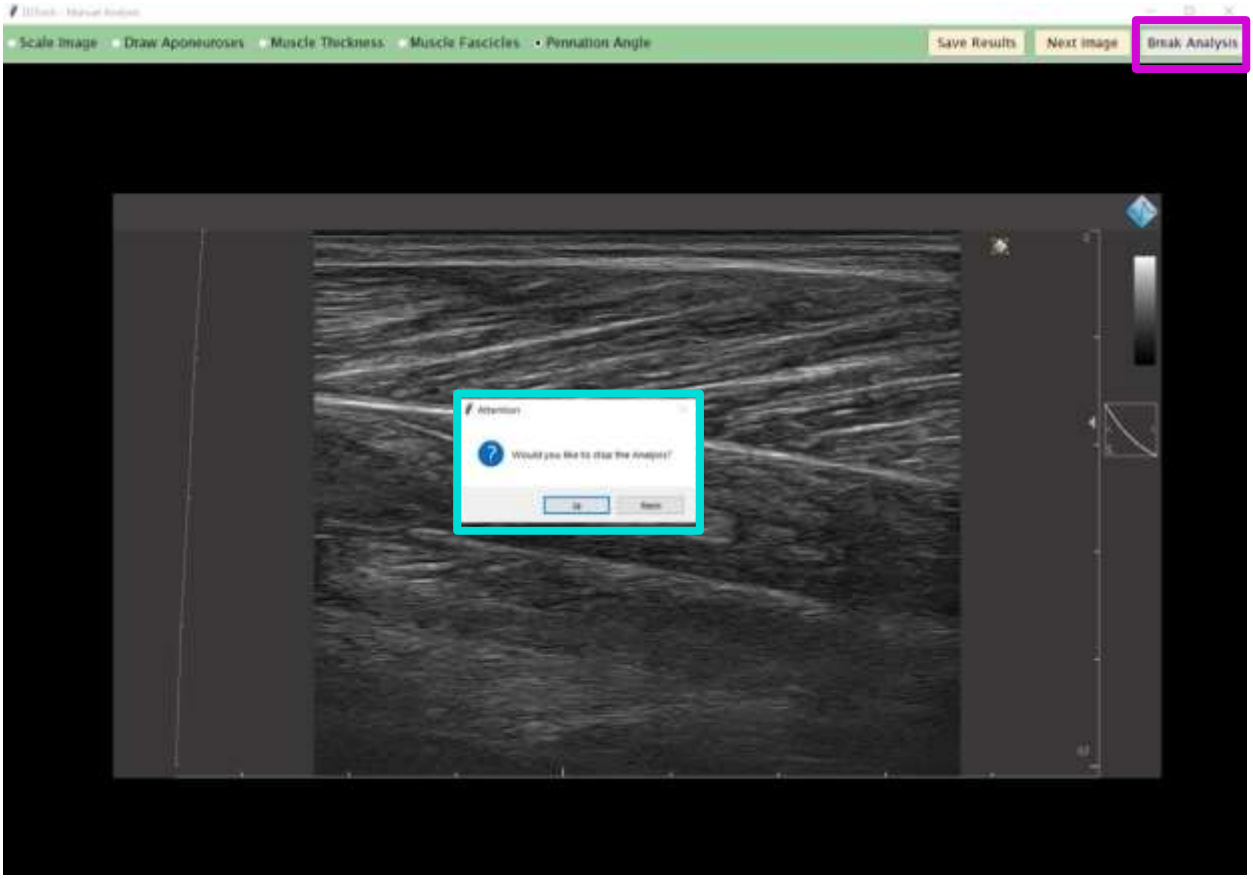
- The first button is the **Save Results** button.
  - The **Save Results** button is a very important button!
  - Press the **Save Results** button once you have analyzed all parameters that you wanted to analyze and **before** continuing with the next image.
  - An excel file with the name Manual_Results.xlsx is saved in the directory of the input images upon pressing the **Save Results** button. Therein, all analysis results are stored. Moreover, by pressing the **Save Results**, a screenshot of your current analysis is captured and stored. (Note: The image may look strange, as we can only approximate the coordinates and size of the manual analysis on your screen.)
  - In your case all files are saved in the “DL_Track_US_example/images_manual” folder.



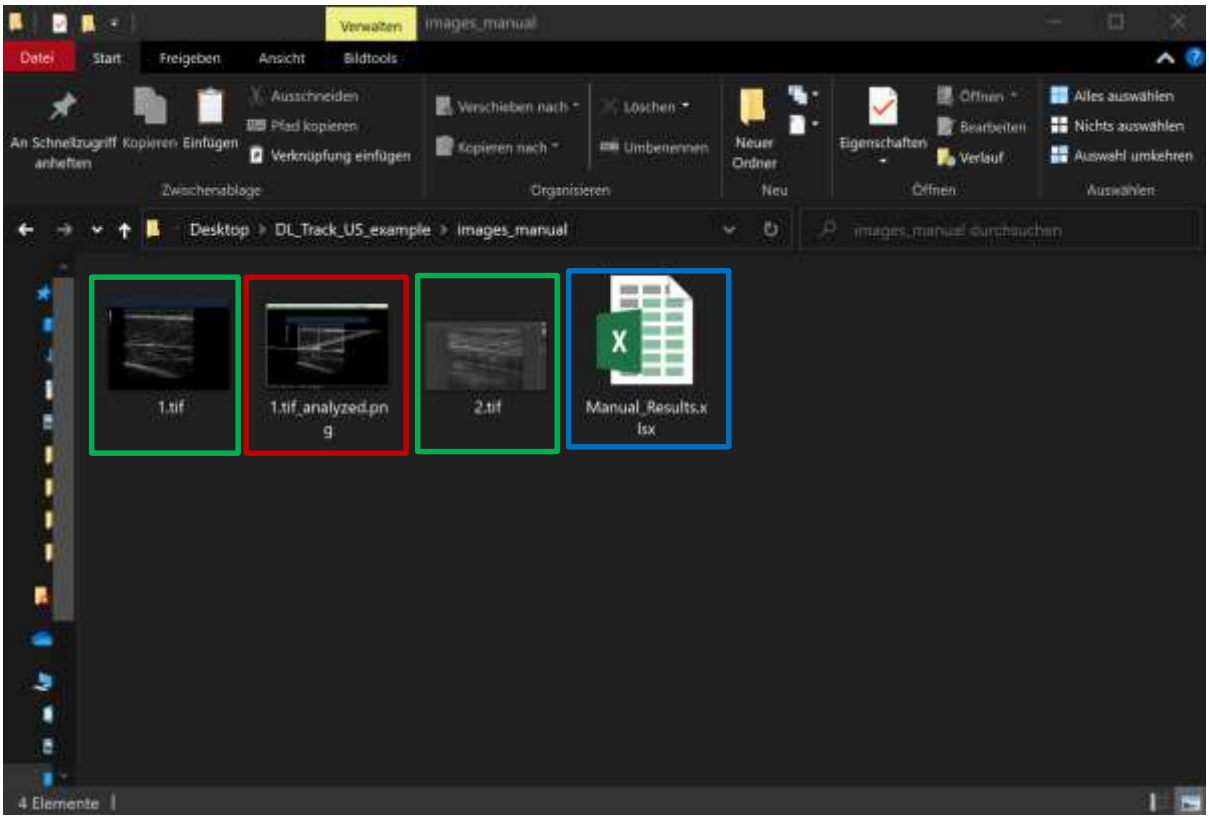
- The second button we haven't explained yet is the **Next Image** button.
  - By clicking this button, you can proceed to the next image in the input folder (in your case the "DL_Track_US_example/images_manual" folder).
  - Please remember to press the **Save Results** button prior to proceeding to the next images, otherwise you analysis results for this image will be lost.
  - When the **Next Image** button is pressed, the displayed image is updated.



- The last button we need to explain is the **Break Analysis** button.
  - Pressing this button allows you to terminate the analysis and return to the main GUI window.
  - A **messagebox** will appear asking you if you really want to stop the analysis.
  - Once the **Break Analysis** button is pressed and you answered the messagebox with "YES", the "Manual Analysis window" will be automatically closed.

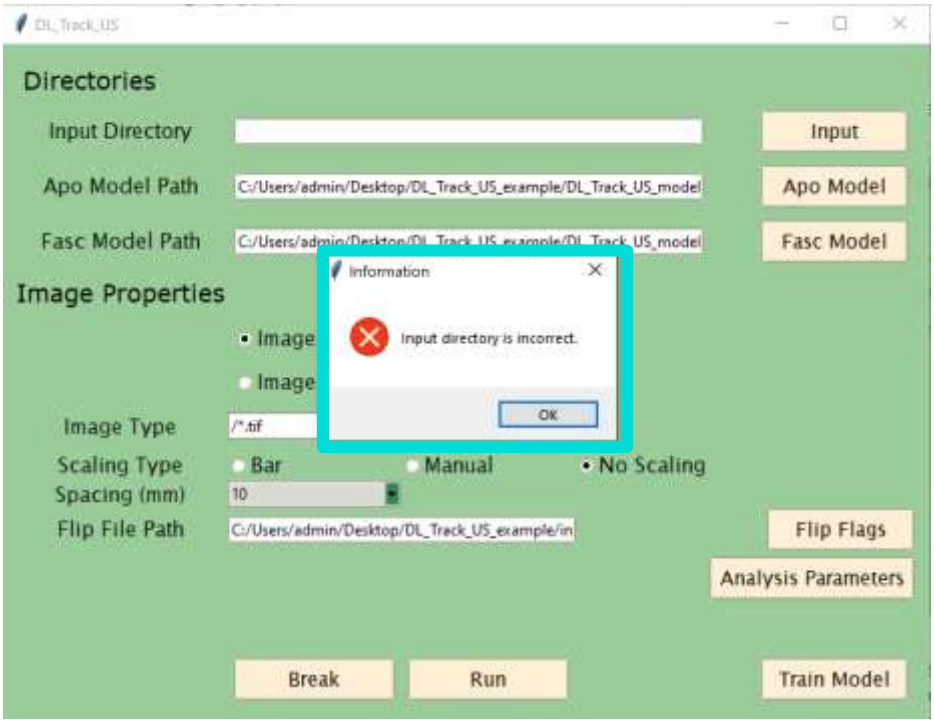


When you have saved your results clicking the very important button and followed our instructions during this tutorial, your input directory "DL_Track_US_example/images_manual" should look like this. It should contain **the images**, saved **screenshots**, as well as the **Manual_Results.xlsx** file.



6. Error Handling

Whenever an error occurs during the manual image analysis process, the DL_Track_US GUI will open a **messagebox**. This looks always similar to this:



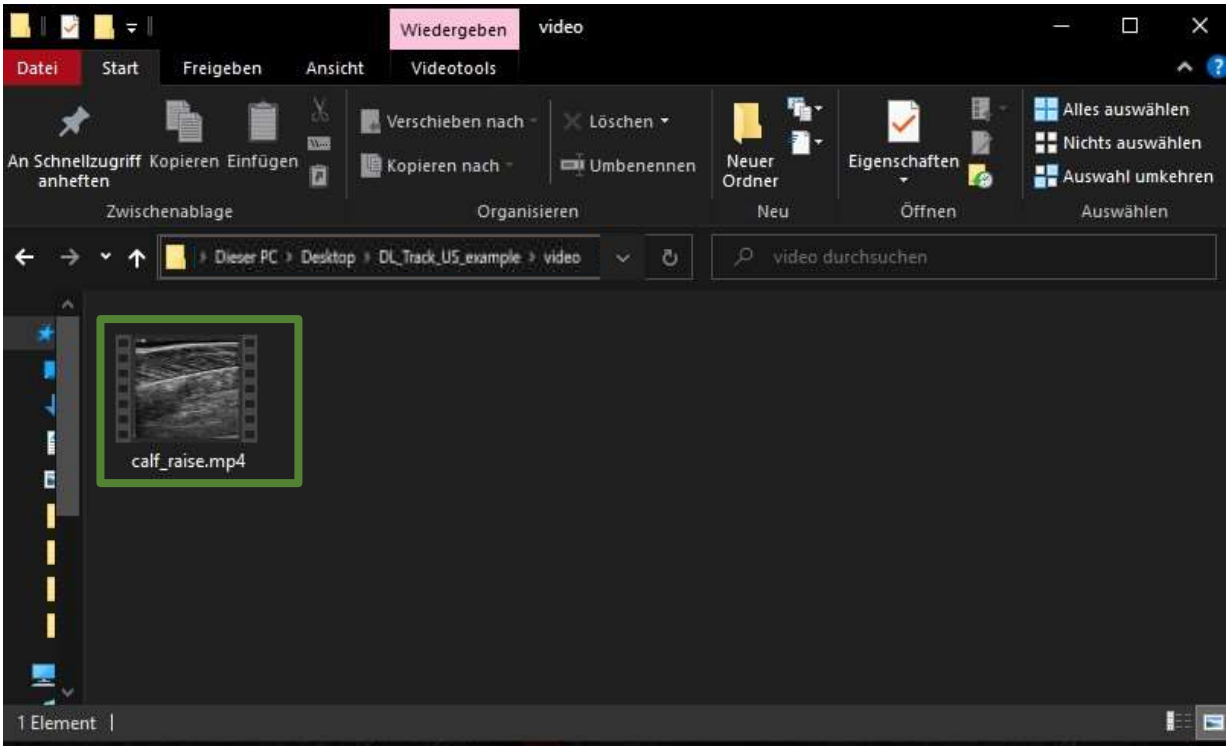
We tried to formulate these **messageboxes** as concise as possible. Just follow their instructions to fix the error and run the analysis anew. In case an error occurs that is not caught by an error **messagebox,** don't hesitate to report this in the Q&A section in the DL_Track_US discussion forum. Please take a look here how do best do this.

Automated Video Analysis

This section of the tutorial covers the automated video analysis. The videos are evaluated without user input and may be scaled. The videos should be contained in a single folder, like in the “DL_Track_US_example/videos” folder. If you haven’t downloaded this folder, please do so now (link: DL Track US - Examples & Models | Zenodo). Unzip the folder and put it somewhere accessible. We will make use of the included example files extensively during this tutorial. The automated video analysis is very similar to the automated image analysis. In fact, the inputted video is analysed frame by frame and each frame is therefore treated like an independent image. Moreover, only few analysis parameters are different between both analysis types. Once the analysis of the video file is finished, a „proc.avi“ file will be created at the directoy of the input video. The „proc.avi“ file can be openend with, i.e., VLC-Player on windows and Omni-Player on macOS. In the next few pages, we will look at every required step to successfully perform automated video analysis with DL_Track_US.

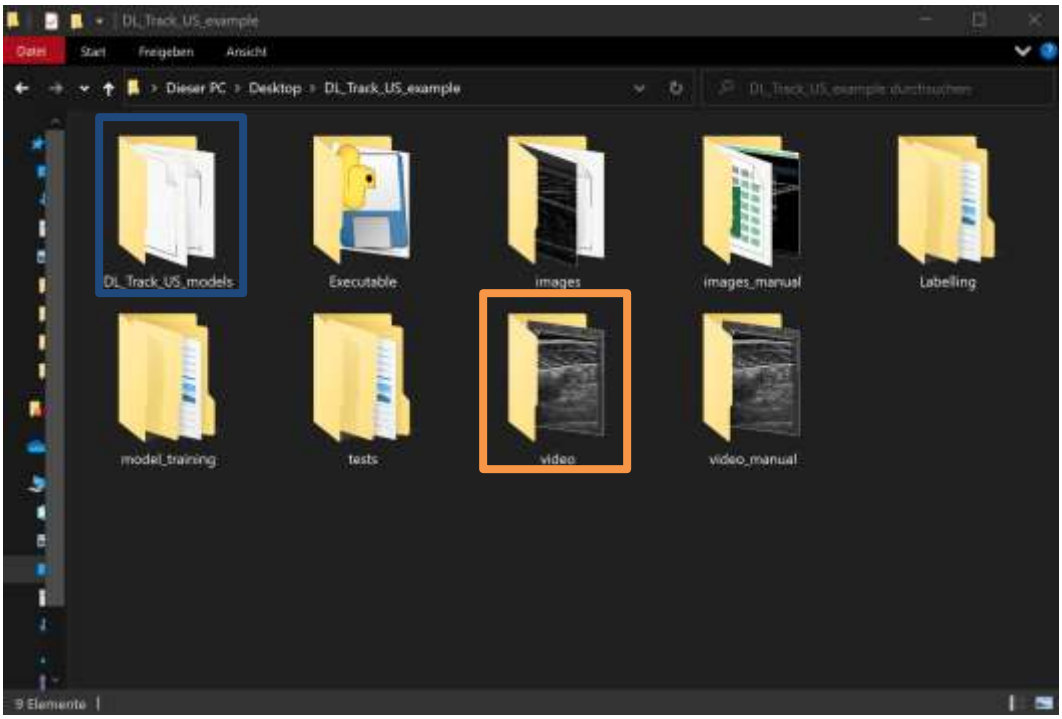
1. Creating Video and Network Directories

- In order for DL_Track_US to recognize your videos, they should best be in a single folder.



- The “DL_Track_US_example/videos“ folder contains **one video.**

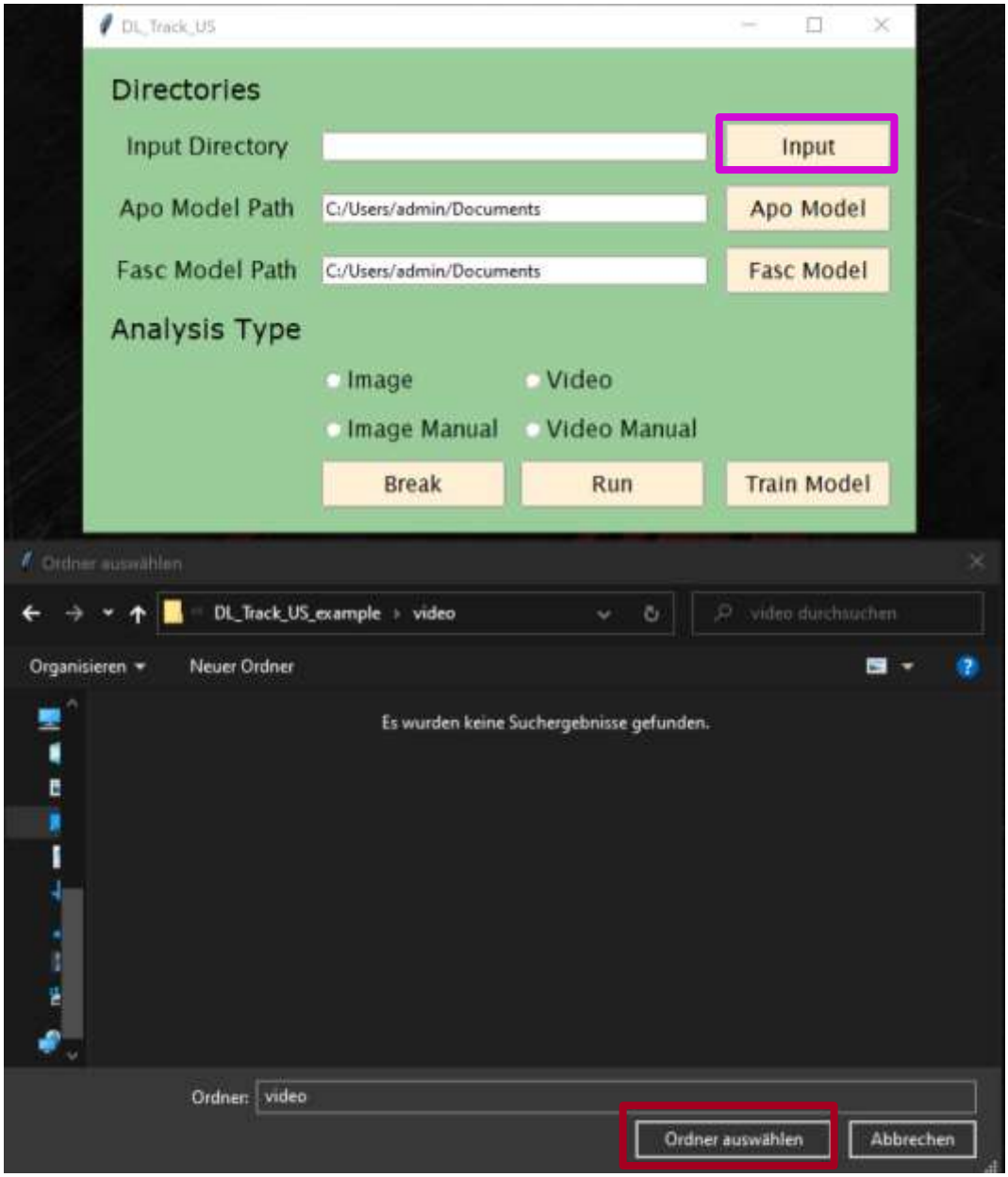
- The **folder containing the video** is the “DL_Track_US_example/video” folder.
- The pre-trained **aponeurosis and fascicle neural networks** are located in the “DL_Track_US_example/models” folder.
- You can make use of these **neural networks** later as well, when you analyse your own videos outside of this tutorial.



