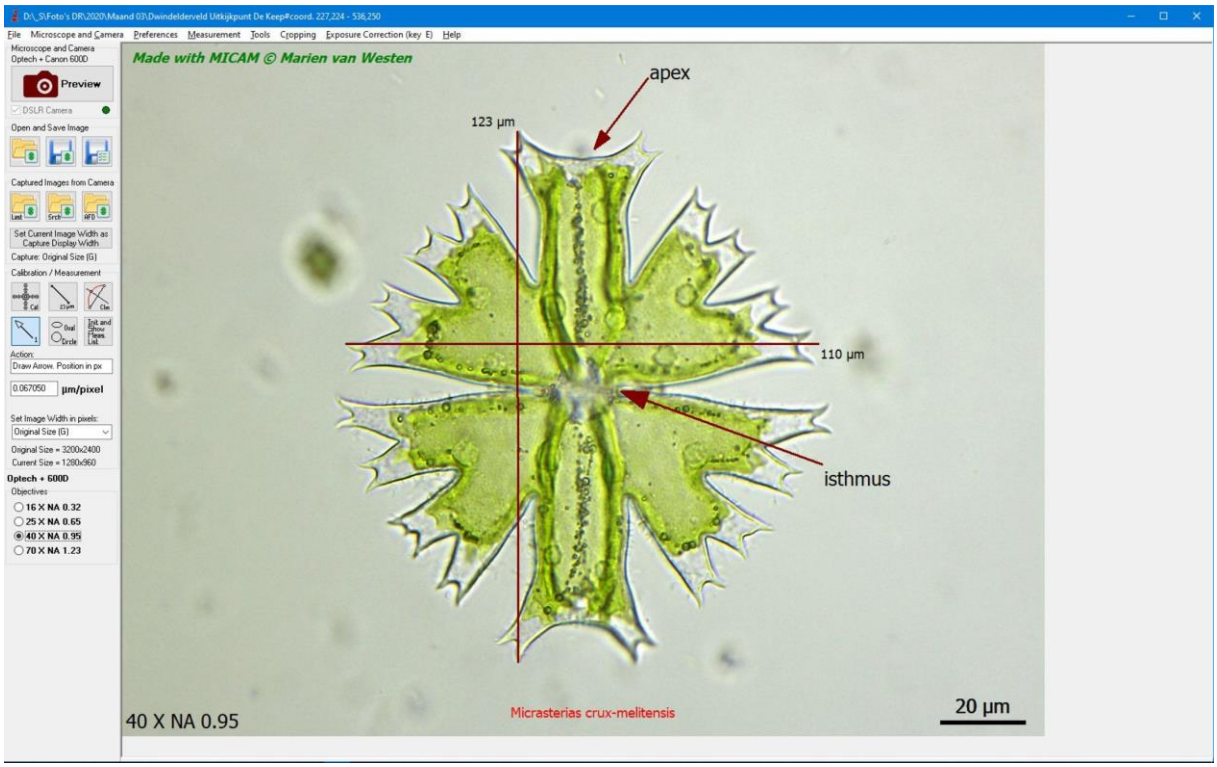
MICAM version 3.0



Marien van Westen
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1. Introduction

I started with microphotography when I was a schoolboy. My experiments were done by removing the ocular and projecting the image of the objective right on the photographic paper. Of course the paper had to be developed immediately and everything had to be done in the dark, with only some red light.
I managed to make some photographs, but the microscope was more a toy and the darkroom equipment was very primitive. I got other interests and I forgot about microscopy and photography.

After many years I started with microscopy again. Times have changed, so I wanted to use a digital camera and a computer for photography.
I attached a webcam to my microscope. I removed the ocular of the microscope and the lens of the webcam (a ToUCam) to project the image from the objective right on the CCD like I did in the old times with the photo paper.
The lens of the camera was replaced by an adapter with UV + IR blocking filter. This way no dust can enter the webcam. Fortunately in my set-up the CCD came almost right in the focus of the objective (see figure 1.1).

Figure 1.1. The ToUCam used for microscopic photography

I made some photos, but very soon I found out that I wanted to measure the objects I photographed. I also wanted a program in which I only have to press a button to save the captured image with a name that gives me some information about the image (it is a webcam, not a digital camera with EXIF and all kind of information stored in the image). So I searched the internet for suitable software.
There is a lot of capturing software available, but all had some drawbacks (technically, but also in price). I have some experience in programming, so I started programming my ***Microscope Image Capture And Measuring*** (**MICAM**) program. The result was a program that was very easy to use, so I think other users of microscopes with a USB camera (or a digital eyepiece) attached can benefit of this program.

Later on I bought a Tucsen 3 MP camera (see fig. 1.2) and a few years later I bought a Canon EOS 600D (figure 1.3). In the current version of MICAM it is possible to use USB cameras (like the ToUCam and Tucsen) and DSLR camera's with LiveView capabilities. Compared with previous versions of MICAM the amount of options was increased and several bugs were solved.

Figure 1.2. The Tucsen camera

Figure 1.3. The Canon EOS 600D

The USB Camera's like the ToUCam and the Tucsen are operated directly from MICAM.
The Canon EOS Camera was not easy to integrate with MICAM. But the Live View program, supplied for free with this camera, has an option to select a folder where the images you take will be stored. This option made it possible to combine Live View with MICAM.
I have no experience with other DSLR cameras, but I know that the free software digiCamControl can be used with Nikon and Sony camera's in combination with MICAM.

Use of MICAM with Live View or digiCamControl
When MICAM is put in Preview mode the program monitors the MICAM-Temp folder. Normally this folder is empty, but when your camera sends an image to that folder, MICAM will notice this and brings the image to the measurement window. The image in the MICAM-Temp folder will be deleted (or moved to another folder) and the system is ready to receive another image.
One of the benefits of this procedure is that it will work for many cameras that have the possibility of remote shooting, so probably many people will be able to use MICAM.
If your USB-camera is not recognized by MICAM, but if you have software that allows you to take snapshots and store them in a folder of your choice you can also use MICAM. But you must instruct MICAM that your camera is a DSLR.

A cell phone as webcam
If you have a cell phone, you can buy (or make) a cheap adapter to attach the phone to the eyepiece of the microscope. With special software on the phone and computer the phone acts like a webcam. You can use this webcam in MICAM.

Another method
What if your camera has no Live View options and digiCamControl does not work for your camera? Many cameras have the option to connect to the computer via USB. The camera memory then acts like a USB-stick. You can use MICAM to open the image from the memory of your camera and then process this image. This method will be described in more detail in Chapter 2.

Important!
If you use a DSLR like Canon EOS or NIKON:
Both the DSLR camera and MICAM must use the same folder (MICAM-Temp) for storing the images. Only then MICAM will be able to grab them from this folder.

If you use a USB-Camera:
Make sure the Direct Show drivers for your camera are installed.

Note
In this manual the word image is used to indicate a photograph (photo) that is processed by a computer

The pictures in this manual were made on a computer running Windows 10.

2. Getting started with MICAM

When you run MICAM for the first time, the program doesn't know what kind of camera you are using and what kind of microscope you have. So before you run the program make sure the camera is attached to the computer and does function with other software. If you have a DSLR like the Canon EOS make sure the LiveView function works.
Then start the program. You will see a warning (figure 2.1). After clicking OK the main window shows up (figure 2.2).

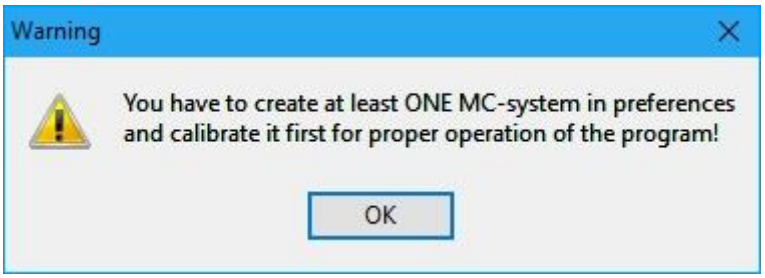

Figure . 2.1. The warning you will see when you start for the first time

2.1 Microscope and camera setup

The first thing you have to do is to prepare the program for your microscope and camera. MICAM allows you to use several Microscope and Camera combinations (MC-systems) up to 16, but for now we assume you have one microscope and one camera.
In the menu bar select ***Preferences*** (or use Alt-P). Select the second tab ***Microscope and Camera*** (see figure 2.3).

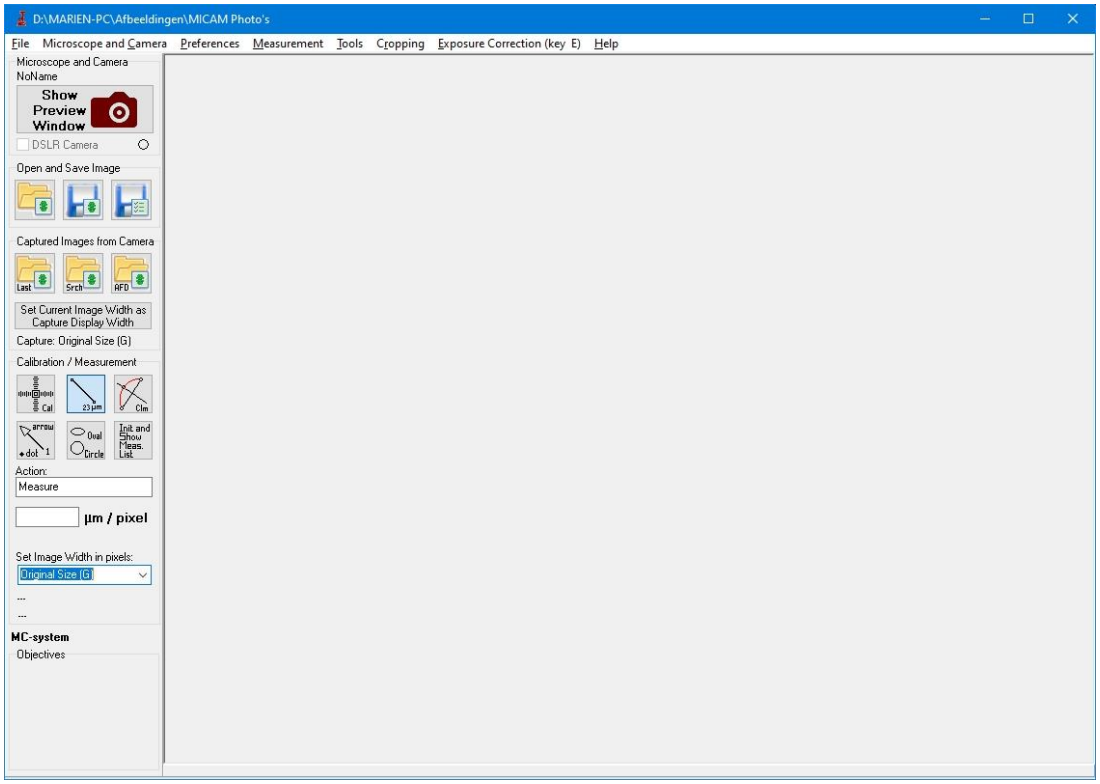

Figure 2.2. MICAM, the main window

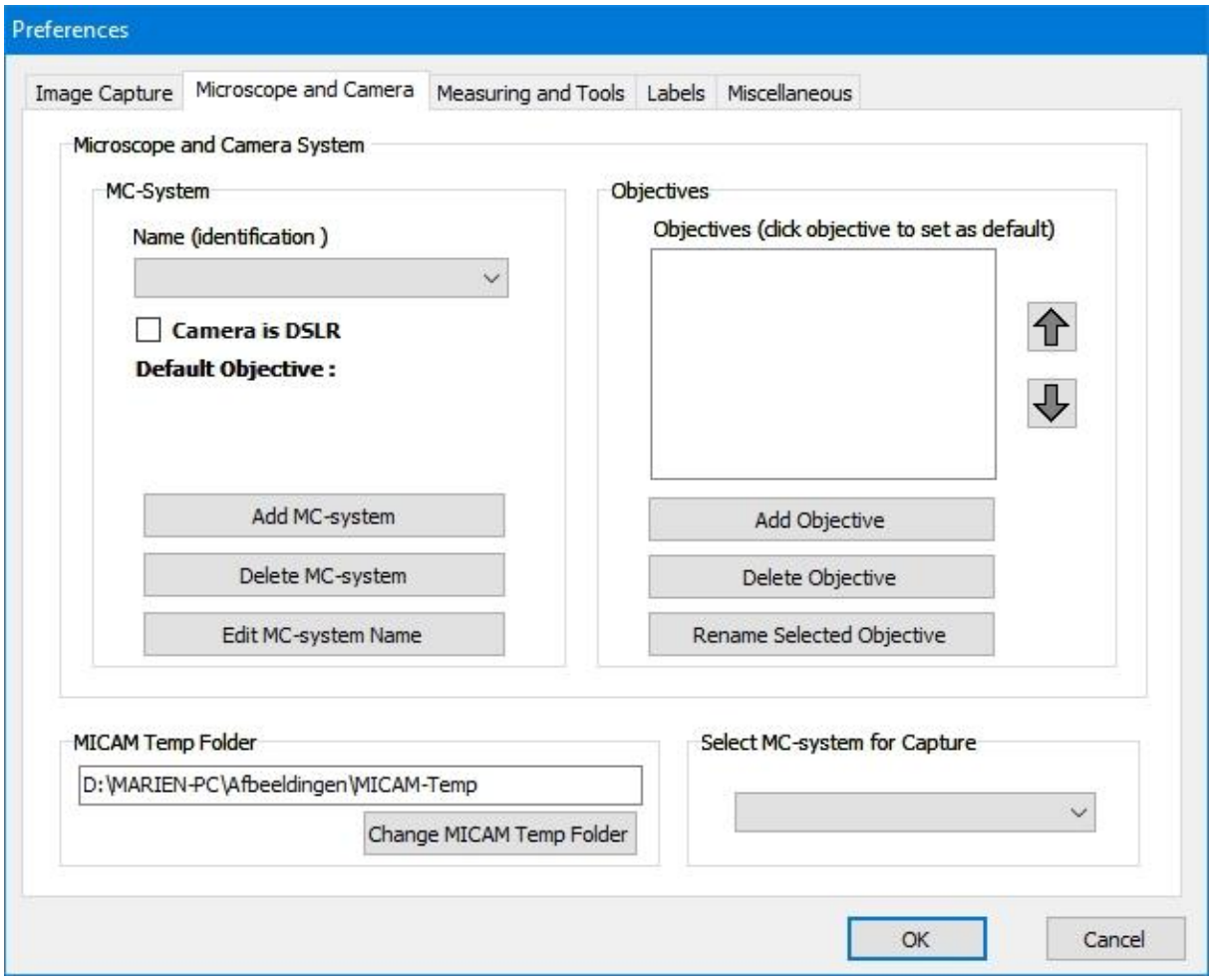

Figure 2.3. The Preferences window showing the Microscope and Camera tab.

Now you can add an MC-system. I give you an example for my Tucsen USB-camera and for my Canon EOS 600D.

We haven't defined any MC-system yet, so we click on ***'Add MC-system'***, enter a name and click OK. In the next window you have to enter a name for an objective. Choose 4 (if you have an objective with 4x magnification). Then we go to ***'Add Objective'*** to add values for the other objectives: 10, 40 and 100 for instance.
The objective is identified by a number (its magnification), but if you enter a space after this number, you can add some comment (see figure 2.4).

In the Microscope and Camera System Overview you see all the objectives of your camera. You can now select the default objective. If you switch from one MC-system to another you will always start with the default objective of that system. As the Tucsen isn't a DSLR I leave the **'Camera is DSLR'** checkbox unchecked. See figure 2.4 for the settings of the Tucsen camera.

On the bottom right you can select the default MC-system. This can be different from the one you just edited.

N.B. you can create 16 MC-systems with each as a maximum 32 objectives. This looks a bit overdone, but I use MICAM also to measure on SEM images which are taken with a

magnification of 1200, 2000, 4000, 6000, 8000, 12000, 16000 and 20000 times (and many more) so once I have calibrated for all those 'objectives' I can easily measure my images.