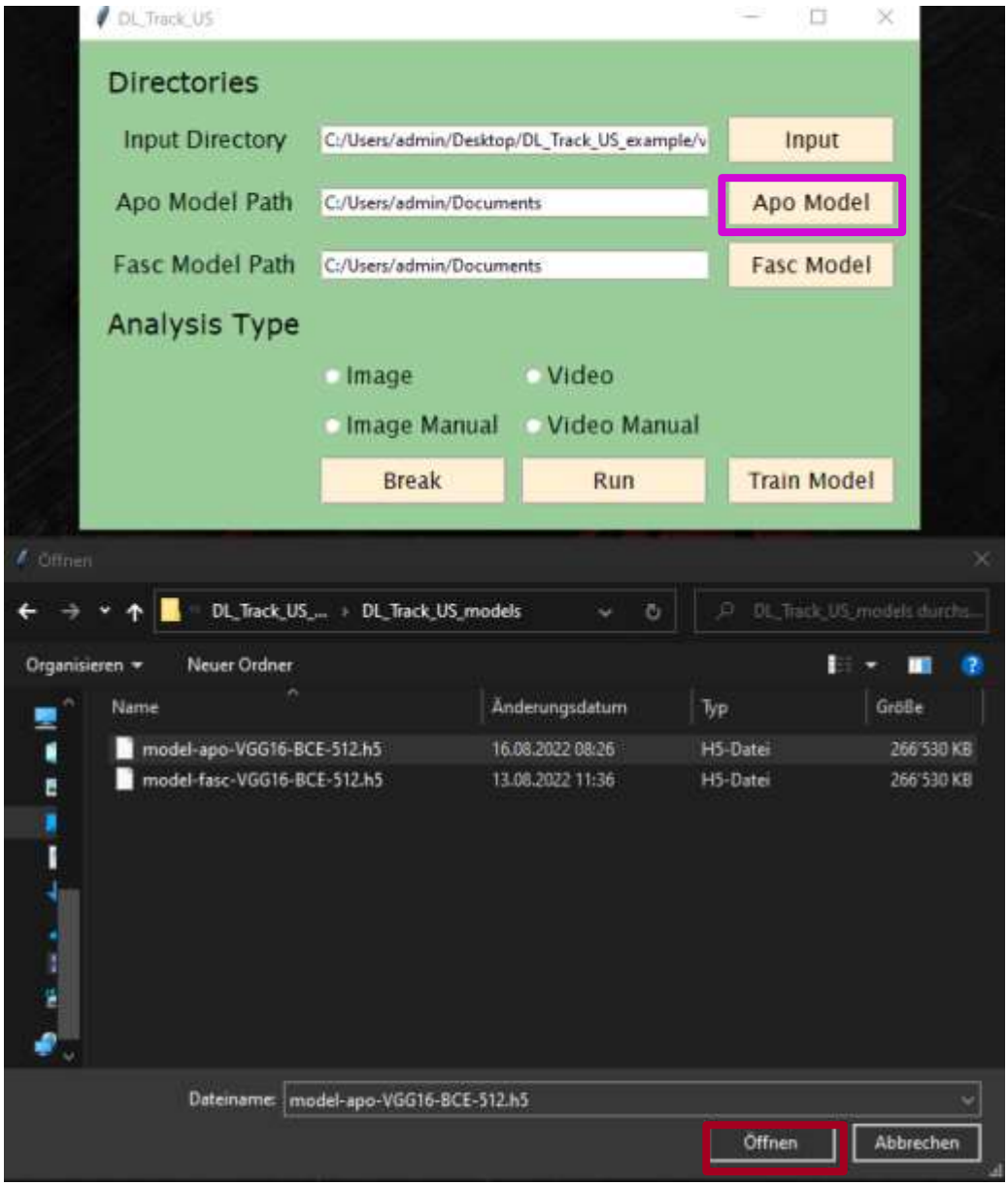
2. Specifying Input Directories in the GUI

Once the GUI is openend, the first step of every analysis type in DL_Track_US is to specify the input directories in the graphical user interface (GUI).

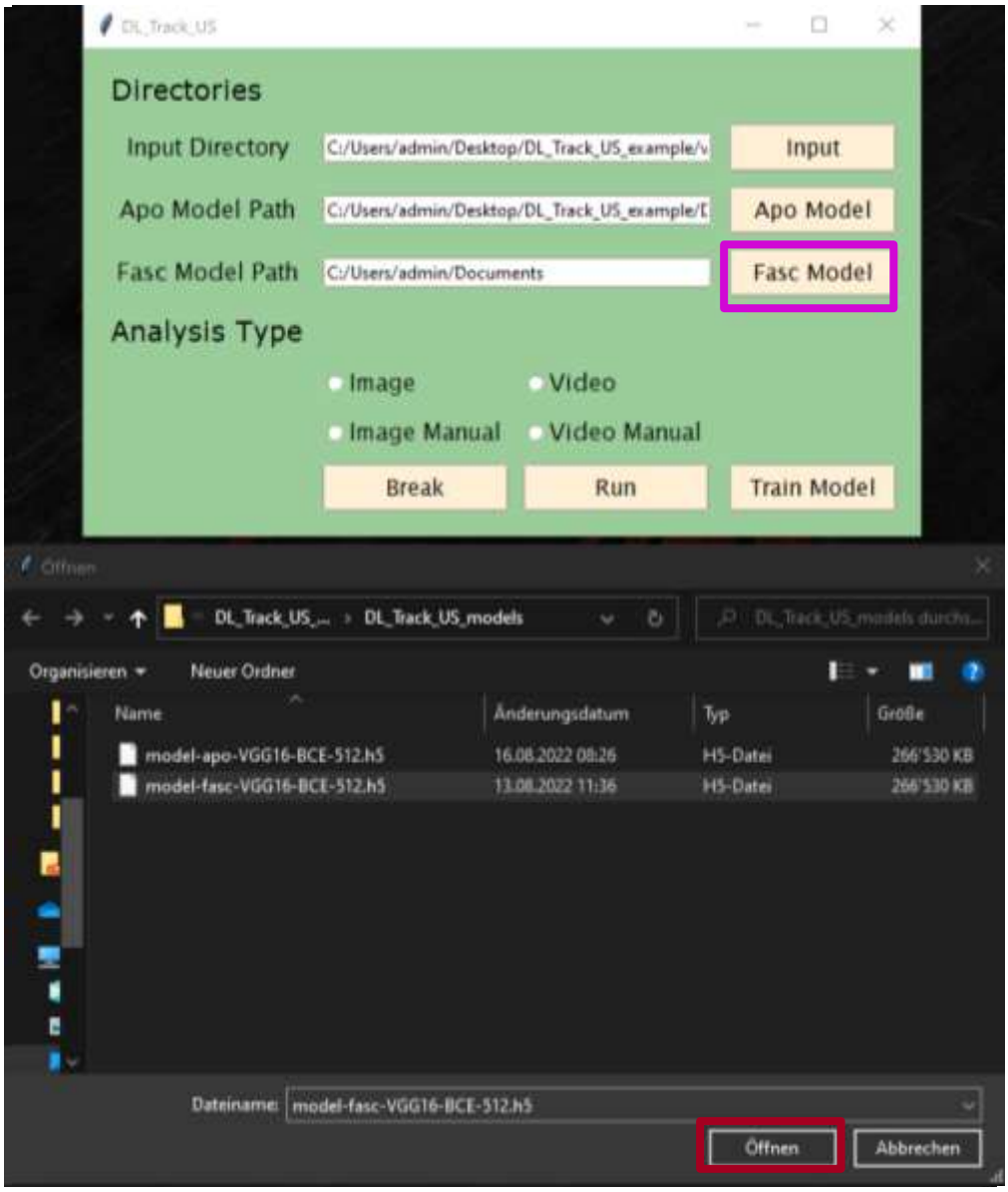
- Start the analysis with specifying the path to the folder containing the **video** to be analysed.
    - Remember this was the folder "DL_Track_US_example/**video**". By clicking on the **Input** button in the GUI a selection window opens were you need to select the images folder.
    - Click **select folder** to specify the path in the GUI.



- Now, you will specify the absolute path to the **aponeurosis neural network**.
  - Remember that the model is in the “DL_Track_US_example/**models**” folder.
  - By clicking on the **Apo Model** button in the GUI a selection window opens were you need to select the **aponeurosis neural network** in the models folder.
  - Click **open** to specify the path to the **aponeurosis neural network** in the GUI.



- Next, you will specify the absolute path to the **fascicle neural network**.
  - The model is in the "DL_Track_US_example/**models**" folder.
  - By clicking on the **Fasc Model** button in the GUI a selection window opens were you need to select the **fascicle neural network** in the models folder.
  - Click **open** to specify the path to the **fascicle neural network** in the GUI.

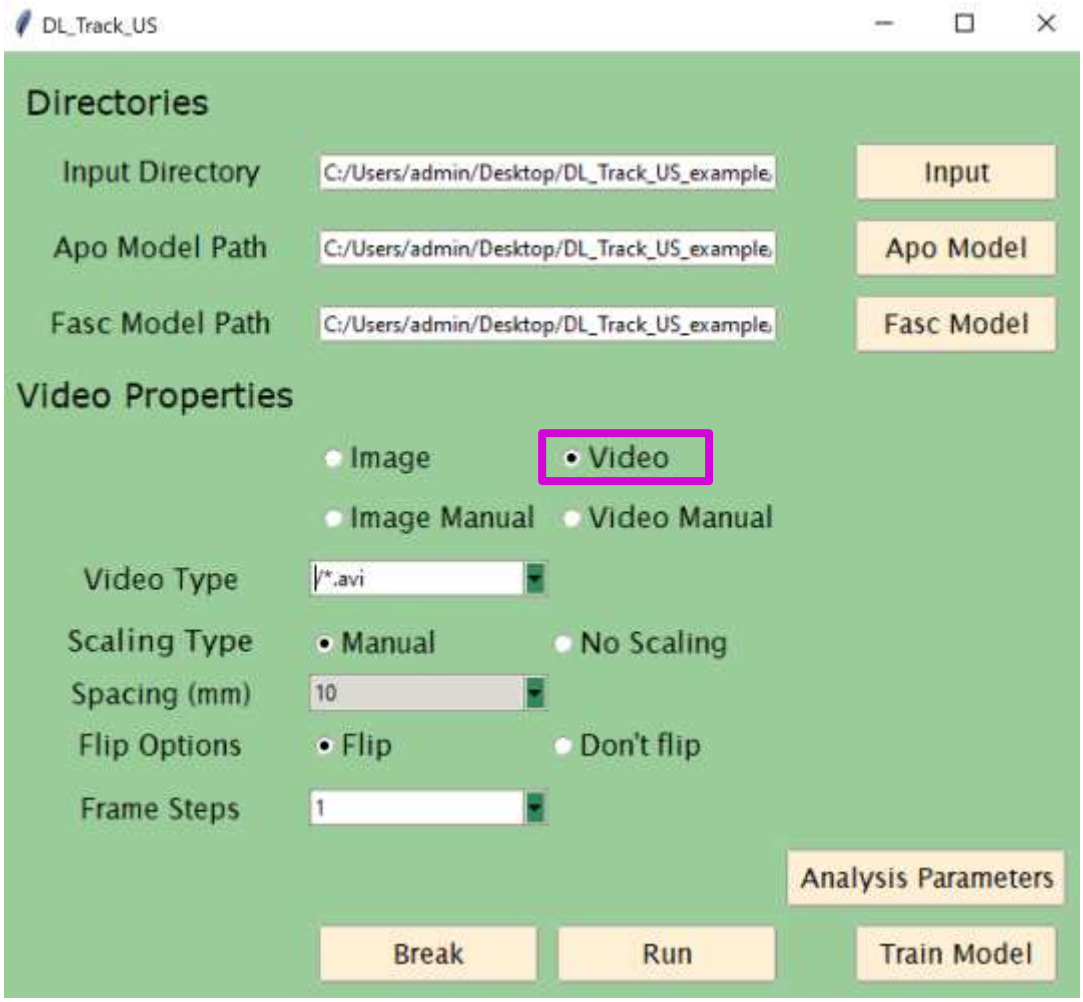


In the next section you will specify all relevant analysis parameters, including the analysis type. We will also explain what each parameter is used for.

3. Specifying Relevant Parameters

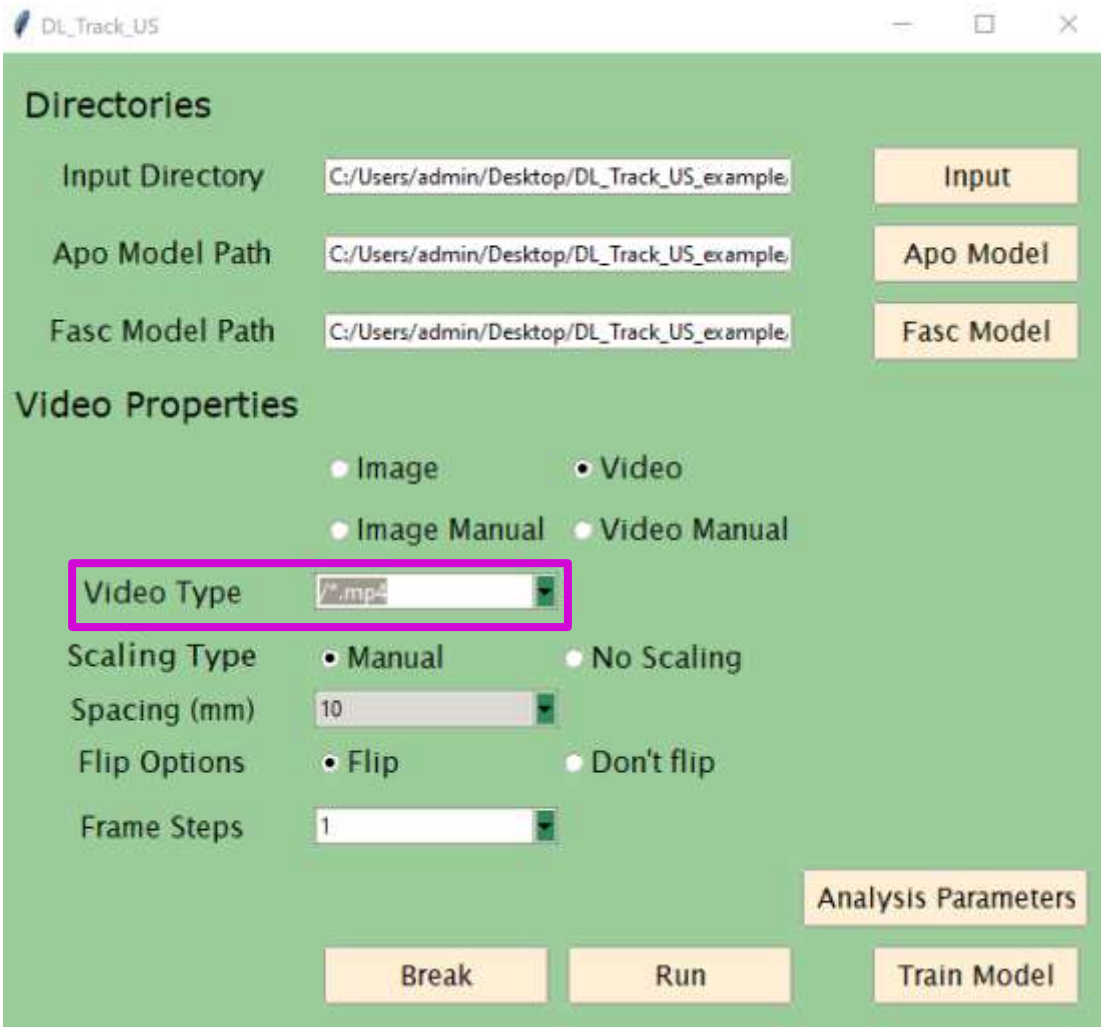
As a first step, you will select the right analysis type in the GUI.

- Please select the **Video** radiobutton.
- You can see that the GUI unfolds and several other parameters appear.
- You will set those in the next steps on the next page.



You now need to specify the **Video Type**.

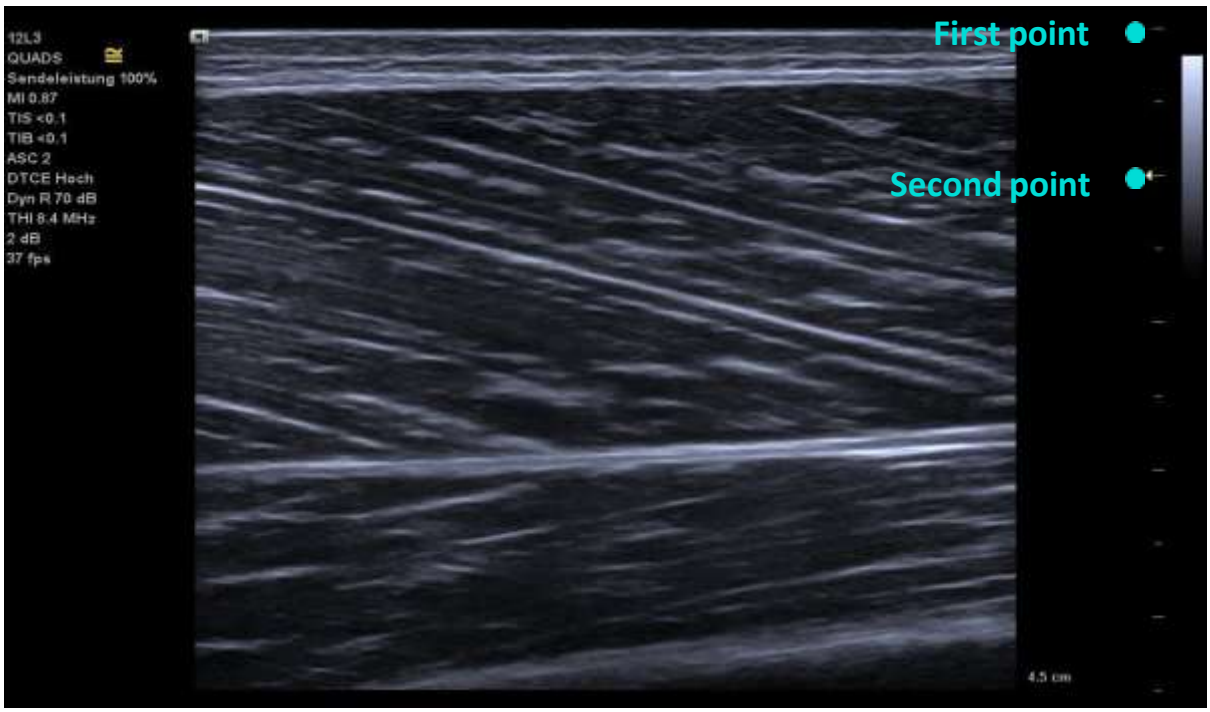
- The ending of the **Video Type** must match the ending of your **videos**, otherwise no files are found by DL_Track_US.
- You can either select a pre-specified ending from the dropdown list or type your own ending.
- Please keep the formatting similar to those **Video Type** provided in the dropdown list.
- The **video** in the "DL_Track_US_example/**video**" folder are of the **Video Type** ".mp4". Thus, you should select the "/*.mp4" **Video Type.**



Subsequently, you need to specify the video **Scaling Type**.