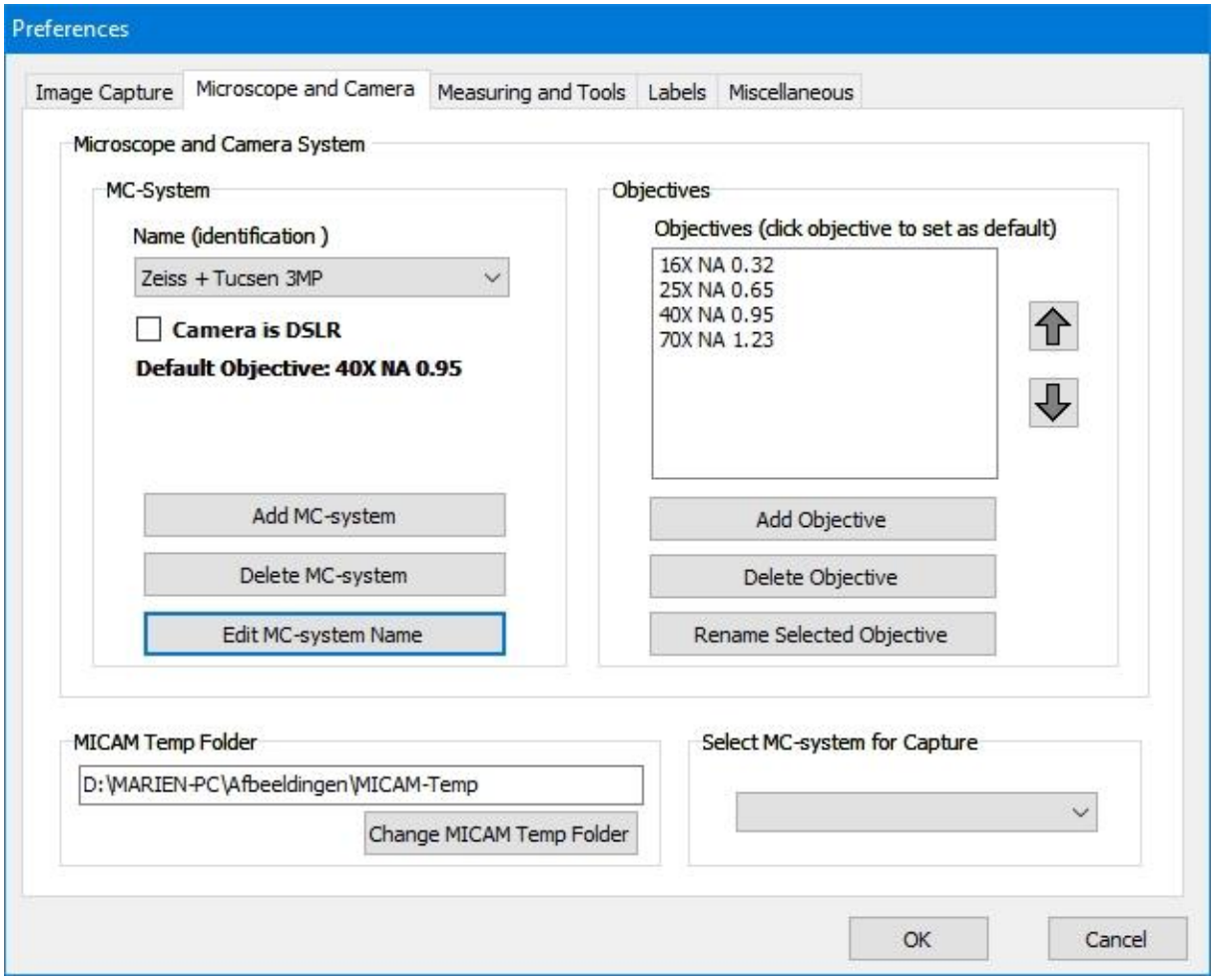

Figure 2.4. The settings for the Zeiss microscope with a Tucsen 3 MP camera

For a DSLR camera you set thing up in a similar way. The only additional thing you have to do is to check the Use DSLR Camera checkbox (see figure 2.5).

Important setting!

A very important thing is the setting of the *MICAM-Temp* folder if you use a DSLR camera. By default the program creates this folder in your *Pictures* folder, but you can select any folder you want.
This folder is important; because that is the folder were your DSLR camera will have to store the images temporarily. So in your Live View program you have to use that folder to store the images from your camera.

Also note that this MICAM-Temp folder is not the folder where you store your images after editing!
The name used for the folders in previous versions of MICAM caused confusion for some users. I hope the new names are less ambiguous.

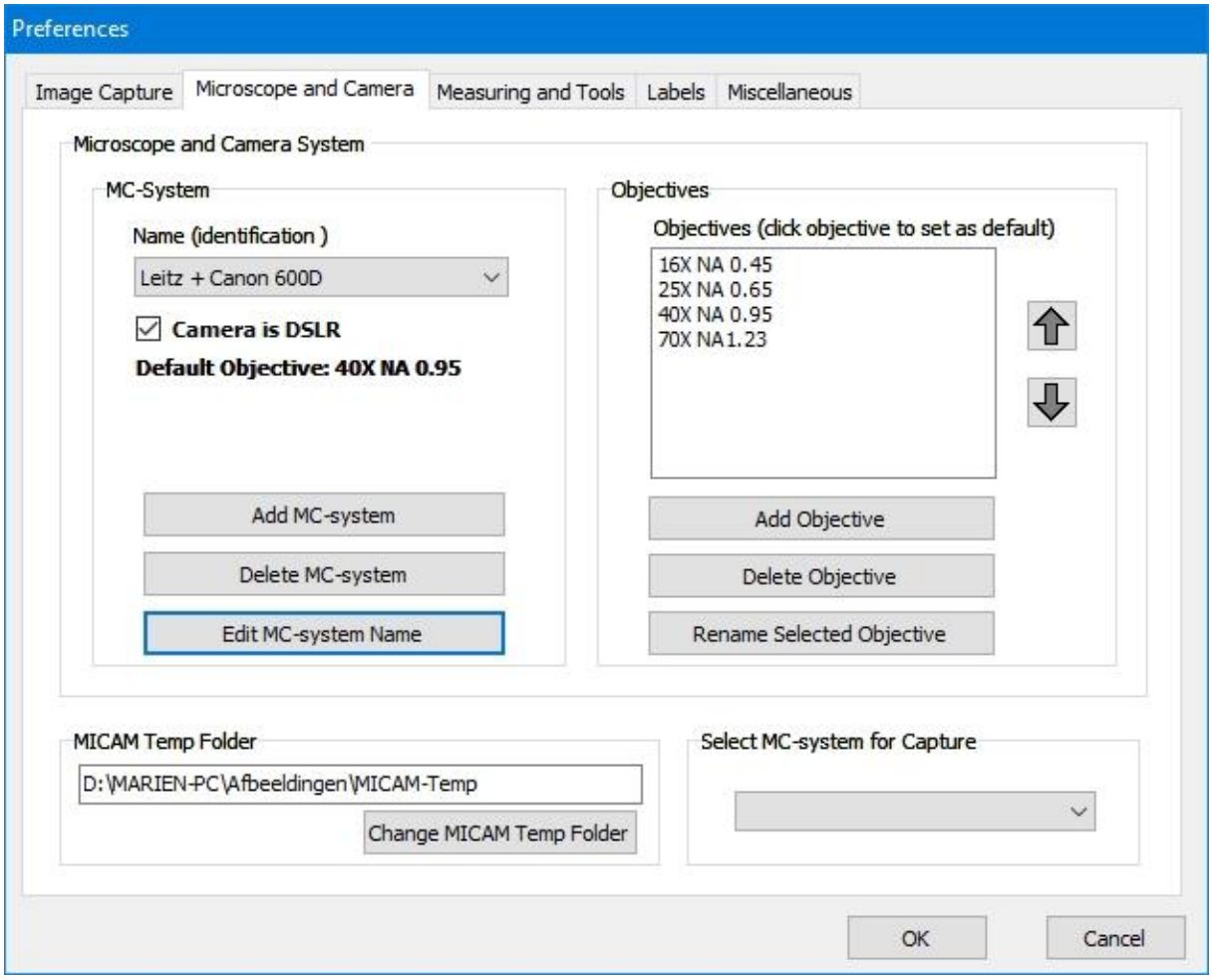

Figure 2.5. The settings for a Leitz microscope with the Canon EOS 600D camera

2.2 Setup of the Photo Folder

Now move to the ***Image Capture*** tab.
Here you set the folder were you store you images after you have done some cropping, colour corrections, inserting a scale etc. You can use the ellipsis buttons (the buttons with three dots ...) to select a different folder. By default this will be the folder ***MICAM*** in the ***Pictures*** folder. Note that in a localized Windows system this folder will have a different name. In Dutch the ***Pictures*** folder will be named ***Afbeeldingen*** (see figure 2.6).
You can also choose the kind of file type you want to use for your images:
BMP is without losses, but gives you large files.
PNG uses some compression and is also lossless.
JPG normally isn't lossless and uses compression, so you will get smaller images. I have chosen a jpg-format with minimal loss.
TIF is also lossless.

Figure 2.6. Part of the Image Capture tab in Preferences

Furthermore you can choose between three kinds of default filenames. This name will be the default name the system selects. Choose what suites you best. The number is always chosen so that the filename will be unique in that folder. See figure 2.7 for details.
When the Capture folder name contains a #-sign, the folder name will be used in the comment that is saved with every image you take:
The folder name is split into two parts. The part before the #-sign will be the "Location" comment, the part behind the # will become the second line (Coordinates).
So when you create a folder with name: 'Assen garden pond#km 255,744-541,280', every image will be saved with Location as 'Assen garden pond' and Coordinates as 'km 255,744-541,280' (see figure 2.7).