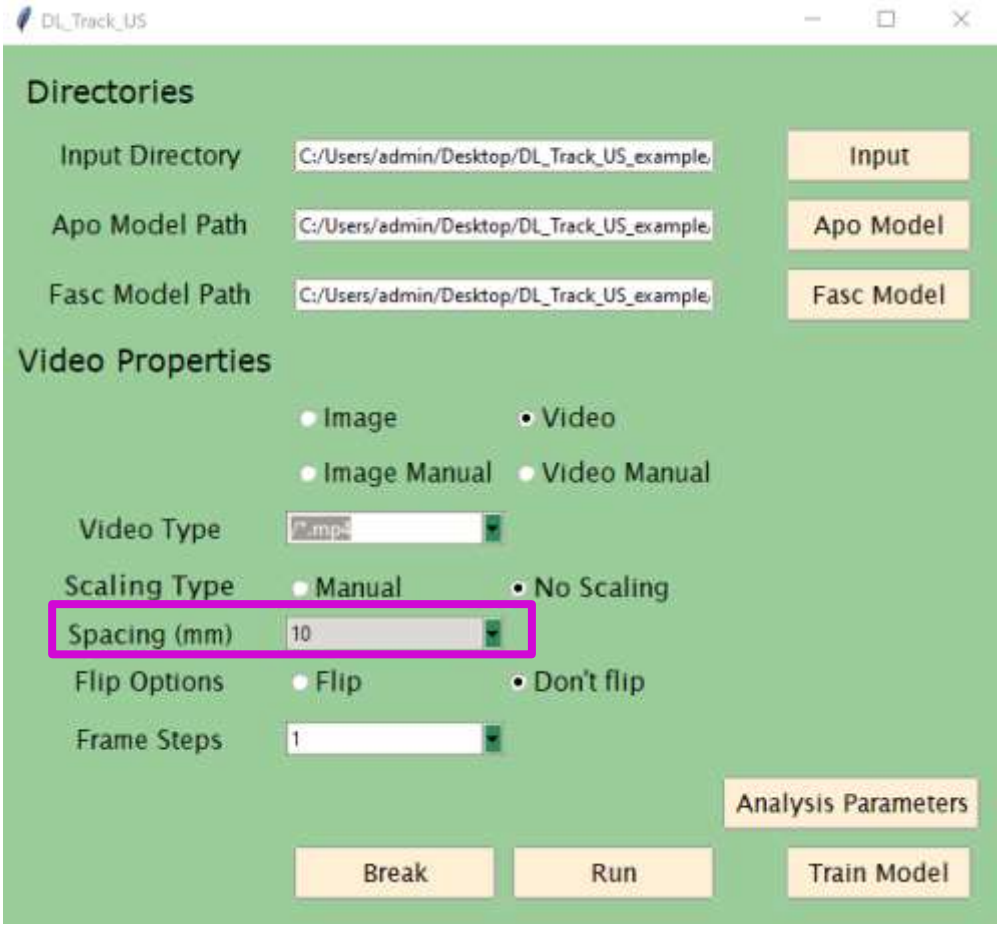
- Scaling in general has the advantage that the resulting estimated muscle architectural features are in centimetre units rather than pixel units.
- There are two **Scaling Types** in the DL_Track_US package.
- For this tutorial however, you will select the **"No Scaling"** option as displayed below. We will explain the other **Scaling Type** on the next.

- The other **Scaling Types** is “Manual”.
  - This **Scaling Type** requires input from the user.
  - When you choose “Manual” as your Scaling type, you need to manually place **two points** on the first video frame using the left mouse button.
  - This step is similar to the “Manual” scaling option for automated and manual image analysis.

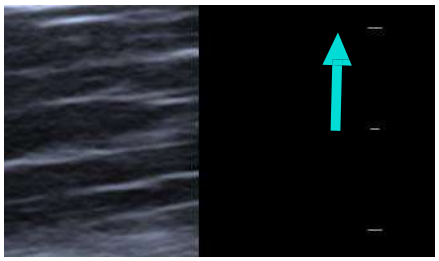


  - Just click one time with your left mouse button to record the **first point** (nothing will be displayed on the video frames during actual analysis).
  - Place the **second point** at a known distance of either 5, 10, 15 or 20 millimetre.
  - The distance you chose must be represented in the Scaling (see next page) parameter in the GUI.

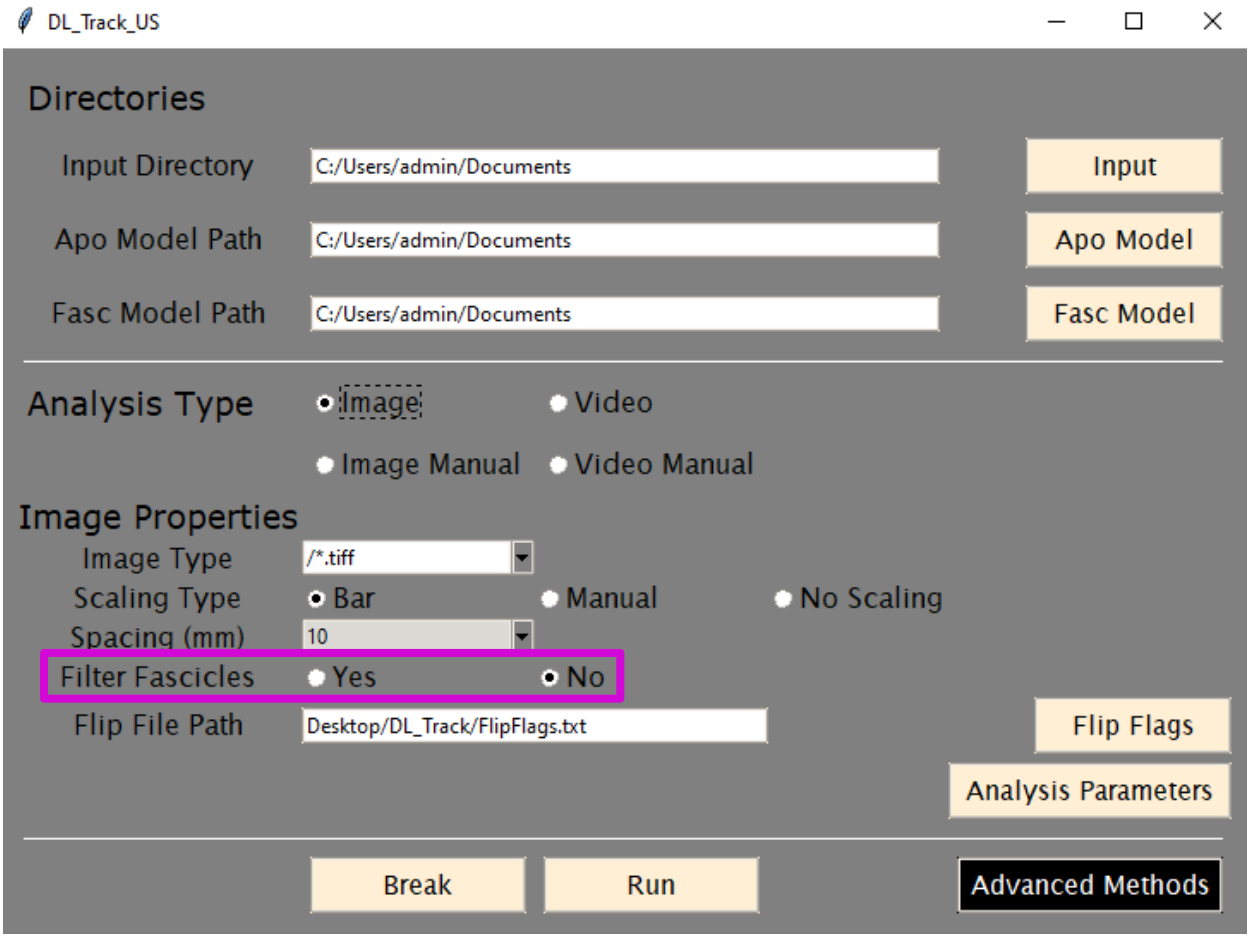
- Whenever you use **“Manual”** as your Scaling Type, please make sure that the minimum distance between the scaling bars or the known distance between your manually specified points is represented in the **Spacing** parameter.



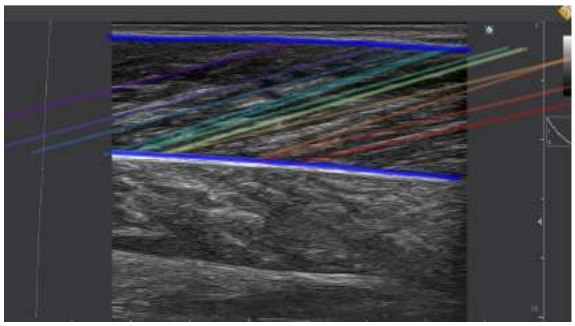
- You can select the **Spacing** parameter only from the dropdown list as 5, 10, 15 or 20 millimetre. For this tutorial it is not necessary to select anything, as the **Spacing** parameter is not used during an analysis with Scaling Type **“No Scaling”**.
- The minimal distance is simply the **distance** in millimeter between the two nearest scaling bars in the frame. If you do not know this **distance**, please use **“Manual”** or **“No Scaling”** Scaling Type. For example in the frame from before, the **distance** between the nearest bars is 5 millimetre.

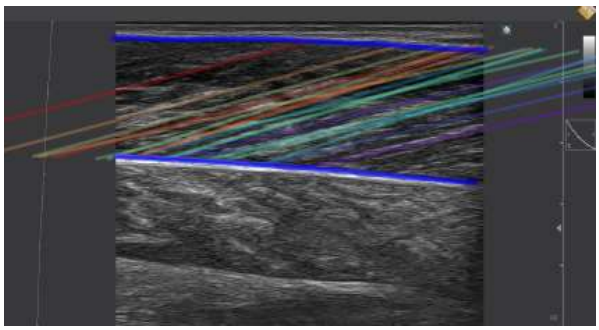


- In version 0.2.1 we introduced a new feature to DL_Track_US, called the **Filter Fascicle** option.
- Here, you have two options, **“YES”** or **“NO”**.
- Using **“YES”** all fascicles that overlap will be removed.



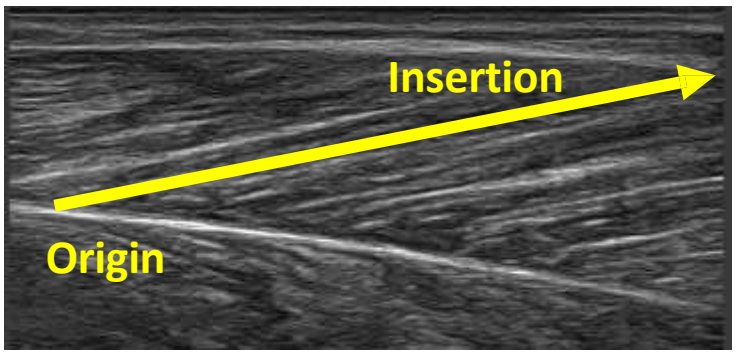
Here are some results demonstrating the difference in an image, for video frames the effect would be similar.





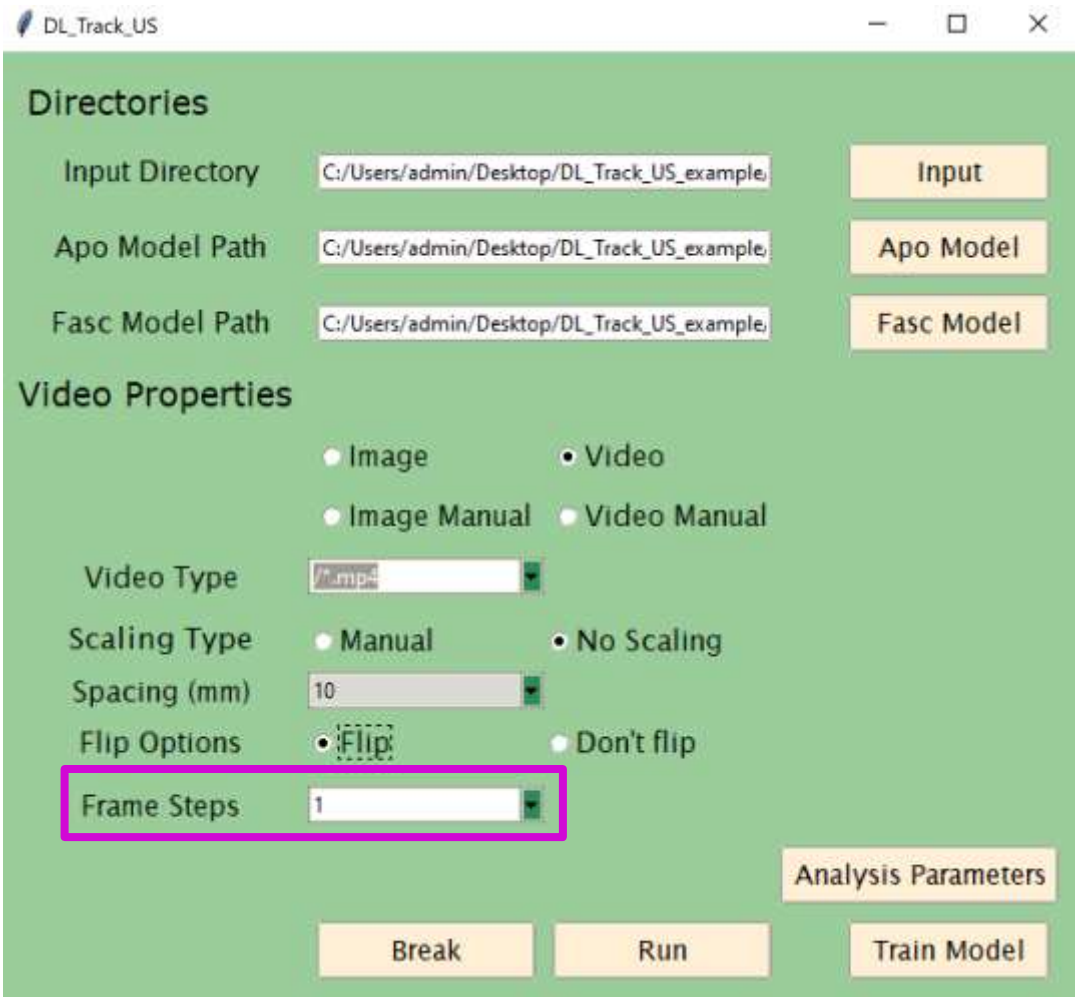
Another parameter that you need to specify is the **Flip Options** parameters.

- The **Flip Options** parameter determines if the whole **video** is flipped along the vertical axis. **"Flip"** stands for flipping the video, whereas **"Don't Flip"** means please do not flip the video.
- The example **video** must be flipped.
- Its fascicle orientation is **incorrect**, with fascicles originating at the bottom right and inserting on the top left.
- Below is a visual representation of a **correct** fascicle orientation.
- The fascicles are originating at the bottom left and are inserting on the top right.
- Note that all videos in the specified input folder, in this case the DL_Track_US_example/**video**" folder, MUST have the same fascicle orientation, since the **Flip Option** is applied to all of them.



The next step is to specify the **Frame Steps**.

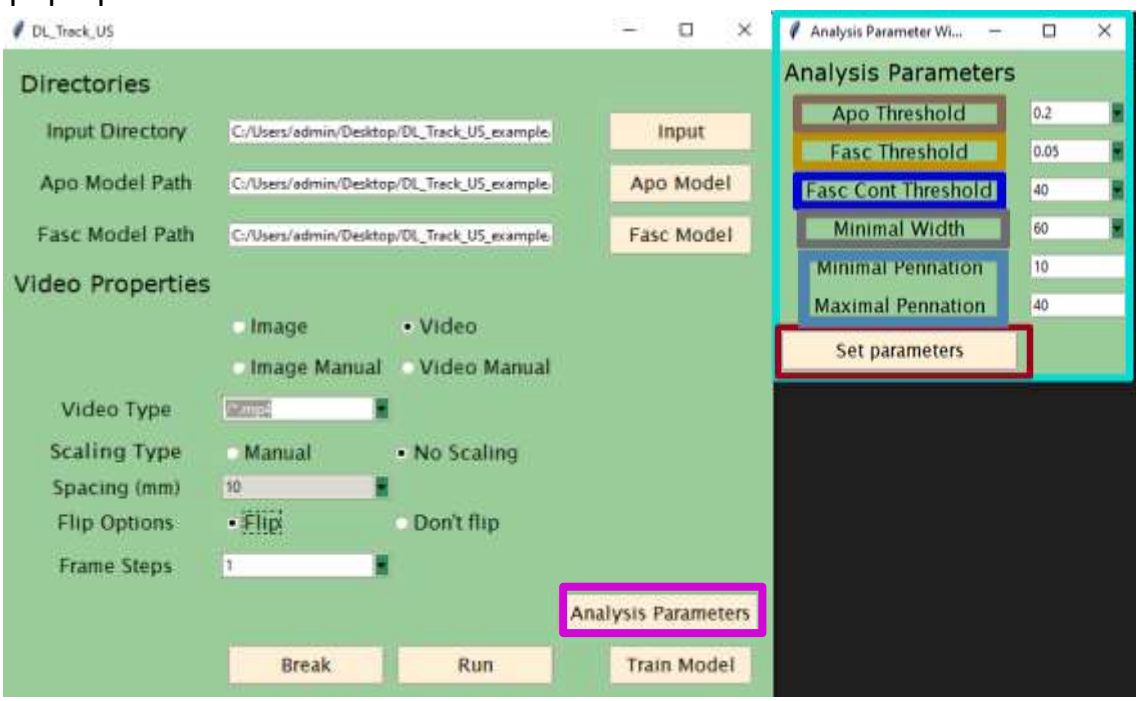
- You can either select a pre-specified **Frame Step** from the dropdown list or type your **Frame Step**.
- The **Frame Step** is used during the analysis as a step size while iterating through all the frames in a video.
- In this tutorial you should specify a **Frame Step** of 1. This means that every video frame is analysed. With a **Frame Step** of 3, every 3rd frame is analysed. With a **Frame Step** of 10, every 10th frame an so on.
- Although **information is lost** when you skip frames during the analysis, it also **reduces the overall analysis time**.



4. Specifying Analysis Parameters

As a last step, you need to specify the analysis parameters for the **aponeurosis and fascicle neural networks.**

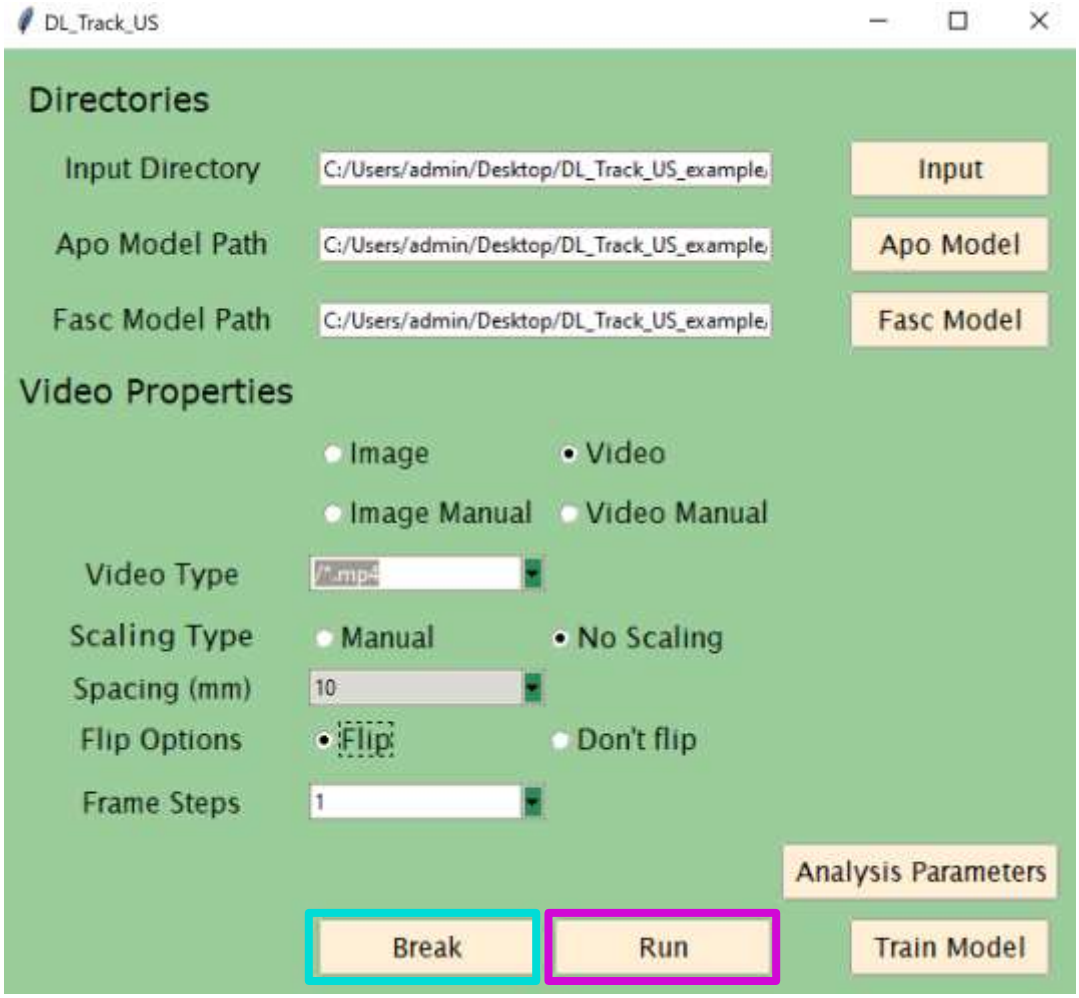
- When you press the **Analysis Parameters** button, a separate window will pop-up.



  - The **Apo Threshold** parameters determines the threshold of the minimal acceptable probability by which a pixel is predicted as aponeurosis. The lower, the more pixels will be classified as aponeurosis.
  - The **Fasc Threshold** is the same thing just for fascicle segments.
  - The lower the **Fasc Cont Threshold**, the shorted the minimal acceptable length of detected fascicle segments to be included in the results.
  - The **Minimal Width** determined the minimal acceptable distance between superficial and deep aponeurosis.
  - **Minimal and Maximal Pennation** describe the respective minimal and maximal pennation angle that is physiologically possible in the analysed video frame/muscle.
- For this tutorial, you can leave all parameters the way they are.
- You can set the parameters by clicking the **Set parameters** button, the **Analysis Parameter window** will then close automatically.
- Please make sure to adapt these parameters according to your images in analyses outside of this tutorial. For future analyses, it's best you test the ideal parameter configuration in a small sample prior to the actual analysis.

5. Running / Breaking DL_Track_US

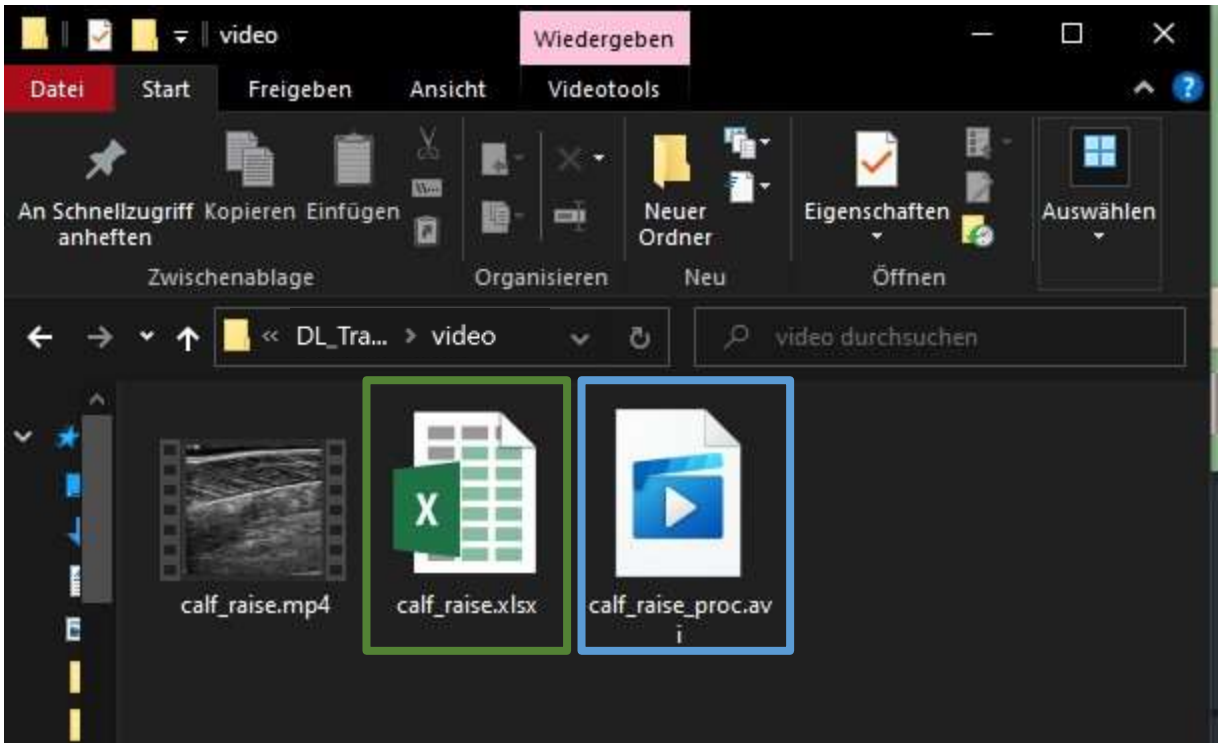
- You can start the analysis by clicking the **Run** button in the main GUI window.



- Moreover, you can see that there is a **Break** button placed in the GUI as well.
- Clicking the Break button allows you to stop the analysis at any point. The currently evaluated frame will be processed and then the analysis is terminated.

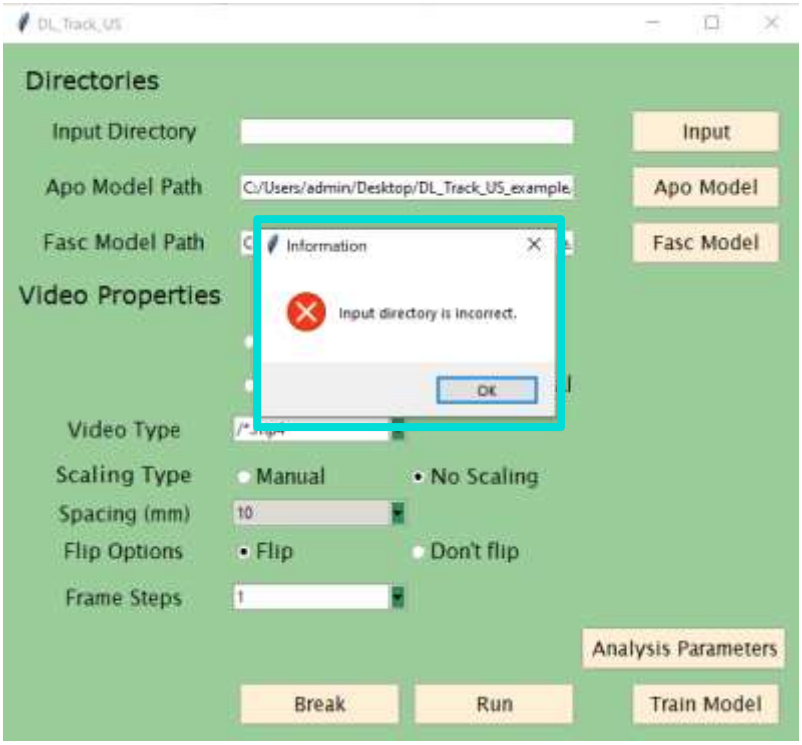
Subsequently to clicking the Run button in the main GUI, navigate again to the “DL_Track_US_example/video”.

- You will see that two files will be / have been created, **calf_raise_proc.avi** and **calf_raise.xlsx**.
- The **calf_raise_proc.avi** file contains each the input video with overlaid segmented fascicles and aponeurosis. This file allows you to visually inspect the model outputs.
- The **calf_raise.xlsx** file contains the actual architectural parameter estimates for each video frame. There, all detected muscle fascicle lengths and pennation angles as well a the calculated muscle thickness will be displayed. Each video frame is displayed in a separate row.
- Note that the **calf_raise_proc.avi** file can be opened only after the **calf_raise.xlsx**. was created.



6. Error Handling

Whenever an error occurs during the video analysis process, the DL_Track_US GUI will open a **messagebox**. This looks always similar to this:



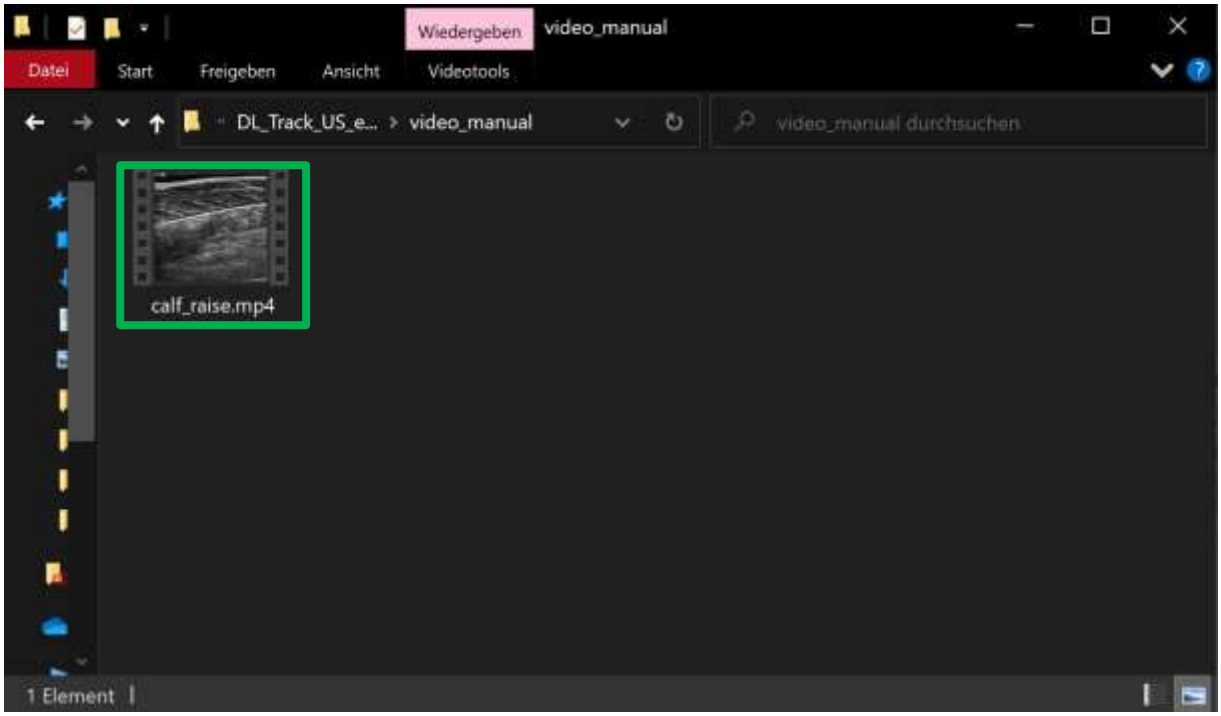
We tried to formulate these **messageboxes** as concise as possible. Just follow their instructions to fix the error and run the analysis anew. In case an error occurs that is not caught by an error **messagebox,** don't hesitate to report this in the Q&A section in the DL_Track_US discussion forum. Please take a look here how do best do this.

Manual Video Analysis

The next and last analysis type this tutorial covers is the manual video analysis. The video frames are evaluated manually by drawing the muscle thickness, fascicle length and pennation angles directly on the Image. For this type of analysis, single videos are a prerequisite. These videos should be contained in a single folder, like in the "DL_Track_US_example/videos_manual" folder. If you haven't downloaded this folder, please do so now (link: DL Track US - Examples & Models | Zenodo). Unzip the folder and put it somewhere accessible. We will make use of the included example files extensively during this tutorial. The manual video analysis type is identical to the manual image analysis type. The only difference is that the absolute video path must be specified instead of the File Type. The video is first converted and all the contained frames are separately stored as single images. Then, each frame image is analysed separately. In the next few pages, we will look at every required step to successfully perform manual video analysis with DL_Track_US.

1. Creating a Video Directory

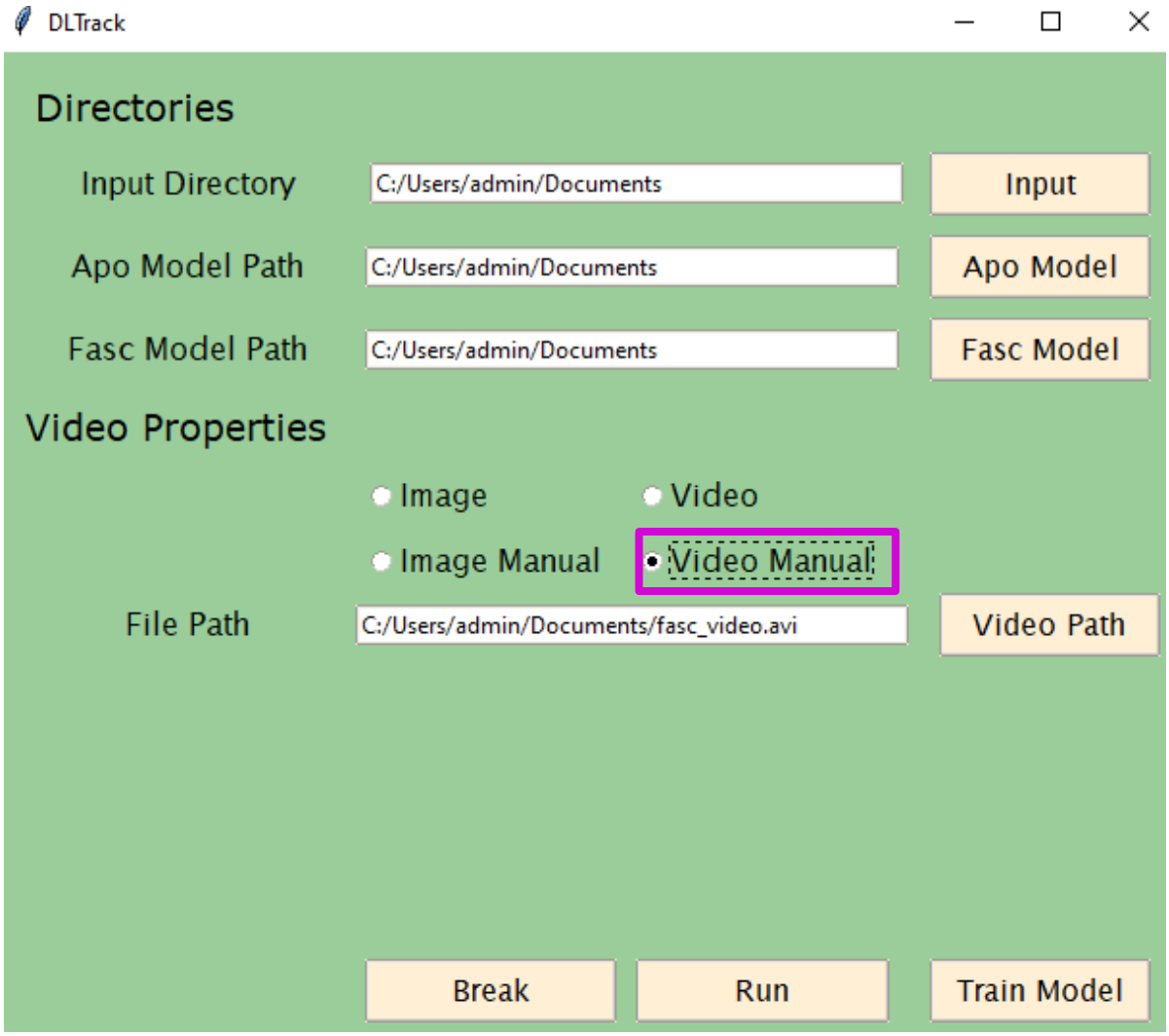
- All **videos** to be analyzed should be in a single folder.



- The “DL_Track_US_example/video_manual“ folder contains one **video** file.