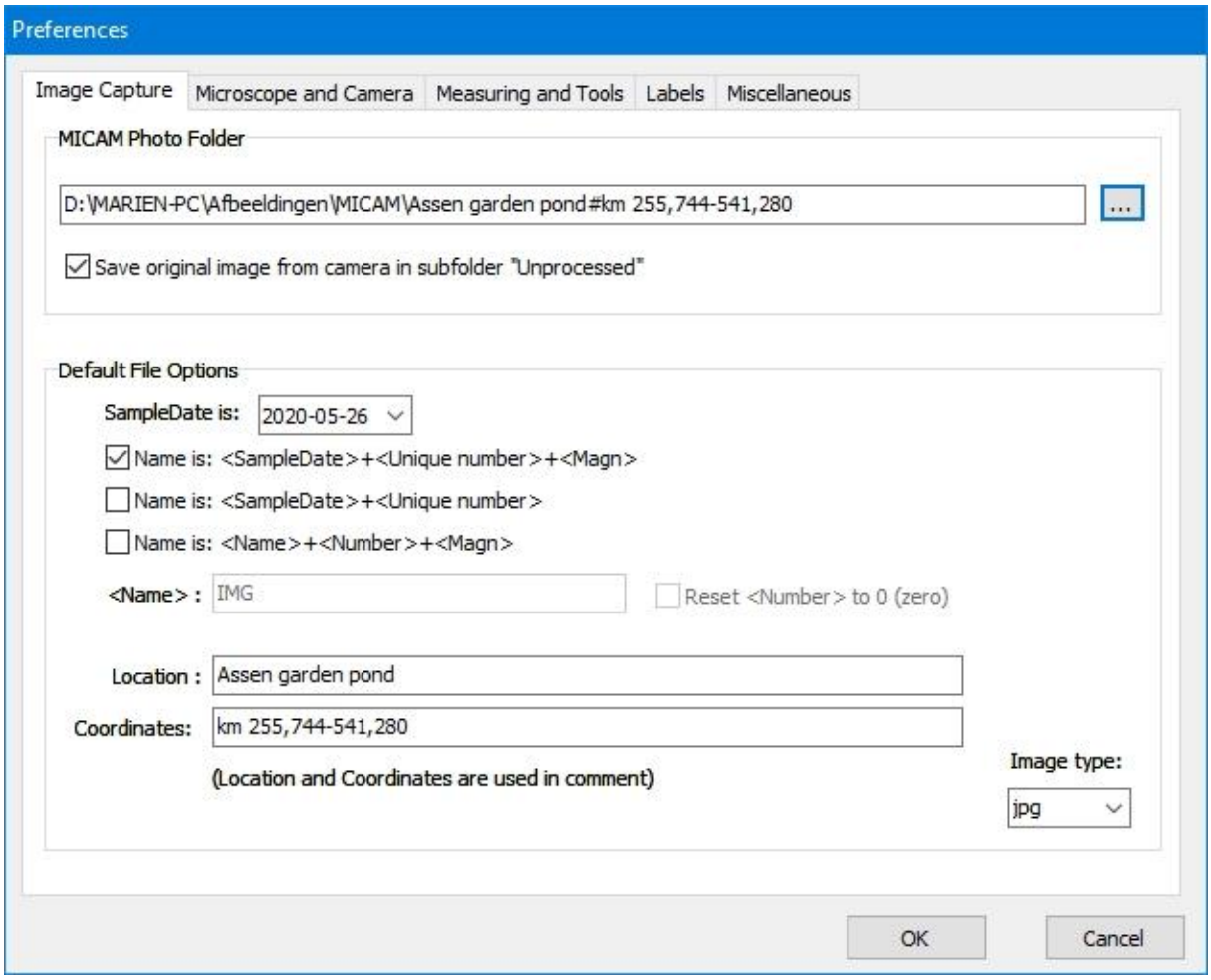

Figure 2.7. Image Capture tab showing all possible settings

The original image from the camera will be lost during the processing of the image. But sometimes during the processing you decide to start all over. Or maybe, much later you want to crop the image to a larger size, but for that you need the original image. So it would be nice if the original image from the camera will be stored as well. For that reason you can make a copy of the original images from the camera in a subfolder ***Unprocessed*** of the photo folder you use. This subfolder will be created automatically when you have selected to save the original image.

When you use PNG, JPG or TIF as image format information about the location, sample date and used microscope, camera and objective will be stored inside the image. When you use BMP the information will be stored in a separate file with the same name, but with the extension '***cmt***'. If you do not want those additional files, you can decide not to store the additional information (see ***Preferences → Miscellaneous***). As BMP files tend to be rather huge, I would recommend not to use BMP at all.

Finally
Click OK to confirm the changes you have made. When you close the program your settings will be saved and used again when you start a new session of the program. So the previous mentioned settings have to be done only once.
The only thing you might have to change then is the folder were you want to store your images, but that setting can be made on the first tab of ***Preference***s.

Now you are ready for the next step.

2.3 Calibrating the system

To perform measurements you have to calibrate MICAM first.
In order to do that, you need an object under the microscope with some kind of calibrated scale on it (a ruler for the 4 X and 10 X objectives maybe). When you go from one line to another on the ruler you know that the distance will be 1 mm = 1000 µm. For the objectives with higher magnification you need a calibration slide. It is easy to find a company on the internet that sells those slides.
In order to calibrate your system you need to capture an image first. The procedure for grabbing images is a bit different for a USB microscope camera and for a DSLR, so this will be handled in separate paragraphs.

2.3.1 Use of a USB camera

First make sure you selected the right camera and the objective setting in the main window corresponds with the setting on the microscope. So if you use a 40 X objective make sure that in the panel on the left the 40 X objective is selected.
At the top left of the main window you have to click the large button with the camera and the text ***'Show Preview Window'***. If you see just ***'Preview'*** then you selected a DSLR camera and you have to go to ***Preferences → Microscope and Camera*** you have to uncheck the ***Camera is DSLR*** checkbox or to select the correct MC-system in the box at the bottom right.
Now the Preview window pops up (figure 2.8). In the Menu option ***'Camera'*** you can select the camera you want to use. Normally there will be only one camera, but if you have more camera's connected to your computer you can select the on you want to use.

When you select ***'Camera Options'*** in the menu bar, you will see extra options. Here you can set the format of the live view and other settings of your camera.

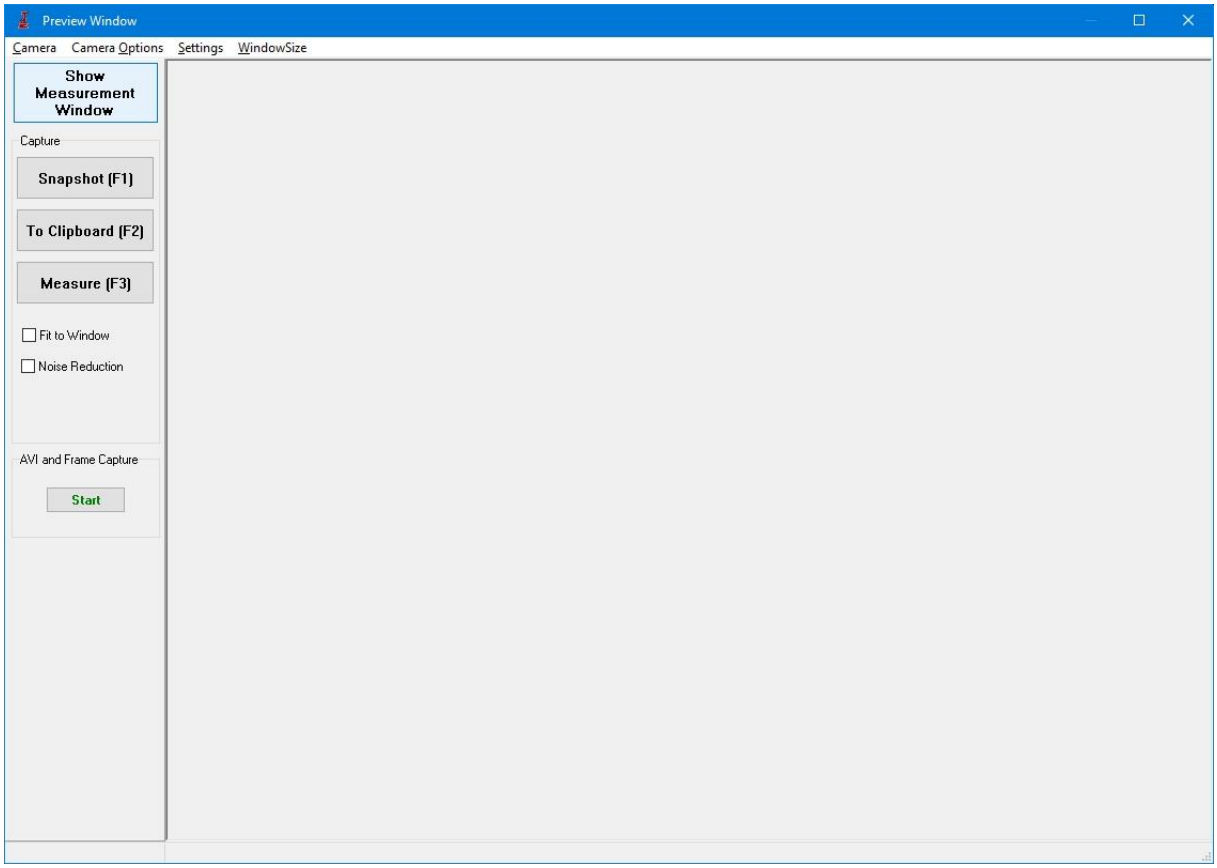

Figure 2.8. The Preview Window for a USB camera

Note! The possibilities depend on your camera, not on MICAM, so you have to find out yourself what possibilities your camera has.
I also found out that my Tucsen camera always shows up in the list of cameras whether it is connected or not. Probably this is an error in the drivers.

After selecting the camera you should see the live view from the camera (Fig. 2.9).
Press the ***'Measure'*** button or ***'F3'***. The program switches back to the Main window and the captured image is shown.

After calibration of the system you can start capturing images by just pressing the 'Snapshot' button (or ***F1***). The system will use the predefined names. The benefit of the snapshot is that you can take many pictures, without bothering about names.
If you want to process an image in other software you also can send it to the Clipboard (***F2***).

As the system has to be calibrated we show an example with a calibration slide first.
Figure 2.9 shows what you see in the preview window of MICAM. Figure 2.10 shows the Main Window after clicking the ***'Measure'*** button or pressing ***'F3'***. The captions showing the objectives are Italic, indicating the system is not calibrated yet.

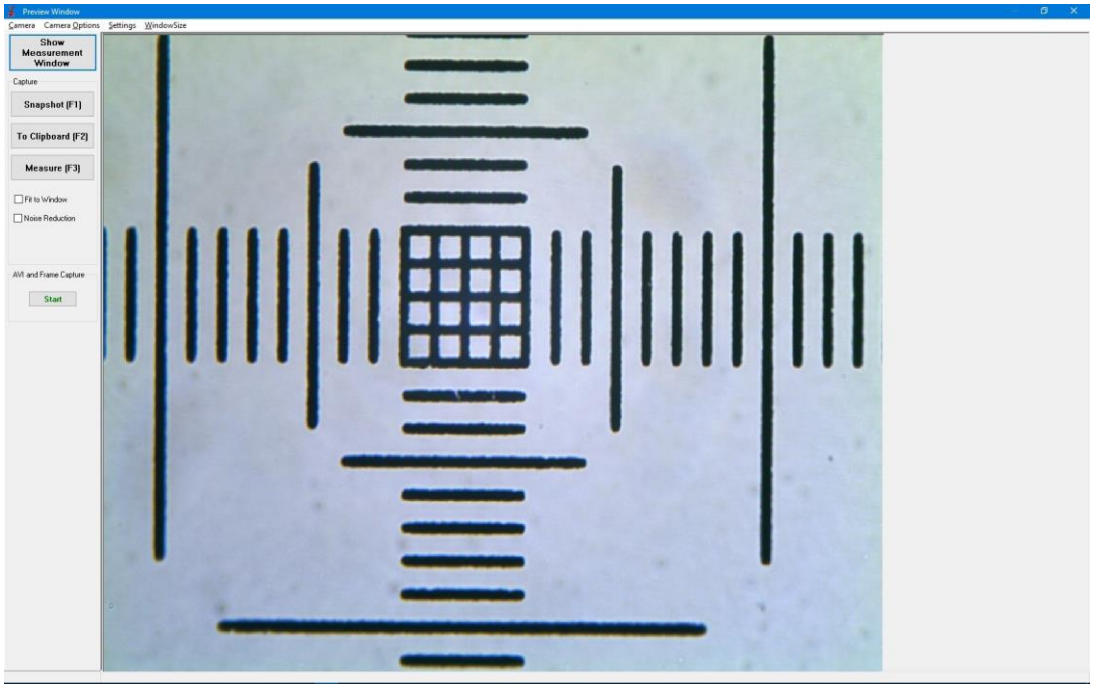

Figure 2.9. The Preview window of MICAM showing a calibration slide

As you can see the image is shown in the original size, but you can easily fit the image to the window by pressing key ***F*** (Fit). See figure 2.10. Going to the original size is possible by pressing ***G*** (oriGinal). Several other image sizes are selectable in the ***Set Image Width*** drop down box.

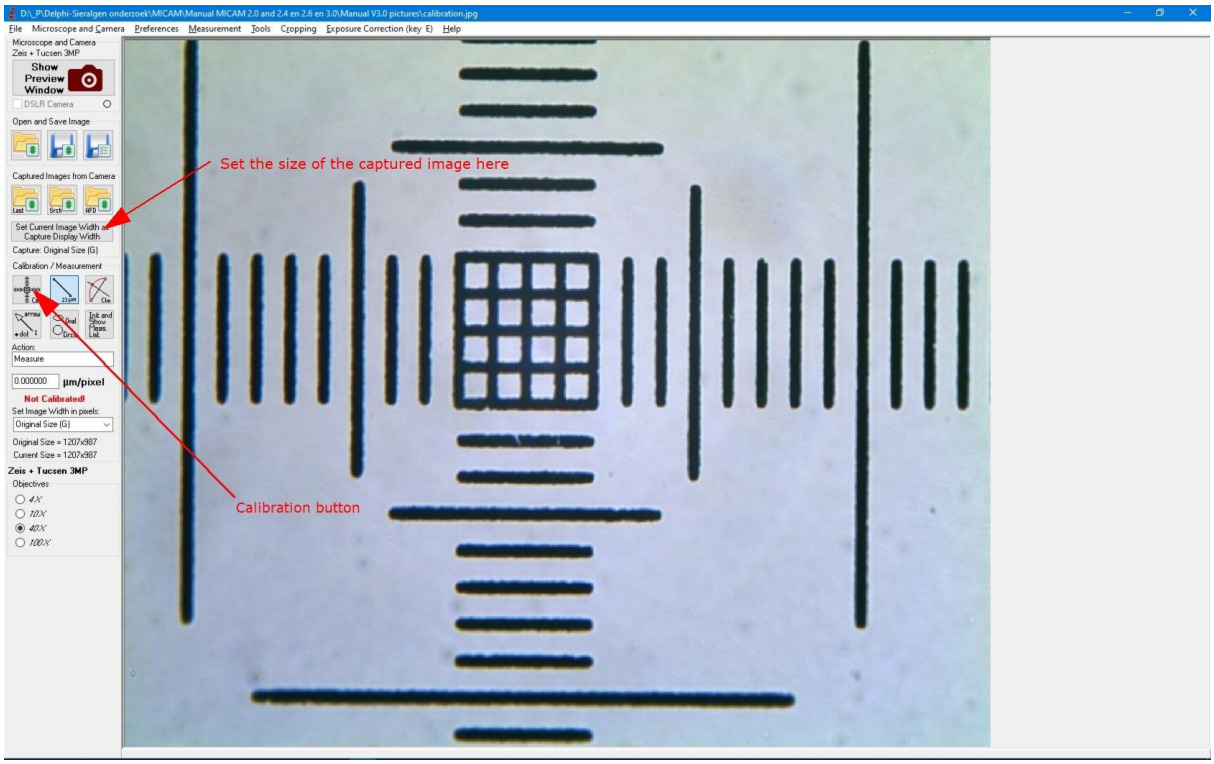

Figure 2.10. The main window with the captured image

Now click the '***Calibrate***' button (see figure 2.10 to locate this button on the tool panel). In the image you go to the start of the known distance. Press the left mouse button and move to the other end of the known distance. Release the left mouse button.

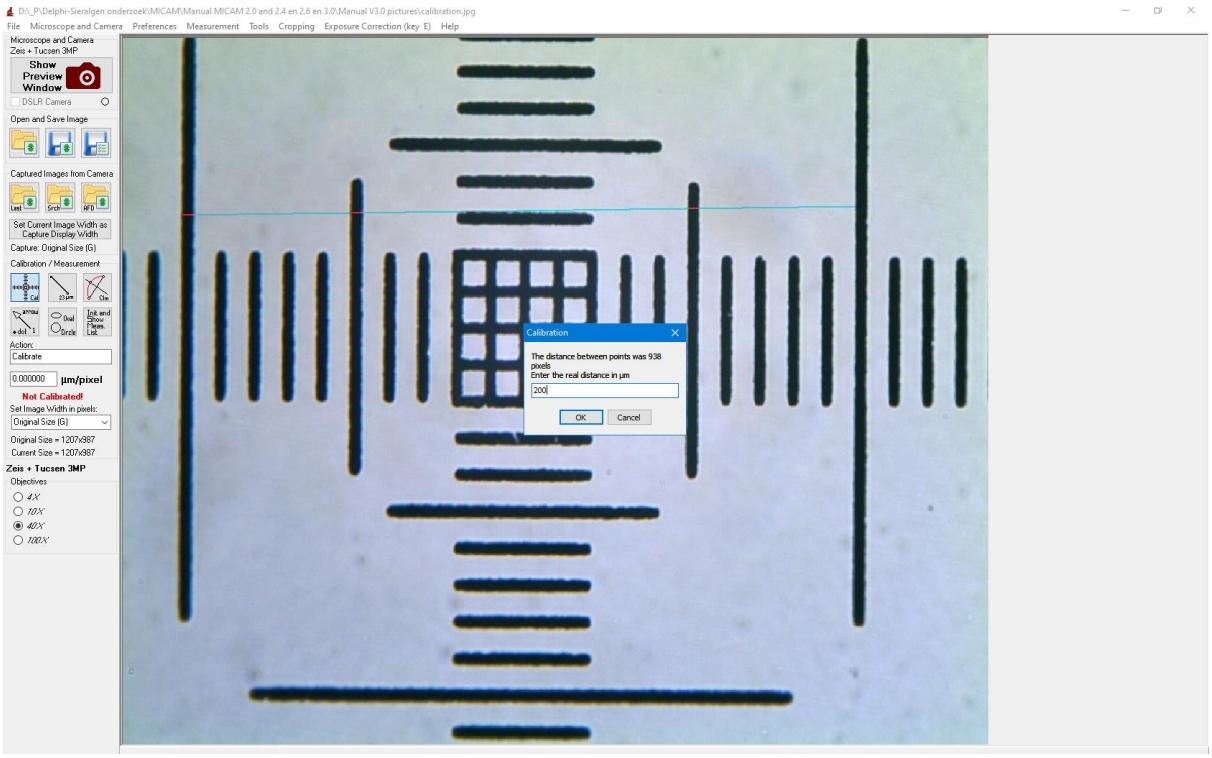

Figure 2.11. Calibrating the system

A small window pops up were you can enter this known distance (see figure 2.11).
In this image you can see the thin line between the two lines that are 200 µm apart. So you have to enter 200 in this pop-up window.
Now the 40 X objective is calibrated. You can see the caption of the 40X radio button is bold face meaning the objective is calibrated. The other objectives now have a calculated calibration value, indicated by captions that show a regular font.
After a calibration of an objective the program makes a guess about the calibration factor for the other objectives in case they are not calibrated yet (I assume that when you calibrated the 10X objective, the 40X objective produces an image almost 4 times as large). I found out that measurements on the other objectives will be rather good without calibrating each objective but it is better to calibrate all objectives. The program will warn you when you use a calculated calibration.