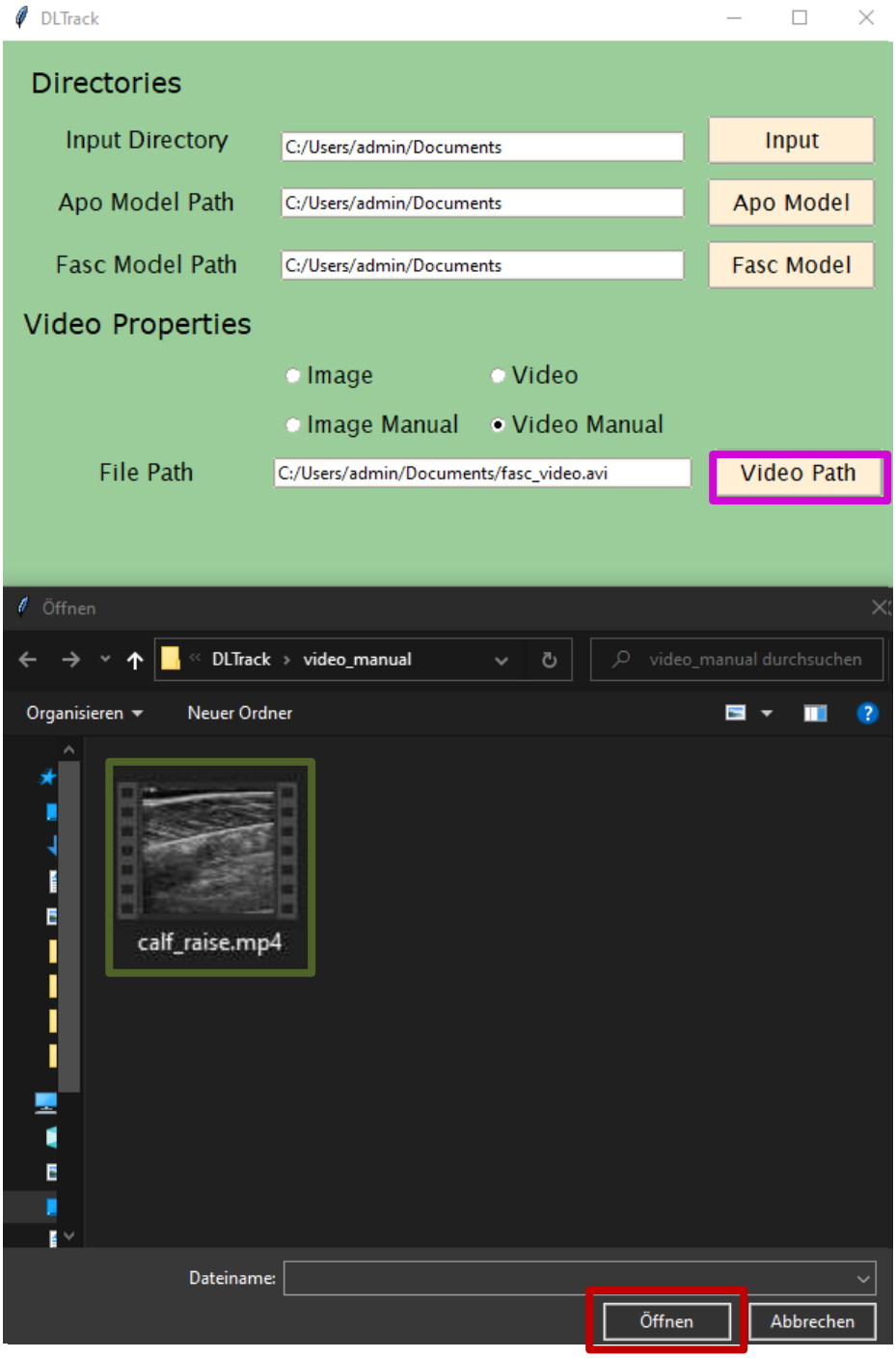
2. Specifying Relevant Parameters

- Please select the **Video Manual** radiobutton.
- You can see that the GUI unfolds and another parameter appears.
- You will set this one in the next step on the next page.

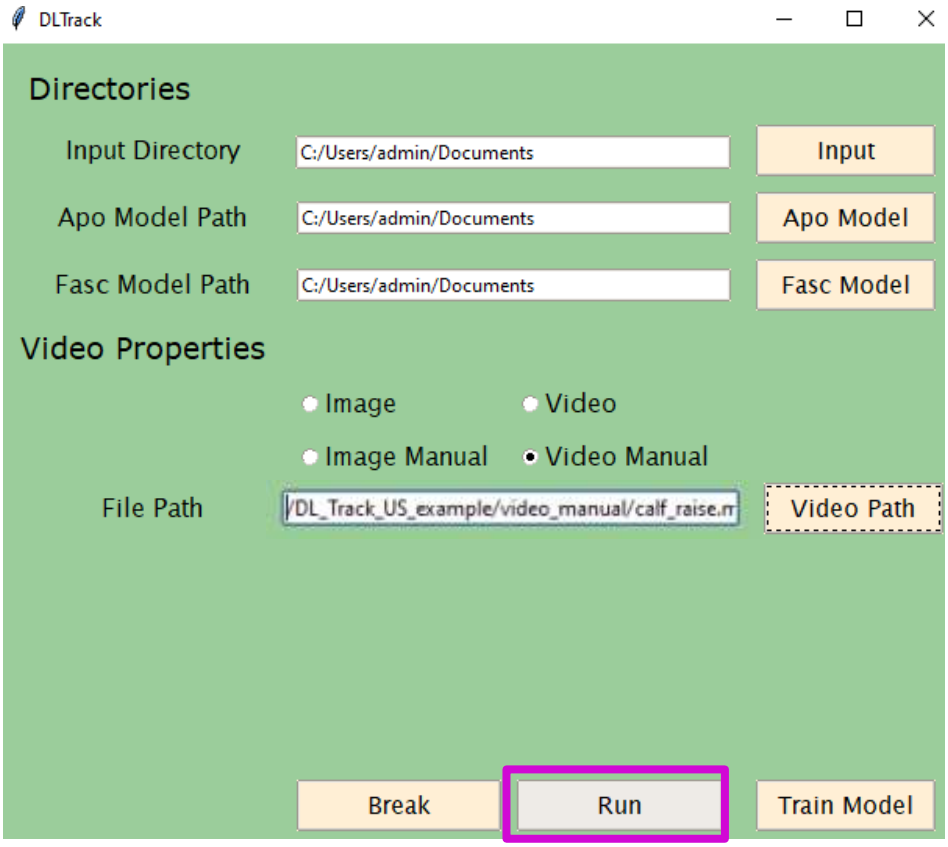


Next, you need to specify the absolute **File Path** of the **video file** to be analysed.

- The example **video file** is placed in the "DL_Track_US_example/**video_manual**" folder.
- By clicking on the **Video Path** button in the GUI, a selection window opens were you need to select the example **video file** in the **video_manual**.
- Click **open** to specify the path to the **video file** in the GUI.



- You can start the analysis by clicking the **Run** button in the main GUI.



- Once you clicked the Run button, the "Manual Analysis window" will pop up.
- From here, all further steps are identical with the manual image analysis.
- The only difference though is that in the folder of the inputted video, a new folder is created containing all the single image frames.
- The scaling of the image, extending of the aponeuroses, single segment muscle thickness measurements, three segment muscle fascicle measurement and two segment pennation angle measurement are identical.
- Saving the results (with the very important button), continuing to the next image frame, terminating the analysis process and error handling is identical.
- **Therefore, we kindly refer you to section 4 of this tutorial Manual Image Analysis** (because we don't want to repeat ourselves) **to see how the all the architectural parameters are analysed.**

Training Your Own Networks

The DL_Track_US package GUI includes the possibility to train your own neural networks. We we will demonstrate how to do this, with a few notes at the beginning:

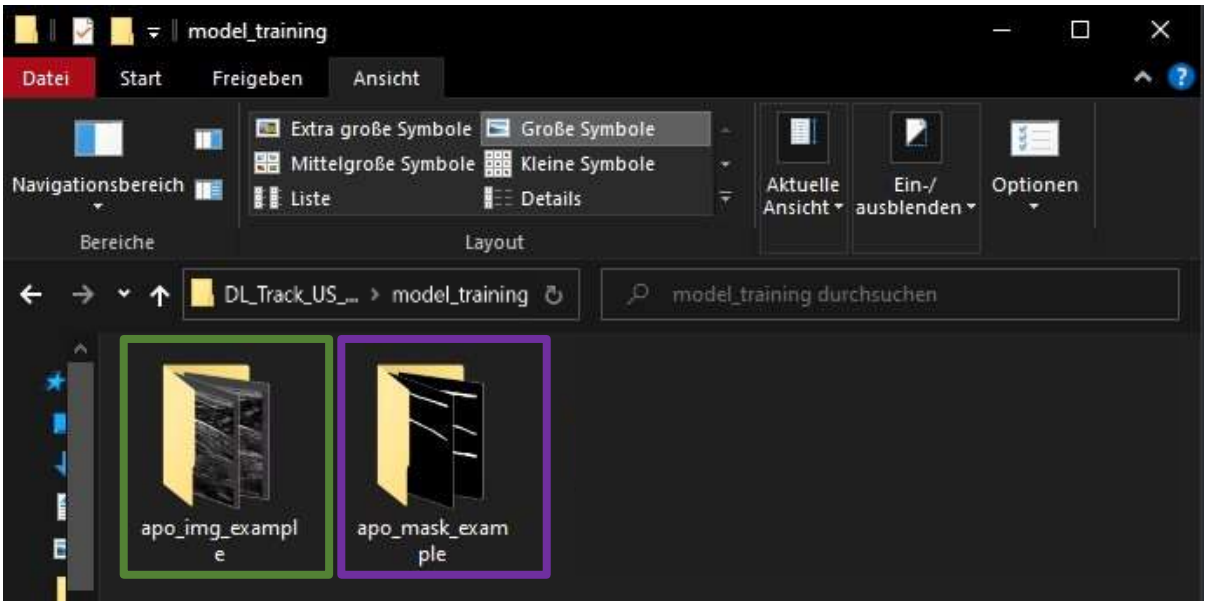
- It is advantageous to have a working GPU setup, otherwise model training will take much longer. Take a look at our Github repository for further instructions.
- If you don't have any experience with training deep neural networks, please refer to this course. We advise you to start with the pre-defined settings. However, DL_Track_US does not allow to change the architecture of the trained neural networks.

The paired original images and labeled masks required for the network training are located in the "DL_Track_US_example/model_training" folder. If you haven't downloaded this folder, please do so now (link: DL_Track_US - Examples & Models | Zenodo). Unzip the folder and put it somewhere accessible. We will demonstrate how to train a model that segments the muscle aponeuroses.

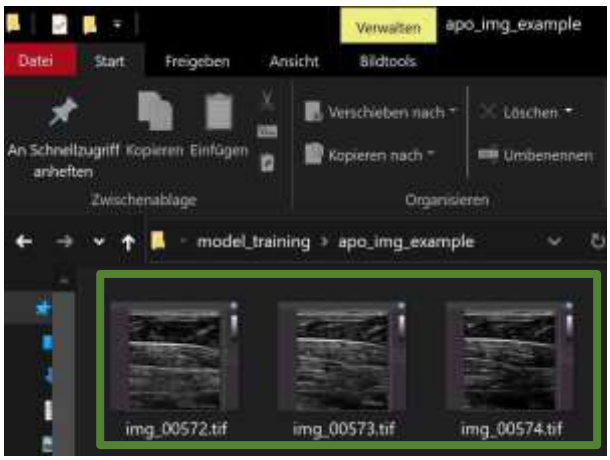
Please keep in mind that the model training process will be illustrated by training a model for aponeurosis segmentation. The process is exactly the same for training a fascicle segmentation model. Solely the images and masks should then contain fascicles and fascicle labels.

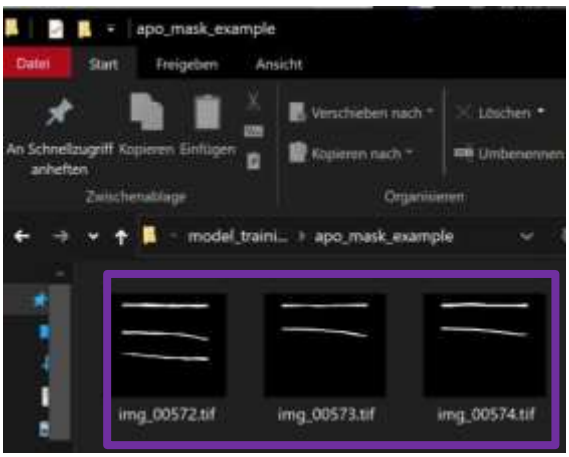
1. Data Preparation and Image Labeling

- Your prepared **aponeurosis images** and **aponeurosis masks** should be in different folders.



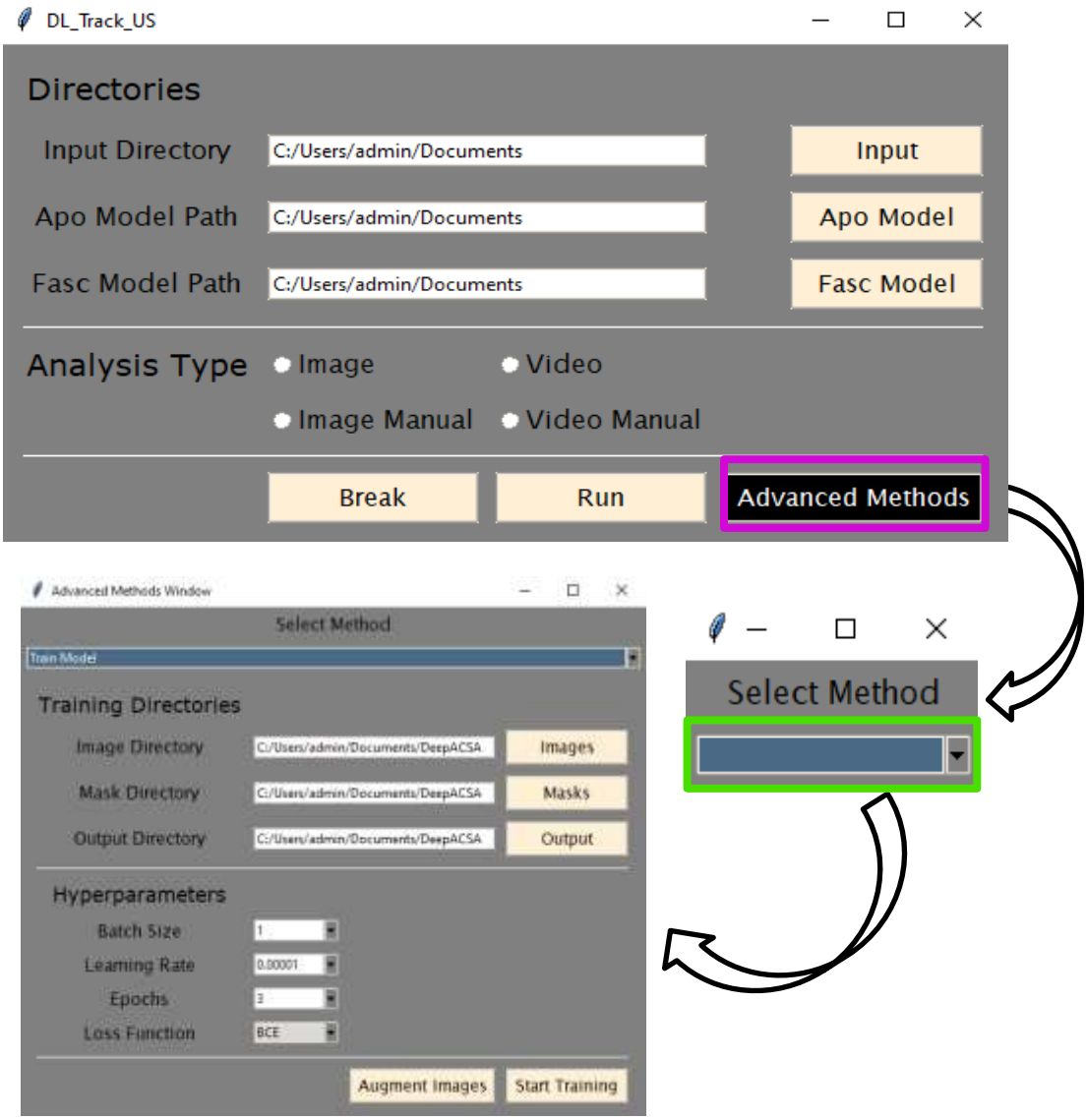
- The «DL_Track_US_example/model_training" folder contains to subfolders, **apo_img_example"** and **"apo_mask_example"**.
- The original images are located in the **"apo_img_example"** folder.
- The corresponding masks are located in the **"apo_maks_example"** folder.
- We advise you to keep a similar folder structure when you train your own models outside of this tutorial.
- Below you can see that the **original image** and the **corresponding masks** have exactly the same name. This is **SUPER MEGA** important. Otherwise, the model is trained using the wrong masks for the images.





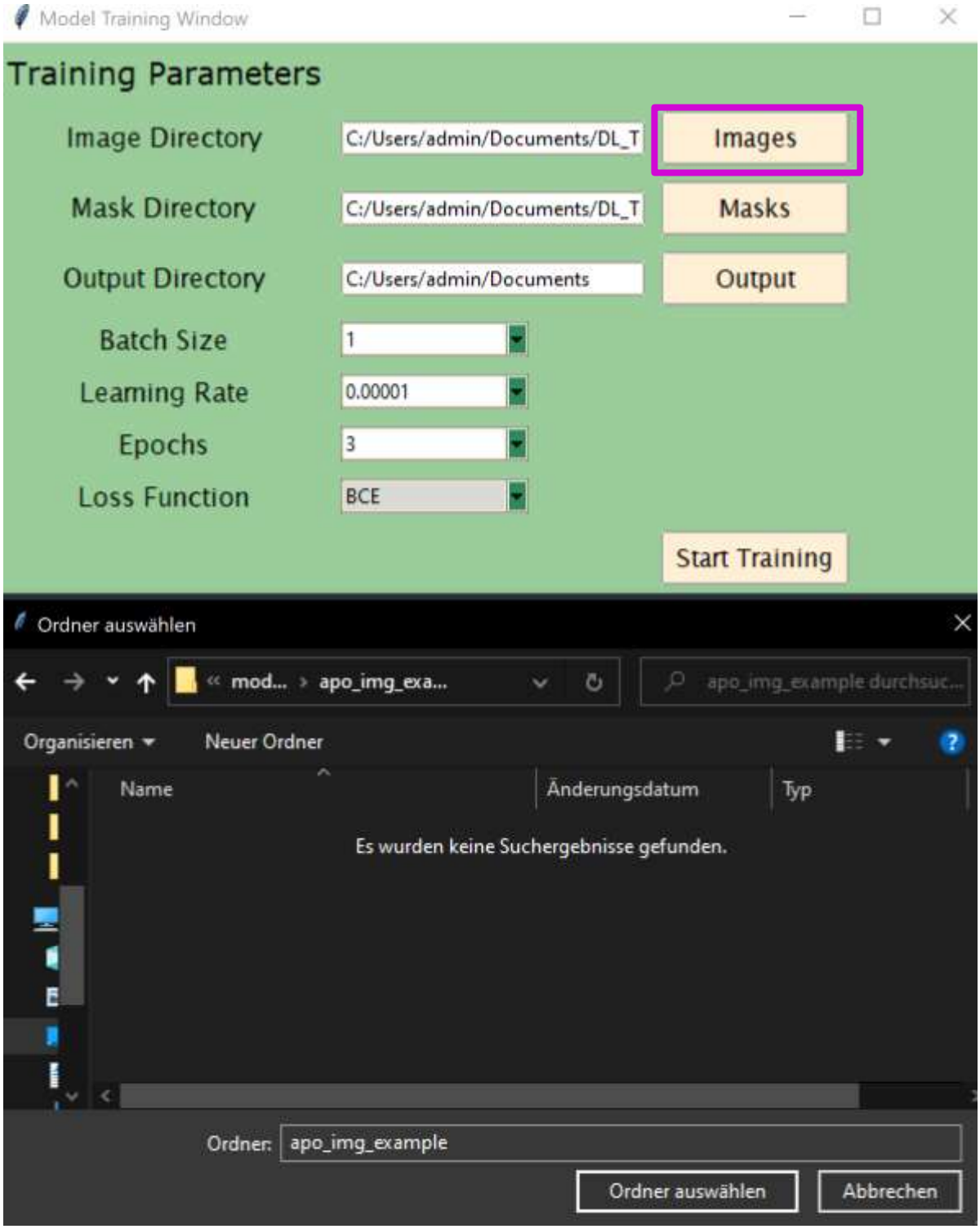
2. Specifying Relevant Directories

- As a next step, you can start the GUI.
- Once you started the GUI and the main GUI window opened, click on the **Train Model** button to select the relevant directories and model training parameters.
- The separate "Model Training window" will pop up.
- We will explain this window on the next page.