Chapter 5 gives a more detailed description of the preview window and its use.

2.3.2 A DSLR camera

The calibration process for a DSLR is almost the same as for a USB camera.

The first thing you have to do is set the ***MICAM-Temp*** folder of your Live View program to the same folder as you have chosen in MICAM. In figure 2.12 you see how I did it for my system with the Canon EOS 600D. I also instructed Live View to download the images from the camera without leaving a copy in the camera memory (in the tab Basic Settings).

In digiCamControl it is a little different, but the principle is the same.

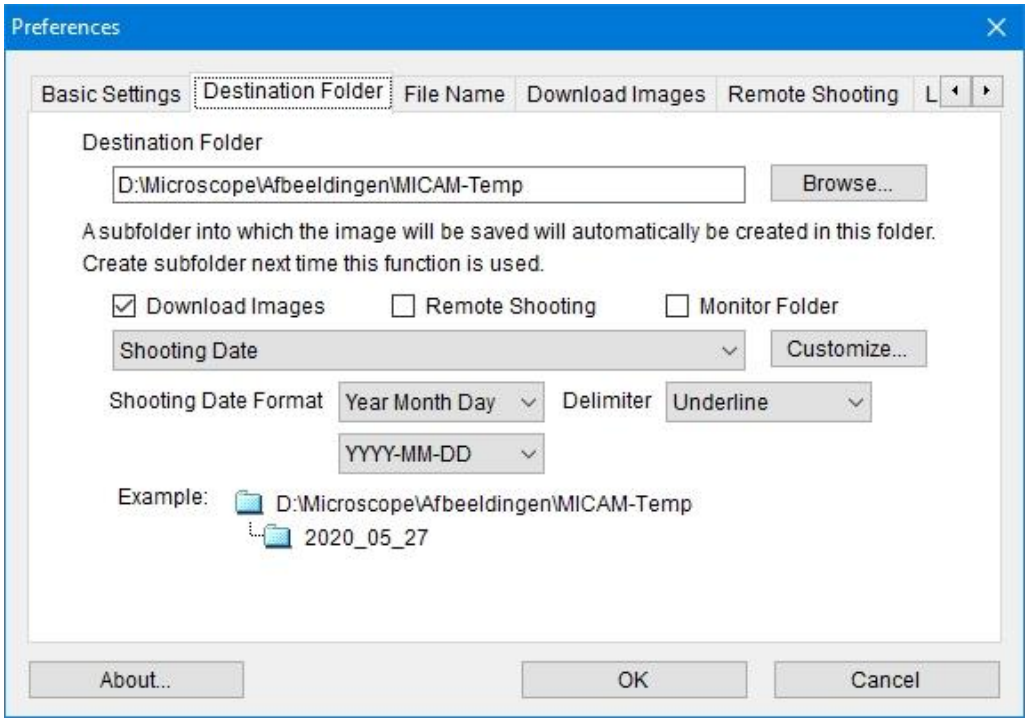

Figure 2.12. The Preferences window of the Canon EOS Camera to set up the Destination Folder

In MICAM you have to setup the same folder in the Preferences window (see figure 2.13). Once this is done, MICAM will monitor the ***MICAM-Temp*** Folder during Preview. When you take a snapshot with your camera, the image will be stored in the ***MICAM-Temp*** Folder and MICAM will get the image from that location.

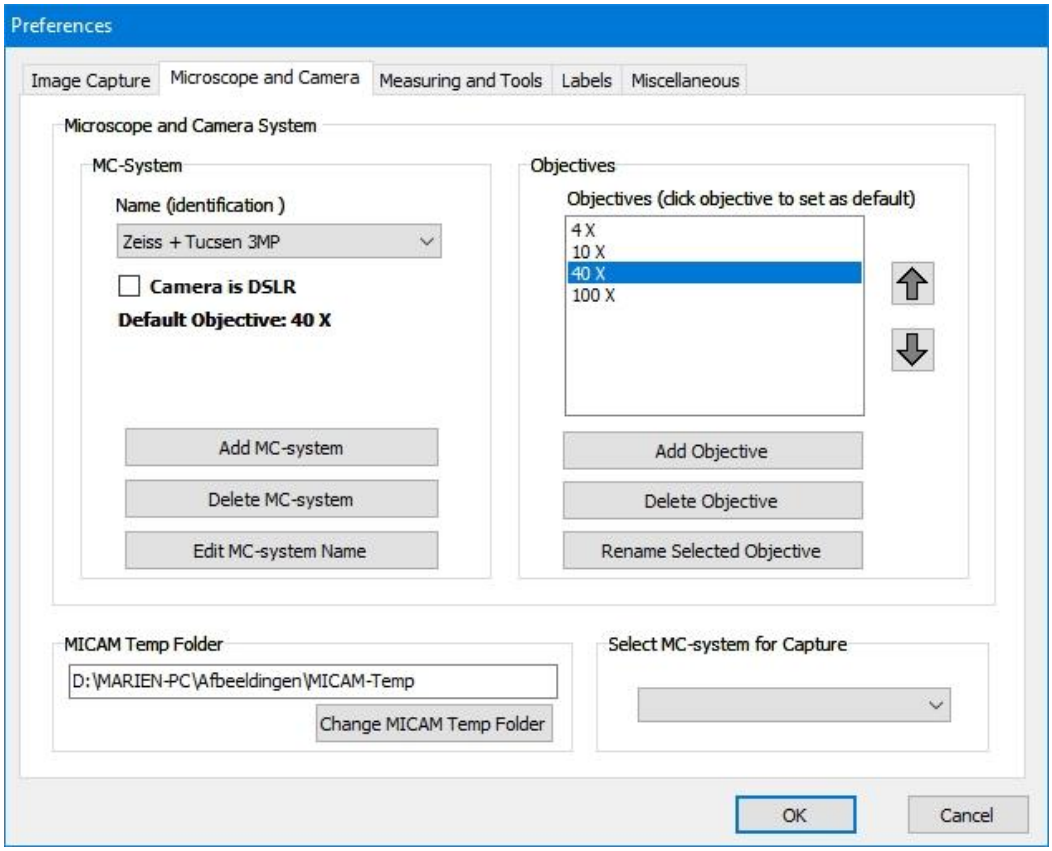

Figure 2.13. The MICAM-Temp Folder setup at the bottom left.

MICAM will be minimized during Preview, so the Live View windows are visible And you can focus on the object you are interested in (figure 2.14). When you have two screens, you can move the Live View controls to the other screen. In that case you don't have to click on the preview button.
When all is set-up well you can press the shutter button in the Live View program and the photo will appear in the measurement window. If the image is to large you can ***Set Image Size*** to ***Fit to Window*** (or just press 'F' on the keyboard). Now you proceed in the same way as described for the USB camera.
When you switch over to Preview mode after processing, the file in the ***MICAM-Temp*** Folder will either be deleted, or will be moved to the '***Unprocessed***' sub folder of your current Photo folder. If for some reason you want to start editing the original Image, it is easy to find it in the '***Unprocessed***' folder.

Most DSLR cameras deliver over 18 megapixel images. This is much larger than the computer screen size. In most situations, initially you want to see the whole image. You can instruct MICAM to show the image from the camera resized, so it fits the window. The first time you capture an image from the camera you have to resize the image with the ***Set Image Size*** drop down list. Then click the ***Set Current Image Width as Capture Display Width*** button. Now all subsequent images will be shown with this size (see Figure 2.10).

Note. Just below the Preview button you see a small grey circle. This circle is green when MICAM is monitoring the ***MICAM-Temp*** folder. When the circle is grey, you can start the monitoring process by pressing the Preview button.
This is all you need to know for capturing images with MICAM. More advanced possibilities will be explained in the next sections of this help file.

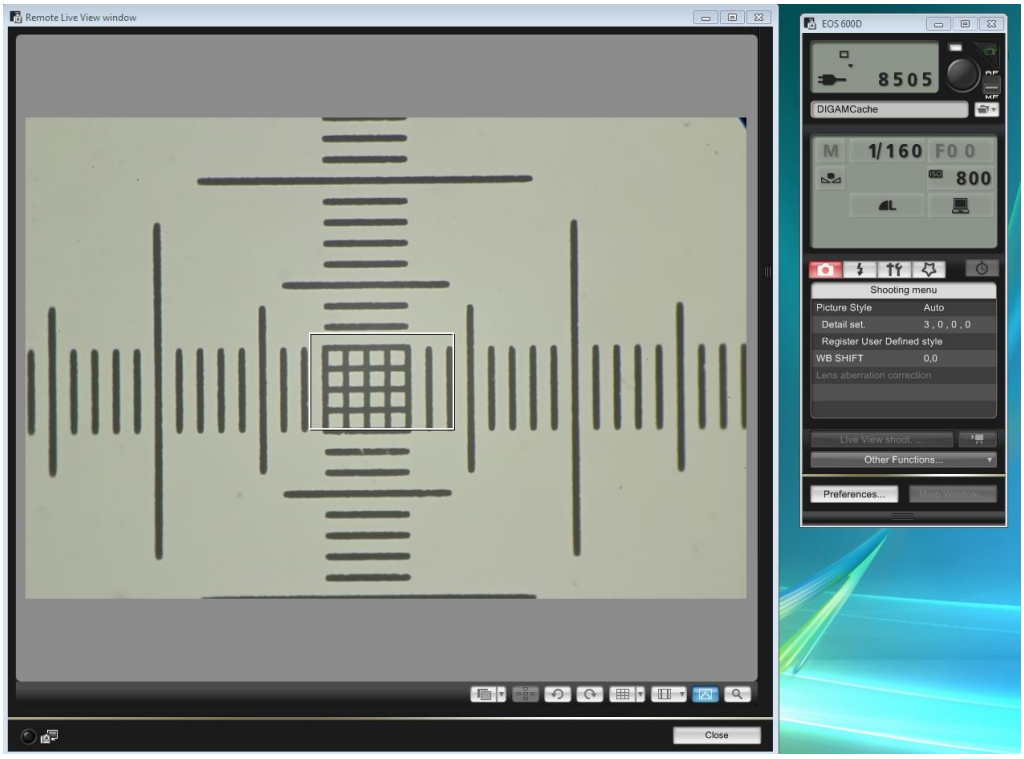

Figure 2.14. The LiveView window of the Canon EOS Camera

A third method of opening image from a camera

MICAM has the option to open an image from a folder that differs from the Photo folder. This can be useful if you can access the camera memory via USB. In that situation the camera memory more or less behaves like a USB-stick.