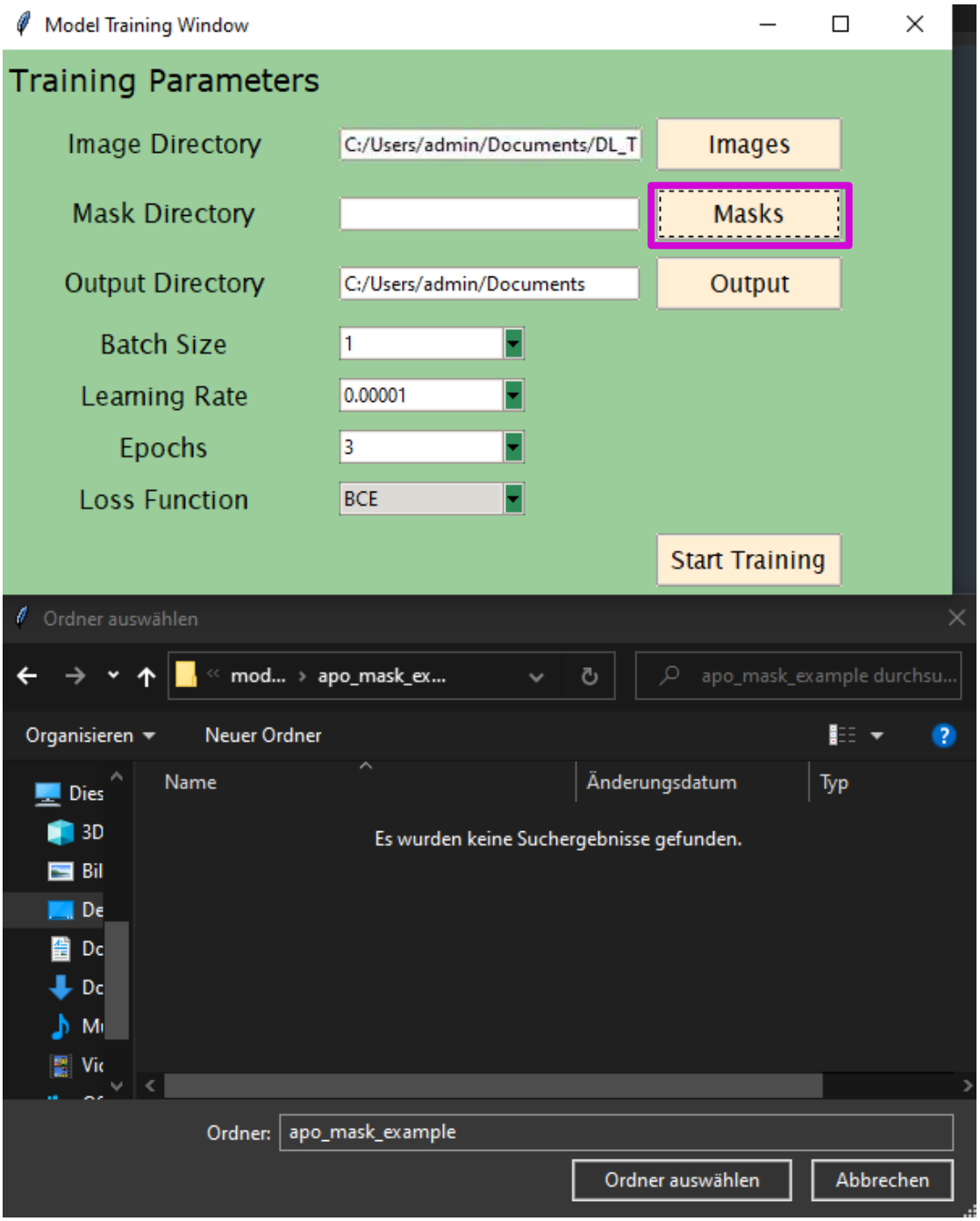
Firstly, select the “Image Directory”.

- Click th button **Images**.
- A selection window will appear and you can select the folder containing the **original images**.
- Select the “DL_Track_US_example/model_training/**apo_img_example**” folder.



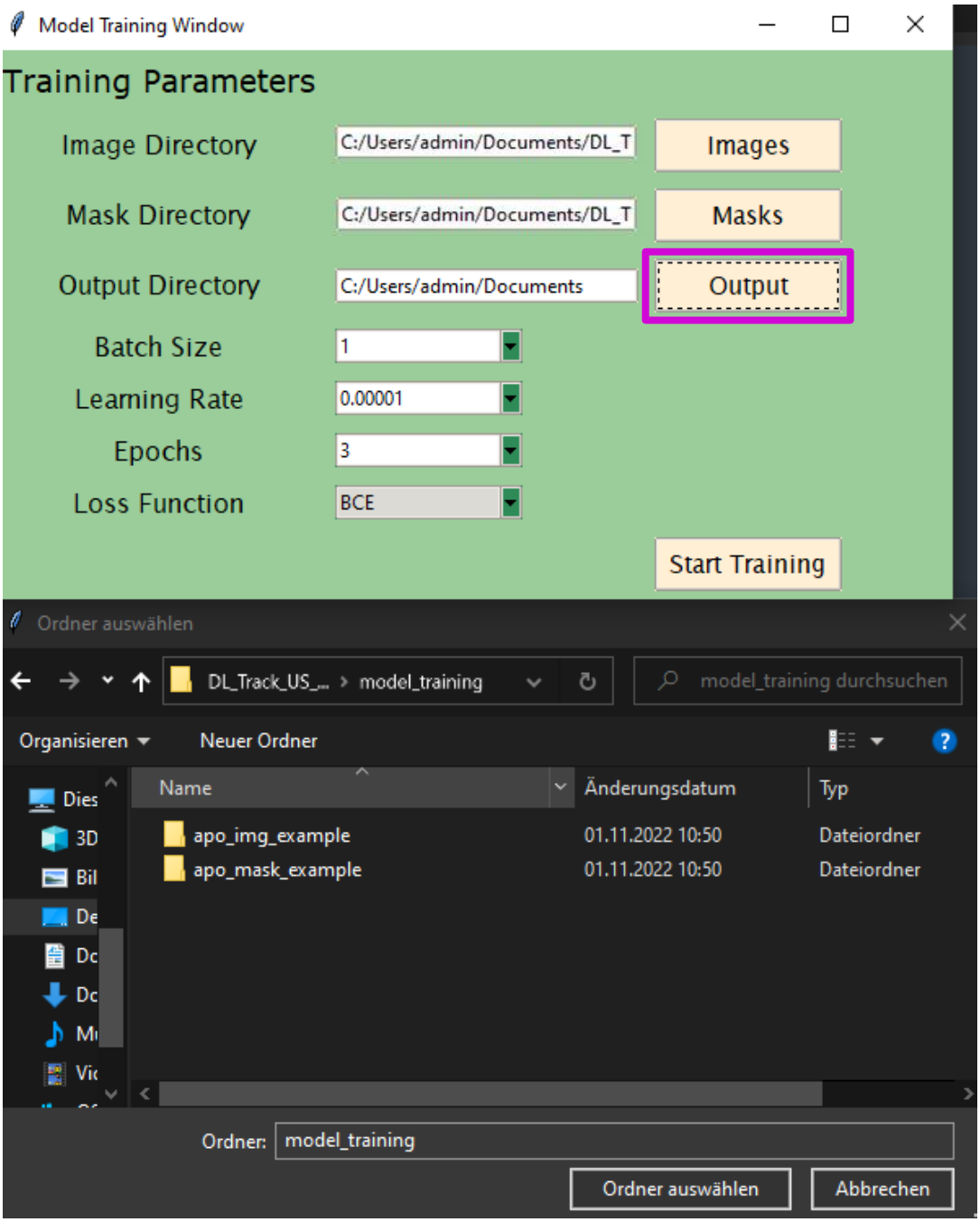
Your next step is to select the “Mask Directory”.

- the button **Masks**.
- A selection window will appear to select the folder containing the **mask images**.
- Select the “DL_Track_US_example/model_training/**apo_mask_example**” folder.



The last directory you need to select for training your own network is the “Output Directory”.

- Click the button **Output**.
- In the **Output** directory, the trained model, the corresponding loss calculation results and a graphic displaying plotting the training epochs against the loss values will be saved.
- A selection window will appear and you can select any folder you like.

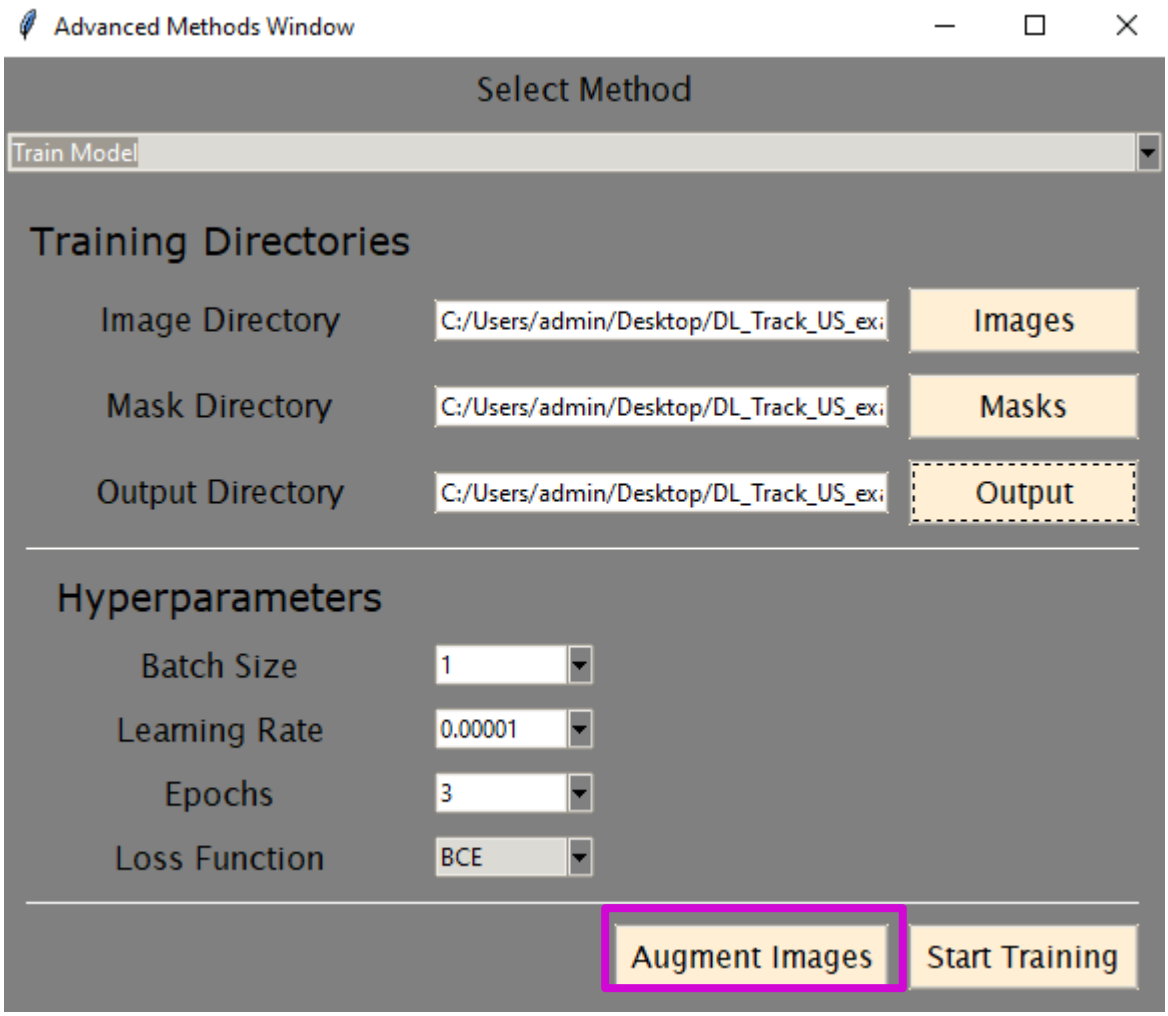


3. Image Augmentation

Image augmentation is a method to artifically increase the size of your training data. In this case, this means multiplying your images and masks based on a generator that changes certain properties of the images. You can find the details of this generator in the code documentation.

Image augmentation optional but advisable if image number is low, i.e. <1500.

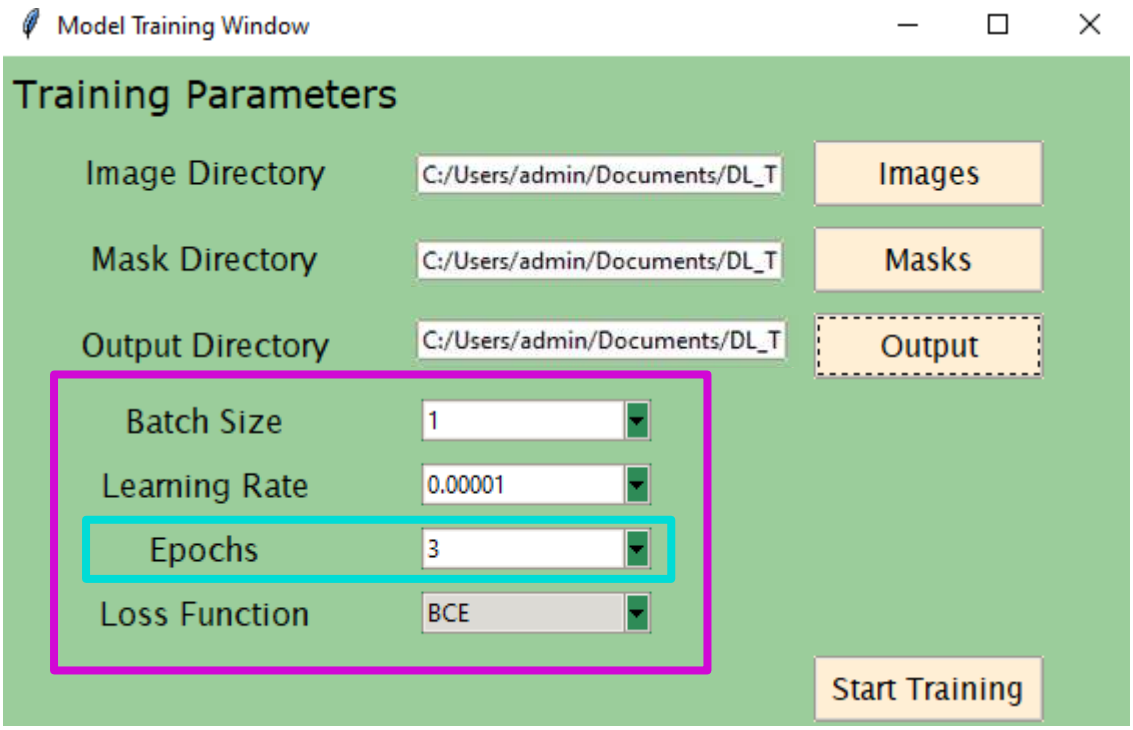
Given you have specified the relevant directories priorly, simply click the **Image Augmentaion** button and see your images being multiplied. A Messagebox will indicate when the augmentation process is finished.



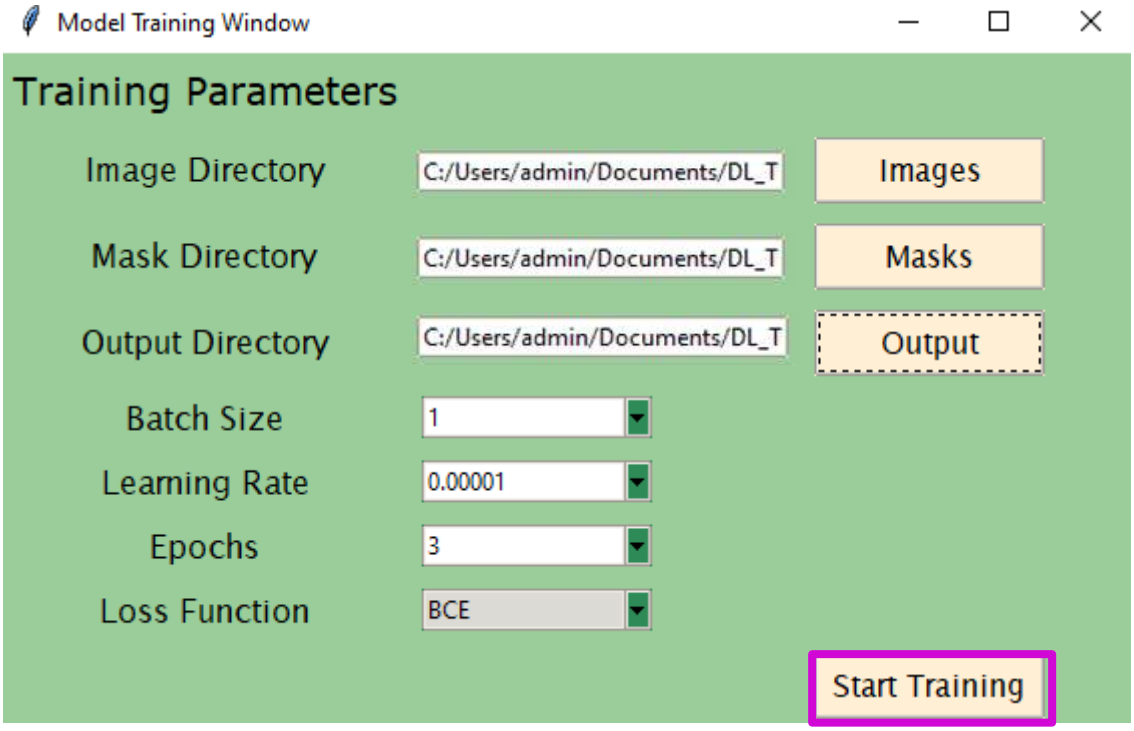
4. Specifying Training Parameters

Now to specifying the **training parameters**.

- For the tutorial leave the pre-specified selections as they are.
- If you do not know what these **training parameters** mean, take a look at this course.
- The only thing we have to say is that you must **NEVER** use only three **Epochs** for actual model training.
- Such a small number of training **Epochs** is only acceptable for demonstration and testing purposes.
- For actual training of your own neural networks, go with at least 60 **Epochs**.



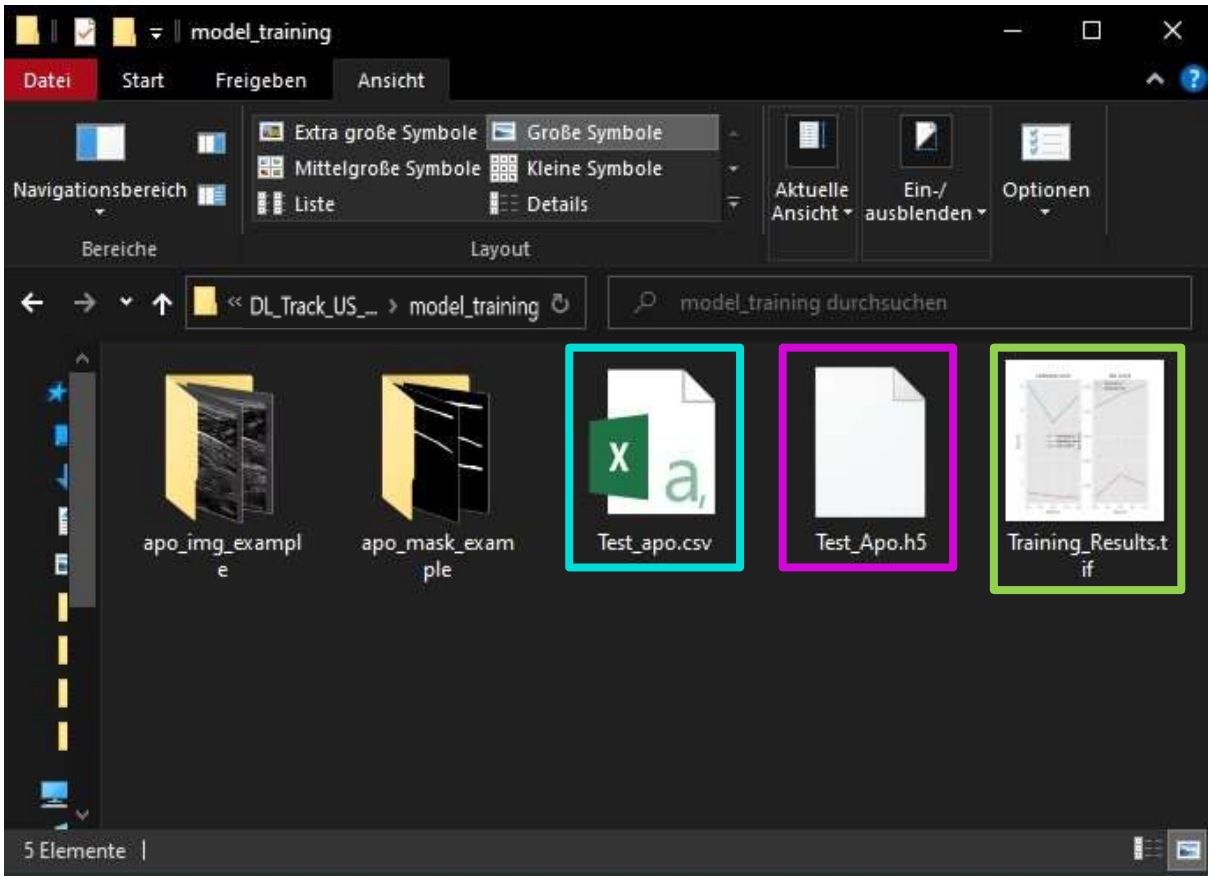
The only thing you have left to do for the training process to start is to click the **Start Training** button.



- During the training process, three messageboxes will pop up.
- The first one will tell you that the images and masks were successfully loaded for further processing.
- The second one will tell you that the model was successfully compiled and can now be trained.
- The last one will tell you that the training process was completed.
- You do have a choice in each messagebox of clicking “OK” or “Cancel”.
- Clicking “OK” will continue the training process, whereas clicking “Cancel” will be cancelling the ongoing training process.

Once the training process in finished, three new files will be placed in your output directory.

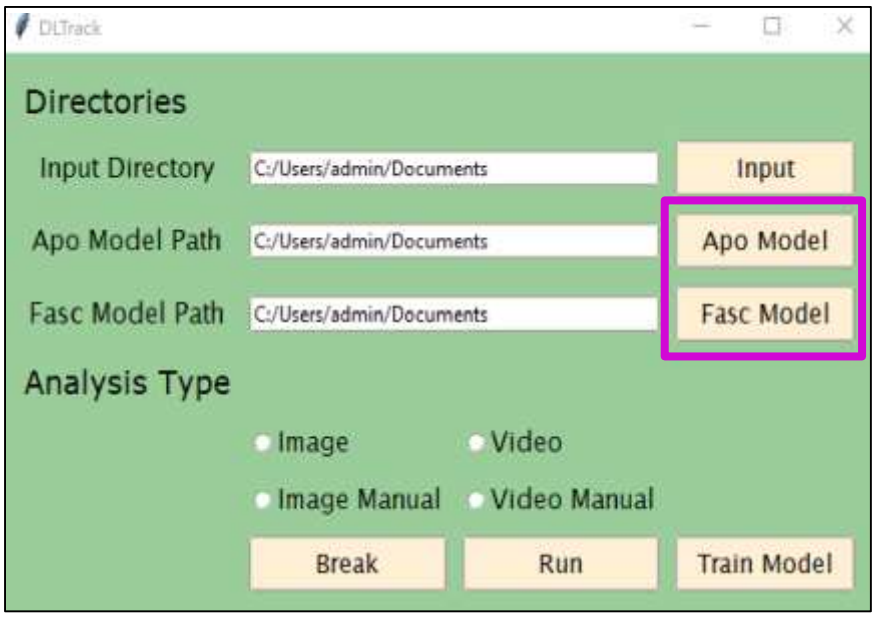
- The **trained model** as Test_Apo.h5 file.
- The **corresponding loss values** for each epoch as Test_apo.csv file
- The **graphical representation** of the training process as Training_Results.tif file.



5. Using Your Own Networks

How do you use you previously trained neural network?

- Simply select the path to your model by clicking the **Apo Model** or **Fasc Model** buttons in the GUI, depending on which model you want to import.
- Subsequently to specifying all other relevant parameters for your analysis in the GUI (as you have learned a couple pages ago).
- DL_Track_US will now analyse your data using your own model.

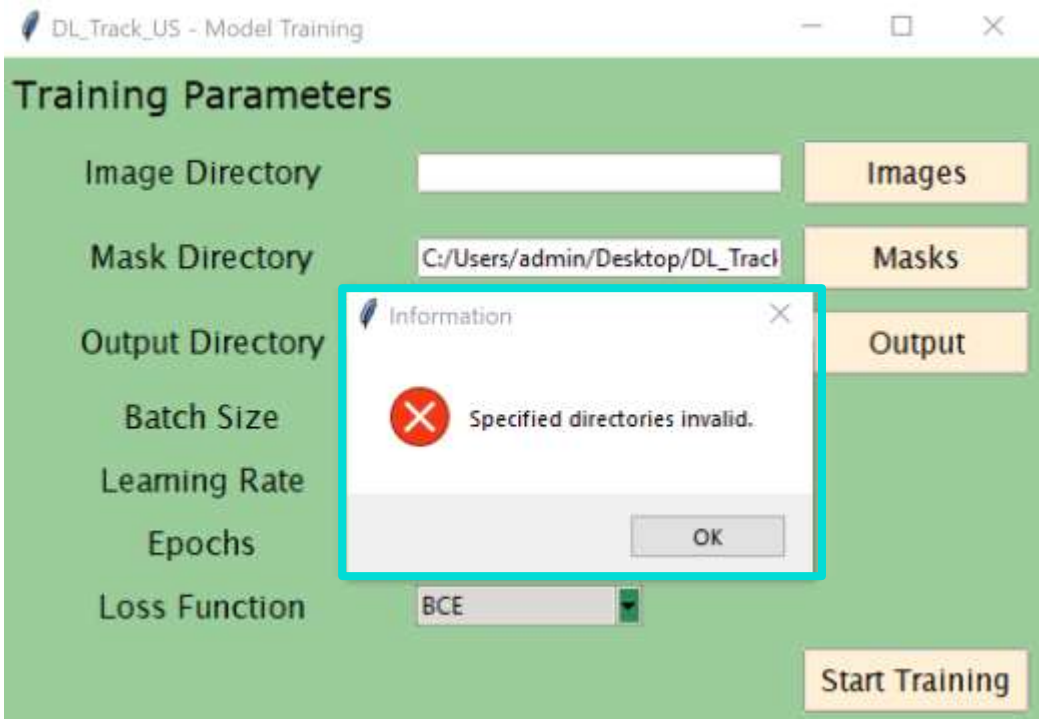


Lastly, a short disclaimer when training your own model.

- It is bad practice using the same images for model training and inference.
- The model should not be used for analysing images it was trained on because it already knows the characteristics of these images.
- **ALWAYS** compare the results of your model to a manual evaluation on a few of your own images. Use different images (best from different individuals) for model training and comparison to manual analysis.
- If this seems strange to you, don't hesitate to ask for further clarification in the DL_Track_US discussion forum.

6. Error Handling

Whenever an error occurs during the analysis process, the DL_Track_US GUI will open a **messagebox**. This looks always similar to this:



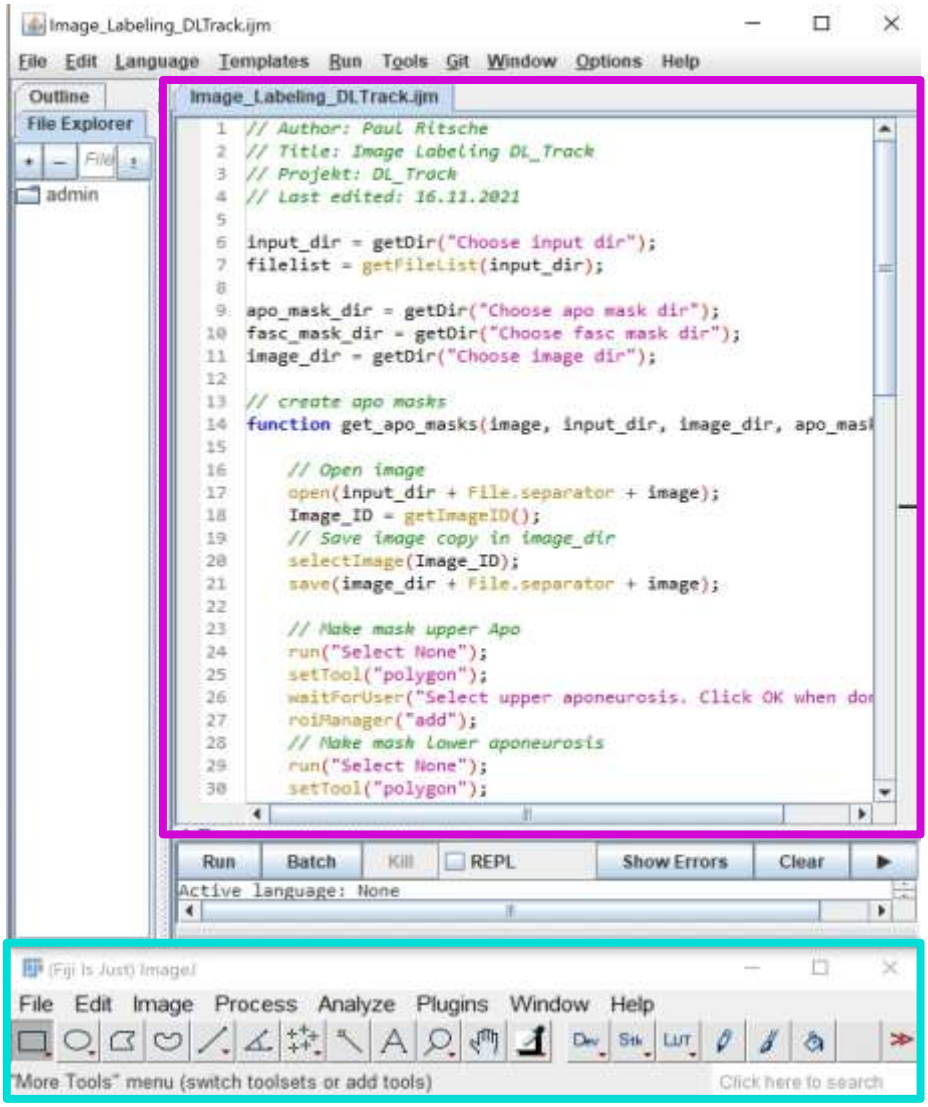
We tried to formulate these **messageboxes** as concise as possible. Just follow their instructions to fix the error and run the analysis anew. In case an error occurs that is not caught by an error **messagebox,** don't hesitate to report this in the Q&A section in the DL_Track_US discussion forum. Please take a look here how do best do this.

This is the end of the main tutorial. The next chapter covers how to label your aponeurosis and fascicle images used for model training.

Image Labels

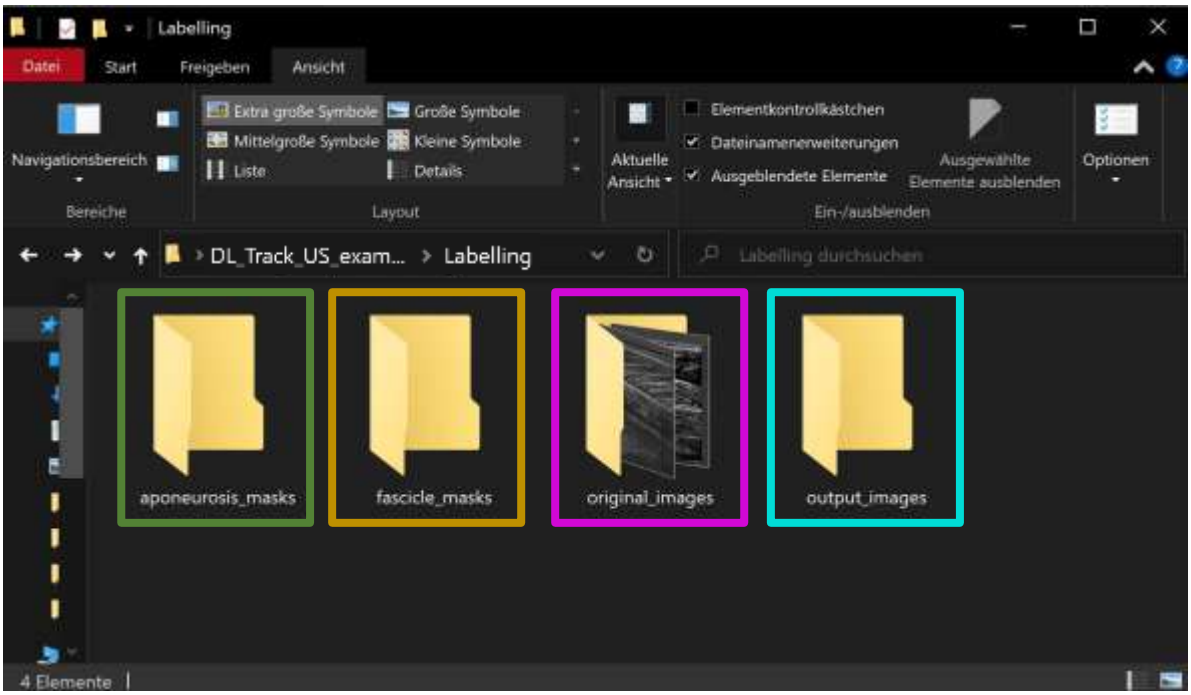
When you train your own networks, you need to label your original ultrasonography images.

- We provide an **automated script** for image labellig.
- This script does not automatically label the images, but automates the selection processes and image / mask saving.
- The software you will perform the labelling in is called **ImageJ / Fiji**. You can download ithere.
- The **automated script "Image_Labeling_DL_Track_US.ijm"** is located in the folder "DL_Track_US/docs/labeling/" in our Github repository.
- The easiest way to run the **"Image_Labeling_DL_Track_US.ijm"** script is by simply drag and drop it in the running **Fiji / ImageJ** window.

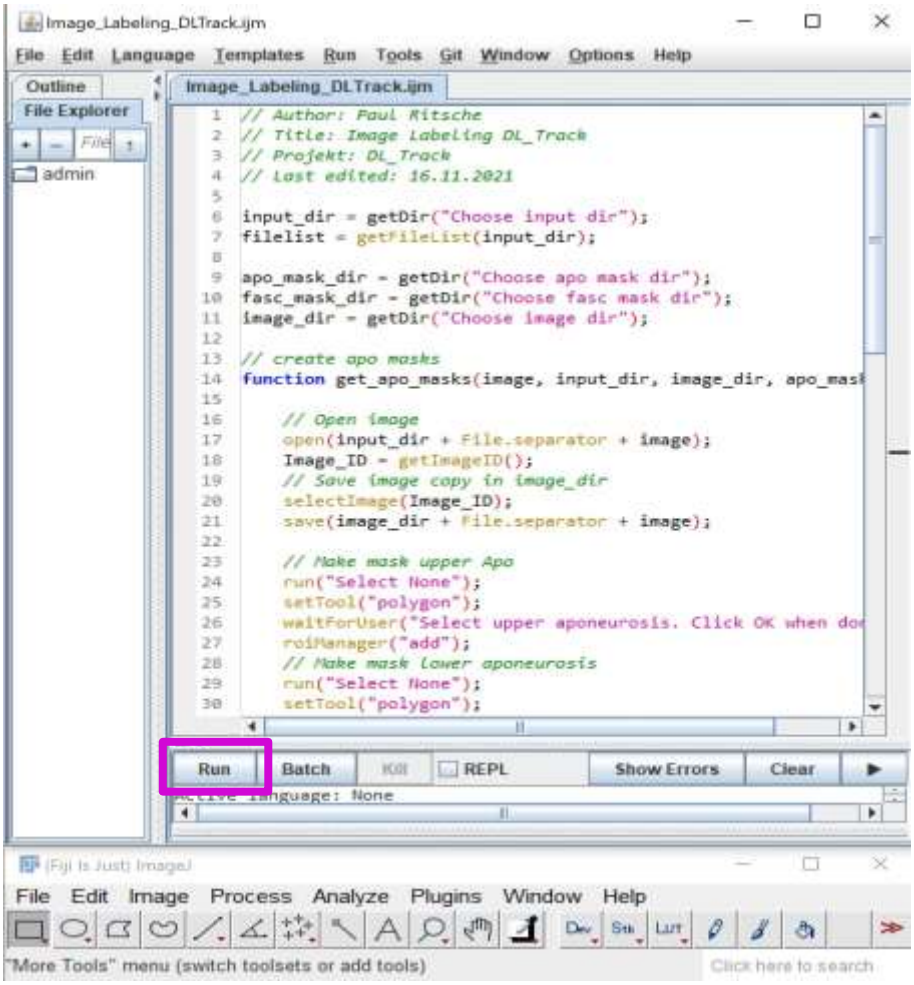


Before you can start the labelling process:

- Create four folders in an easily accessible place.
- One folder containing the **original images** you want to label.
- Then create three more folders, one named **“output_images”**, the second called **“fascicle_masks”** and the third called **“aponeurosis_masks”**.
- In the **“output_images”** the original images are saved with an adapted name.
- In the **“fascicle_masks”** and **“aponeurosis_masks”** folder the respective masks are saved with the same name as the corresponding image in **“output_images”**.



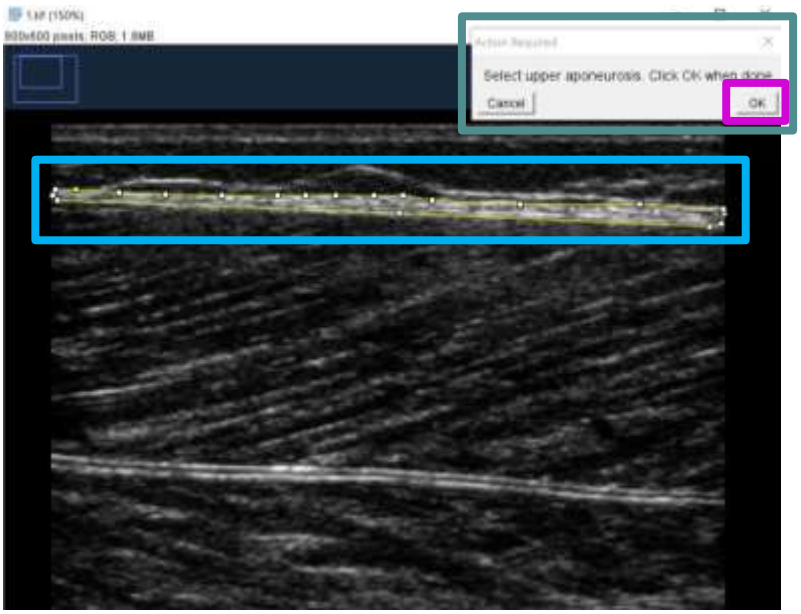
When you have created all folders, press the **Run** button in the Fiji / ImageJ API to start the "Image_labelling_DL_Track_US.ijm" script.