So in MICAM you can open the images from your camera, while the processed images will be saved in the Photo folder.

To open a folder that is different from the Photo folder is possible by holding down the CTRL-key while clicking the Open Image button (CTRL + O key). Initially the folder might be identical to the Photo folder, but after moving to the camera memory folder and opening an image, MICAM will remember the last used location and the next time you press CTRL-O the camera memory will be opened directly.

3. The main operations

The main operations of MICAM, after retrieving images from a camera, are:

1. Set the right size and orientation of the image.
2. Process the image.
3. Save the image to from the hard disk

It is also possible to open an image from your hard disk to do the processing. If this image was processed by MICAM before, the earlier used calibration information will be used. You can send the MICAM images by mail to someone else. If he or she opens this image in MICAM, the calibration information will be available on that computer as well.

The main operations will be described in this chapter.

3.1. The Main operations in detail

MICAM image processing has to be applied in a certain order. **General rule:**
First set the size of the image and adjust colours (exposure correction), then perform the measurements and finally save the image.

1. Select the final size and orientation of the image first. This is the size and orientation that will be used to store the image. Due to the way MICAM is constructed ***all information you placed on the image will be gone when you resize an image afterwards***. This means you have to do the cropping, resizing and rotation first. Then apply the Exposure Correction, although you can apply resizing and exposure correction in the reversed order.
   To Undo changes in this stage use CTRL-U.
2. Now you can apply additional processing of the image. This means:
   a. Calibrate the image when not done automatically.
   b. Perform measurements.
   c. Add a scale.
   d. Place three different kinds of labels on the image.
   e. Measure the length and arc of a special kind of algae called *Closterium.*
   f. Insert an arrow or place dots in the image.
   g. Draw a circle or oval on the image
   h. Save measurements to an Excel file.
   i. Undo or redo each action with CTRL-Z or CTRL-Y.
3. Save the final result to your hard disk.

The details of those processes will be discussed below.

3.1.1 Cropping an image

The menu item ***Cropping*** allows you to crop a part of the image (see figure 3.0). The options you have are discussed below.

Select Crop Area
Sometimes you are interested in a smaller part of a large image, but you want to have a specific width to height ratio (for placing several images in a grid on a website for example).

With ***Select Crop Area (pixels)*** you can select several area widths in pixels (640, 800, 1024 etc.). ***Select Crop Area (units)*** if the area has to be the same amount of µm, mm or mils wide. N.B. in ***Preferences → Miscellaneous*** you can switch between setting the Width to a certain value or the Height of a certain value, depending on your purpose you can select the best method.

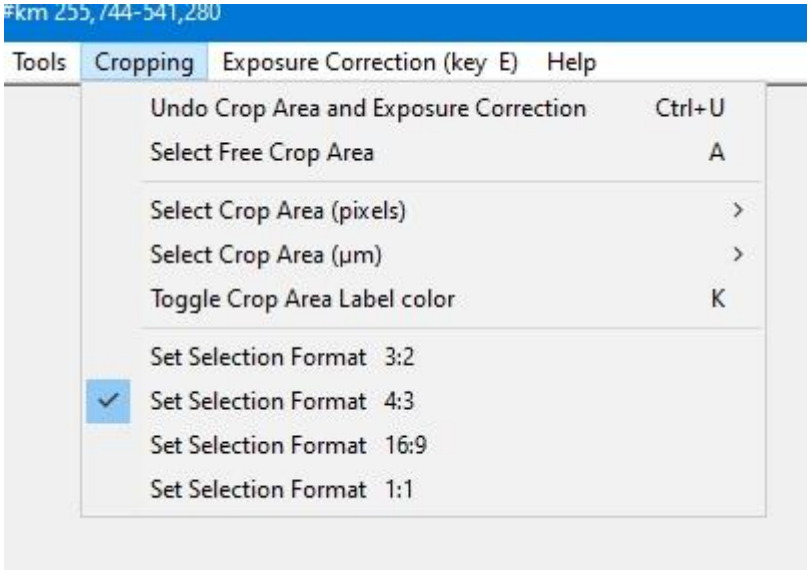

Figure 3.0. The Cropping menu

When you select this option a rectangle will appear with near the centre a label displaying the size of the area covered by the rectangle. When you move the mouse (***don't press any mouse button!)*** you see the selection rectangle following your mouse movements. When the selection area is on the right spot, press the left mouse button and the selection will be cut out with the selected width to height ratio in ***Set Selection Format***. If the selected area is too small or too large, you can use the mouse wheel to increase or decrease the size of the area before clicking the left mouse button. N.B. When you have a large image, but you decided to fit the image to the window, the selected area may look rather small. This is because the selected area refers to the real image and not to the size displayed on screen.
This cropping operation can be undone with CTRL-U.
When during the selection process you decide NOT to cut a selection you can press the RIGHT mouse button or the Escape key to quit the selection process.

By default the selection area is in 'landscape' format (width larger than height). pressing the ***Q-key*** will change this to 'portrait' format.

To select a cropping area it is much easier to right-click on the image. In the pop-up window (see figure 3.1) you can select the appropriate size. Sometimes it is necessary to select a 'portrait' shaped part from a 'landscape' oriented image. In the right-click pop-up window select the item at the bottom to get largest portrait oriented part of the image.

Set Selection Format
When you select a part of the image (for cropping) MICAM will select a part of the image with a predefined width to height ration. Ratio 3:2, 4:3, 16:9 and 1:1 are predefined.

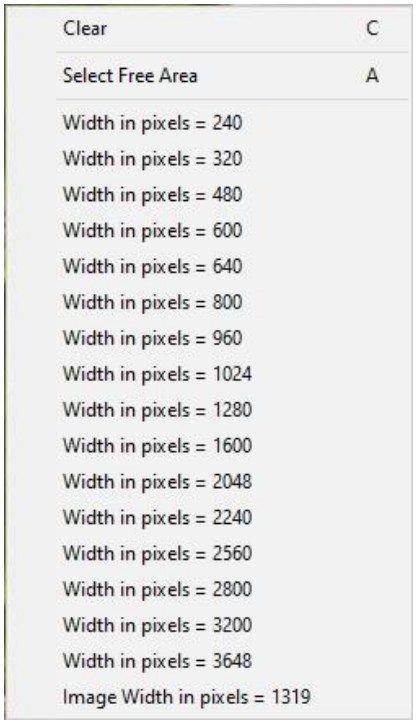

Figure 3.1. The right-click pop-up menu

Select Free Area

When the available options are not sufficient, you can select the size and Width to Height ratio yourself with ***Select Free Crop Area*** (Key A). A 'dragging' cursor will appear if you press the A key. Press the left mouse button to select the upper left corner of the selected area, then (with the left mouse button pressed) move to lower right corner of your selected area. Now release the mouse button. The four sides of the rectangle can be moved with the mouse. Placing the mouse on a corner, two sides can be changed together. When the square fits nicely around the part of the image you are interested in, you can click the image while you press the CTRL key. The result will be an image containing the selected area. The size of the box in pixels and in µm will show up inside the square. The arrow keys, together with the ALT-key, allow you to set the size of the square in multiples of 10 µm (or mm or mils).
The label in the centre of the selected area is Black by default. When the background of your image is very dark, this text will be difficult to read. You can change the colour to White by pressing the K-key. Pressing this key again changes the colour to Black again.

3.1.2 Rotate an image

The image can be rotated over an angle of 90 degrees left with the rotate function in the Tools Menu item (or use the L-key). The 90 degrees right rotation can be done with the R-key.

3.1.3 Exposure Correction

When you select this menu item a new window pops up, were you can correct the gamma, brightness, contrast or saturation of the image. Also some colour corrections can be made. If the original image needs some corrections you can do that in this window (Figure 3.2).