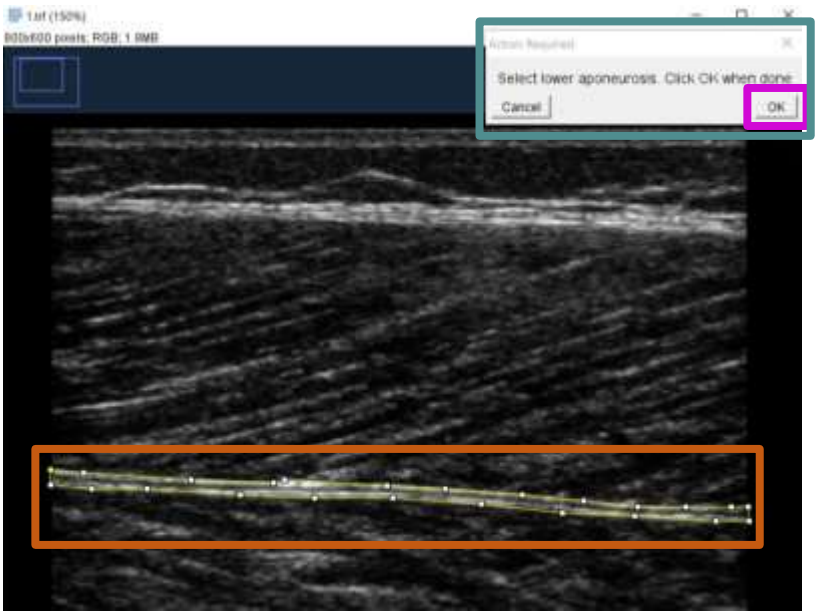


Follow the instructions appearing in the messageboxes.

- To begin with, you need to specify the four directories.
- The first directory you need to select is the original image folder (called input dir).
- The second folder is the "aponeurosis_masks" folder (called apo mask dir).
- The third is the "fascicle_masks" folder (called fasc mask dir).
- The last folder you need to specify is the "output_images" folder (called image dir).
- Subsequent to specifying the directories, you are required to create the masks.
- First the aponeurosis mask, then the fascicle mask.
- How to do this is demonstrated on the next page.

- Firstly, draw the **superficial aponeurosis** using the selected polygon tool by following the instructions in the **messagebox**.
- Draw around the **superficial aponeurosis** (double click to start drawing, click to add a segment, double click do stop drawing).
- Once you are finished, click the **OK** button in the **messagebox** to proceed to the selection of the **lower aponeurosis.**
- Please be careful to only include aponeurosis tissue in your selection and no surrounding tissue.
- The result should look like this for the **upper** and **lower aponeurosis**:

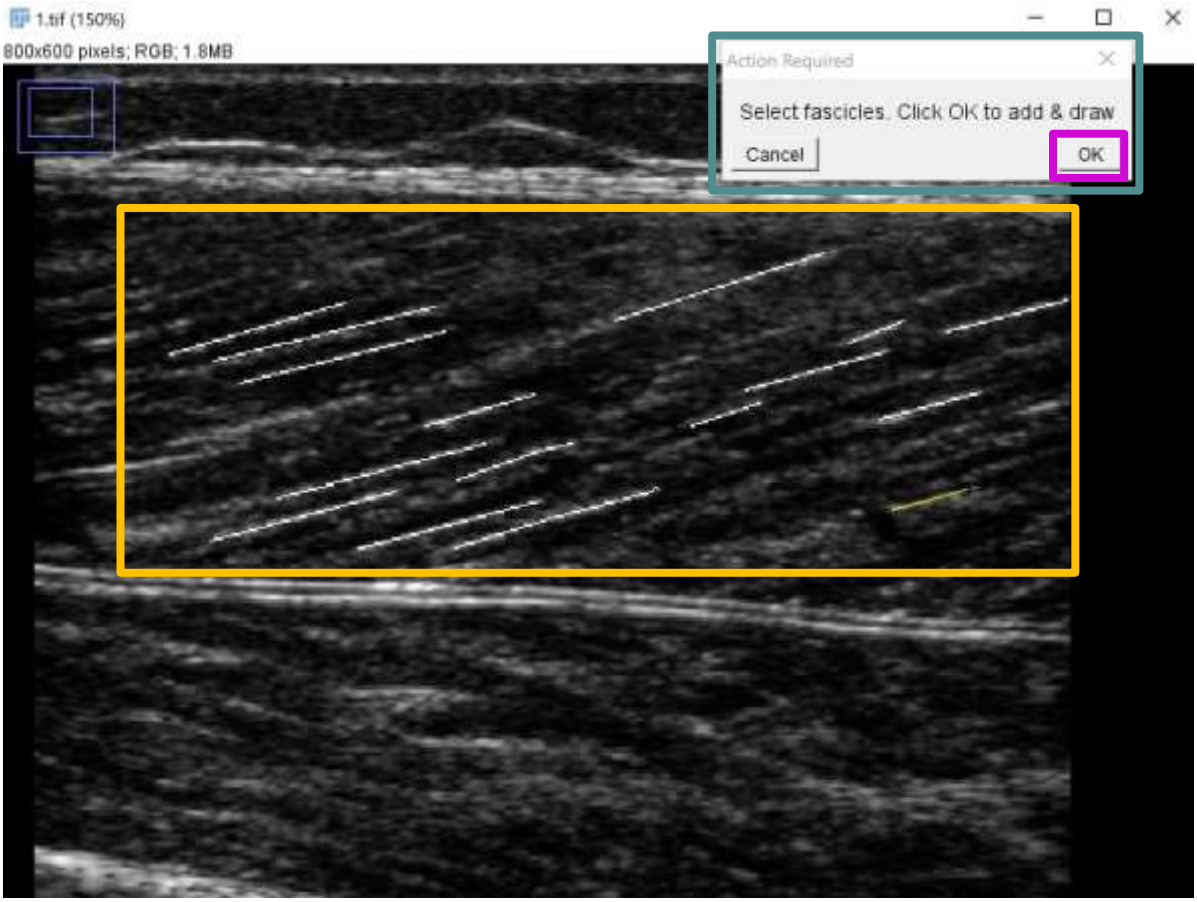




Once you have selected the lower aponeurosis, click the **OK** button in the **messagebox** to proceed to the fascicle labelling. Take a look on the next page to see how this is done.

The segmented line tool is selected automatically for you to follow the visible fascicle segments.

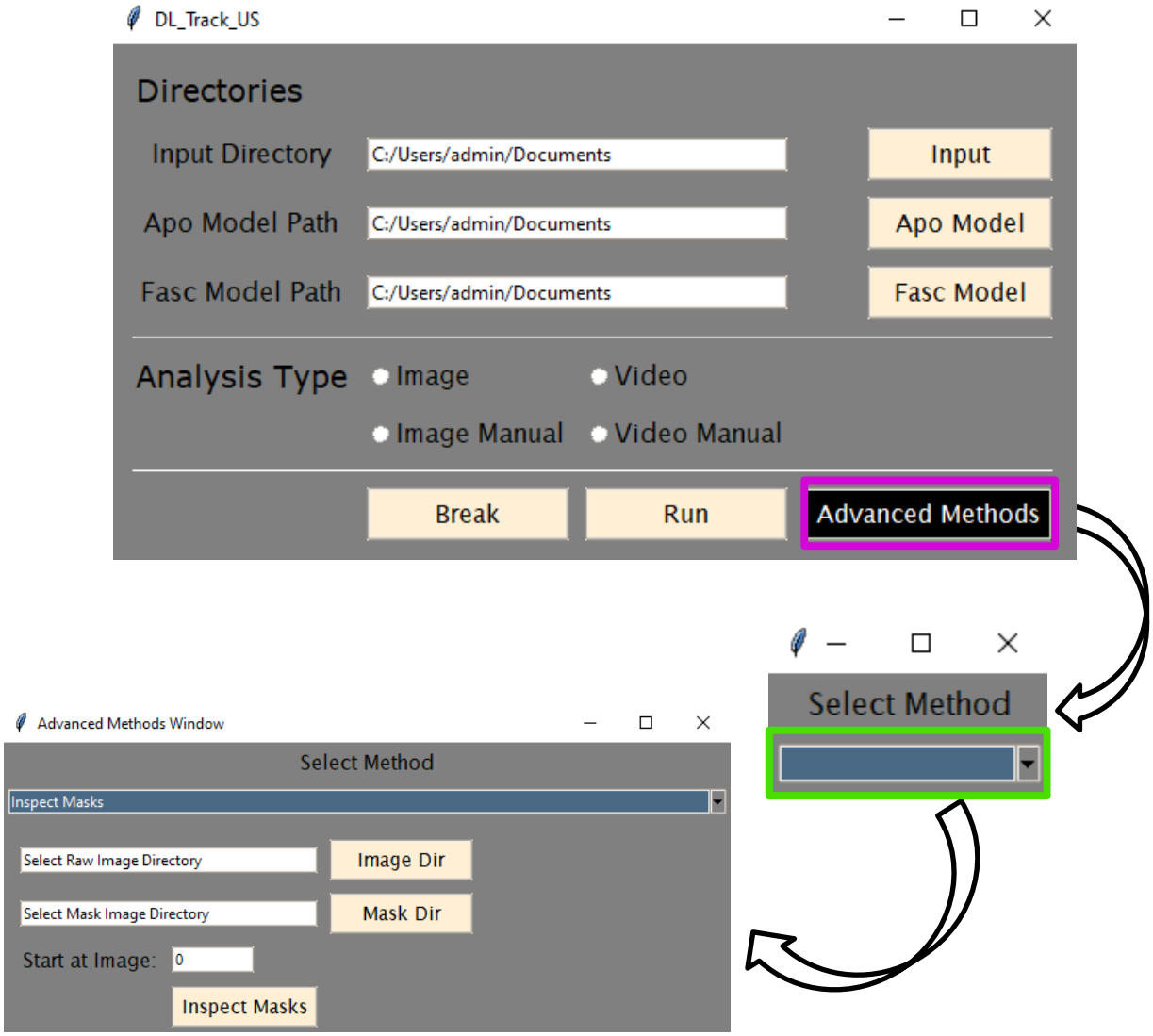
- Follow the instructions in the **messagebox.**
- It is of **utmost importance** that you draw **only over the actually visible parts** of the **fascicle** segment.
- Make sure that you only label bright **fascicle** tissue that is clearly visible.
- Once you drew one **fascicle** with segmented line tool (double click to start drawing, click to add a segment, double click do stop drawing) click the **OK** button in the **messagebox** to proceed to the next **fascicle** segment.
- Draw as many segments as are clearly visible on the image.
- When you press the **OK** button in the **messagebox** without making a further selection, you will proceed to the next image in the original image folder and start again with the aponeurosis labelling.
- The result of you labelling should look something like this:



Inspecting Masks

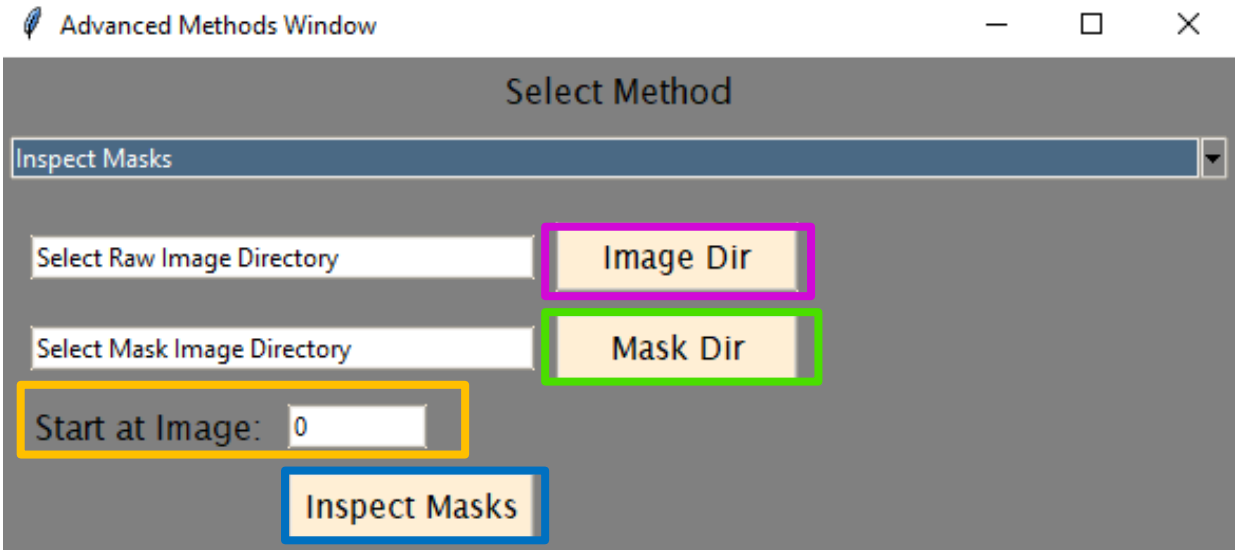
Data quality is of utmost importance when labelling the images. In version 0.2.1 of DL_Track_US we included an option to inspect the labelled images and corresponding masks.

- Once you started the GUI and the main GUI window opened, click on the **Advanced Methods** button to select the relevant directories and model training parameters.
- In the **Select Method** Dropdown select **"Inspect Masks".** The separate "Mask Inspection Window" will pop up. We will explain this window on the next page.



- First, you need to specify the relevant directories for the image/mask inspection.
- Three folders are of relevance here, **“output_images”**, **“fascicle_masks”**, **“aponeurosis_masks”**. They should have been created during the labelling process we explained in the previous chapter.
- Given that the number of fascicle/aponeurosis masks might differ, you can inspect both masks separately.

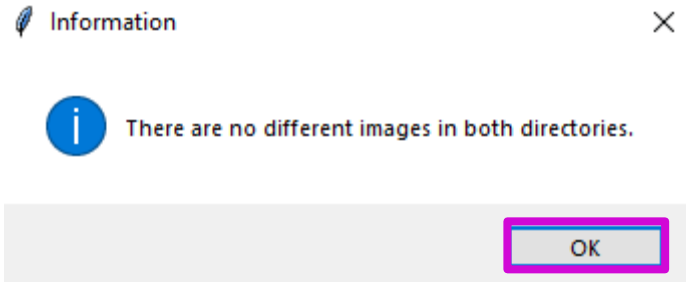
- Specify the directory containing the **“output_images”** clicking the **Image Dir** button.
- Specify the directory containing the respective **“fascicle/aponeurosis masks”** clicking the **Mask Dir** button.
- The **Start Index** allows you to specify the index/number of the image you want to start inspecting.



- Clicking on the **Inspect Masks** button, you will start the inspection process.

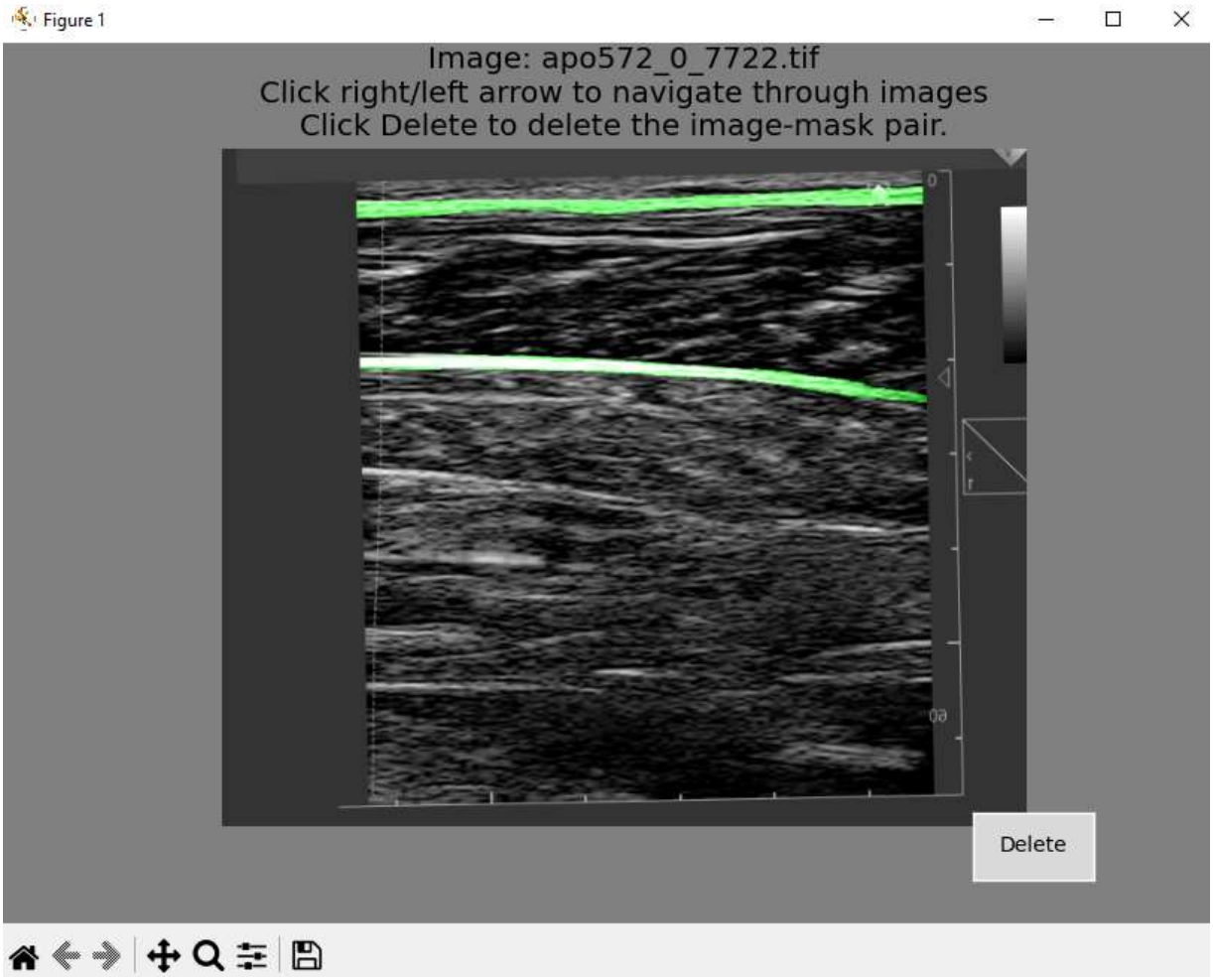
Given that the number of images and masks as well as the names of images and masks must be the same, one of two things will happen next:

1. Number of images and masks is equal and naming is correct. You will see a messagebox telling you so. Click **OK** to continue.



2. Number of images and masks **is not** equal and/or naming **is not** correct. A table will appear telling you which image names are incorrect, in which directory they occur and if the number of images differs between the directories. Based on this, go on to delete/change the images/image names.

Independently of what happened before, the **“Mask Inspection GUI”** will open and the previous windows will be closed.



- You can now follow the instruction displayed in the GUI.
- The labels will be projected on the image in an opague **green**.
- Be aware the the **Delete** button will permanently delete the image/mask pair in the respective folders. Making copies of the folders priorly might be advantageous, in case you want to keep the images/masks for corrections.

Closing remarks

Thanks for checking out the DL_Track_US python package tutorial. We hope you were able to enjoy it a bit. Moreover, we hope it was clear, concise and easy to follow. We tried to put our biases aside and to start from scratch. In case we failed to do so at some point and something was not clearly illustrated, please let us know. Don't hesitate to report this in the Q&A section in the DL_Track_US discussion forum. Otherwise, you can contact us by email at paul.ritsche@unibas.ch.