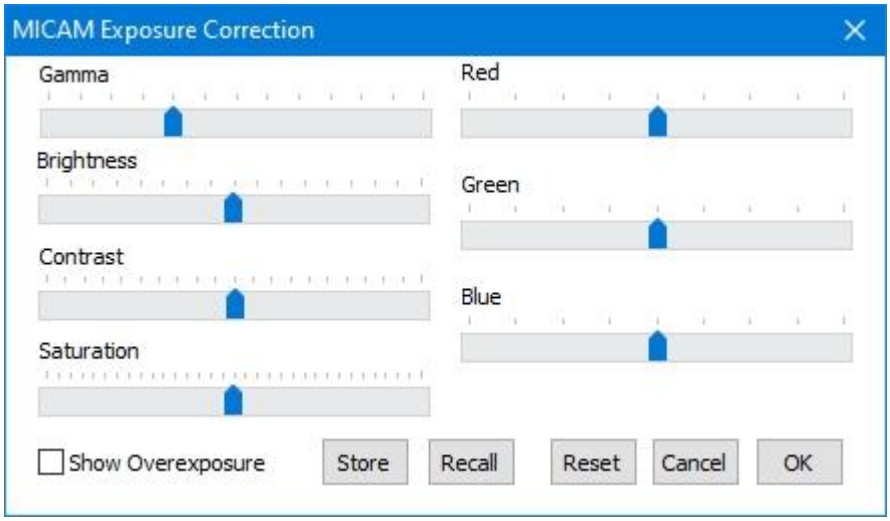

Figure 3.2. The Exposure Correction (or colour correction) form.

If you want to make sure there are no large white overexposed areas in the image you can check the ***Show Overexposure*** box.
In order to process several images in the same way you can ***Store*** the corrections before you press OK. After opening another image you can apply the same changes by pressing ***Recall***.
The keyboard shortcut for this window is the ***E-key***.

3.2. Image tools

Once the image has the correct size and colours, measurements can be done, arrows and labels can be placed and the most important part of all, a scale can be placed on the image.
Many options for the processing tools can be set in ***Preferences → Measuring and Tools*** (Figure 3.3). This menu more easily can be opened directly from the keyboard with the ***T-key***.

3.2.1 Placing a scale

Placing a scale in the image is the most important processing task. Figure 3.3 shows how you can select the colour and height of this scale in ***Preferences → Measuring and Tools***. The height can be set in a certain amount of pixels or as a percentage of the height. The last option can be used when images with different size are handled, but in a publication they will be printed with the same size. Now the scales will have (more or less) the same height in all images. If you only want to show a scale bar in the image you can deselect the option ***Show Scale Text***. When scale Type 2 is selected, you can select the division interval that suits you best. Finally you can select the initial placement of the scale.
The scale and text colour will always be the same. So the ***Color*** selected in the ***Scale*** box will override the colour selected in the ***Scale and Measurement Font*** box!

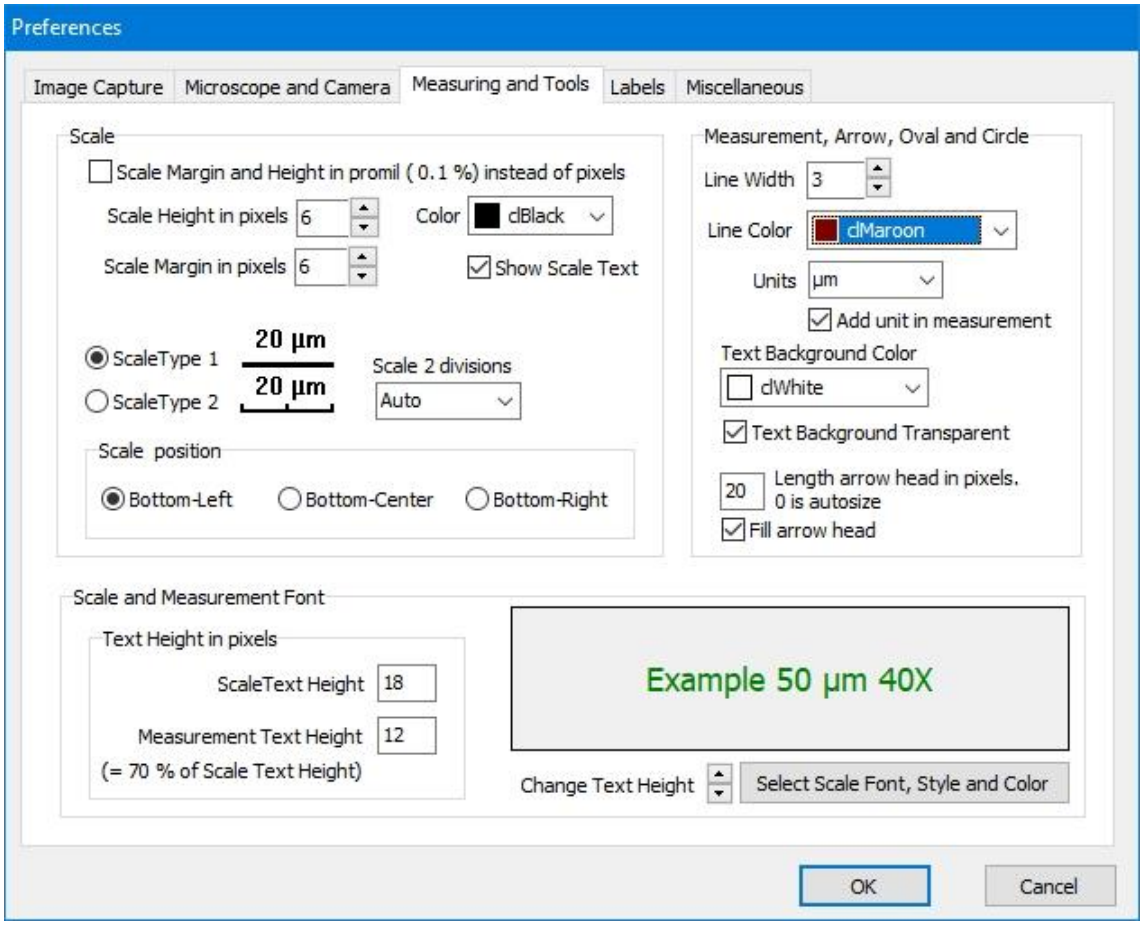

Figure 3.3. The Measuring and Tools tab in Preferences

Scale length can be selected from the Measurement window (Figure 3.4). Or use the accompanying Function Key (***F1*** to ***F7***).

Directly after placement of the scale you can place the mouse cursor on the scale and toggle the Scale Text with the right mouse key.
With the mouse on the label, you can move the scale to any position moving the mouse with the left mouse button pressed. A green light will show when you are near the centre of the image. When you release the mouse button in this situation, the scale will be placed exactly in the centre. Also the amount of pixels from the left margin and above the bottom margin will be shown, enabling precise positioning of the scale.
The scale initially will be placed on a predefined ***Margin*** from the bottom of the image. Dragging the scale below this margin is not possible, but by pressing CTRL you can override this behaviour.

The lines for ***Measurement***, ***Arrows***, ***Ovals*** and ***Circles*** are drawn with a width of 3 pixels in the colour Maroon. The text labels of Measurement lines Arrows and Circles have a green font. The selected background is white, but invisible because the background is transparent (see figure 3.3).

Thera are two options for drawing arrows. You can select the size of the arrow head in pixels, but can allow MICAM to choose a size, depending on the length of the tail of the arrow by entering a 0 for the size of this arrow head. Another option is to choose between a filled or open arrow head.

The labels of Measurement, Arrows and Circles can be edited right after placement by clicking them with the right mouse button. For long texts you can choose to wrap the text to occupy several shorter lines.

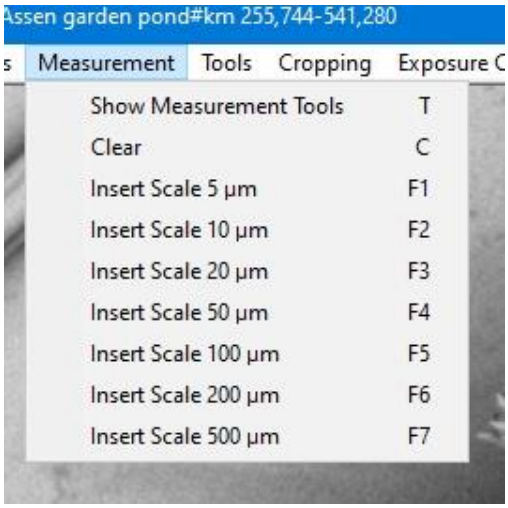

Figure 3.4. The Measurement menu item

Pressing the C-Key will remove all processing that has been done after selecting the size, orientation and exposure correction will be wiped out. So use this key with care.
It is better to use CTRL-Z to undo the last action in most cases. CTRL-Z can be applied 16 times when necessary.

3.2.2 Perform calibration and measurements, place arrows, circles etc.

Measurement lines, arrows, ovals and circles are drawn with a predefined Width and Colour, so you can have different line widths and colours for the scale and the measurement lines..
Select a convenient Font in the ***'Scale and Measurement Font'*** box on the ***Measuring and Tools*** tab in ***Preferences***. Use the ***Up-Down*** buttons to increase or decrease the font size (actually the height of the font. With the Font Select dialog you can select a Font (name), Style and Colour.
Every line or arrow has a label. This label can have a coloured background (so it stands out) or be transparent.

The Tools Panel
Several tools can be selected from the Tool Panel (see fig. 3.5)

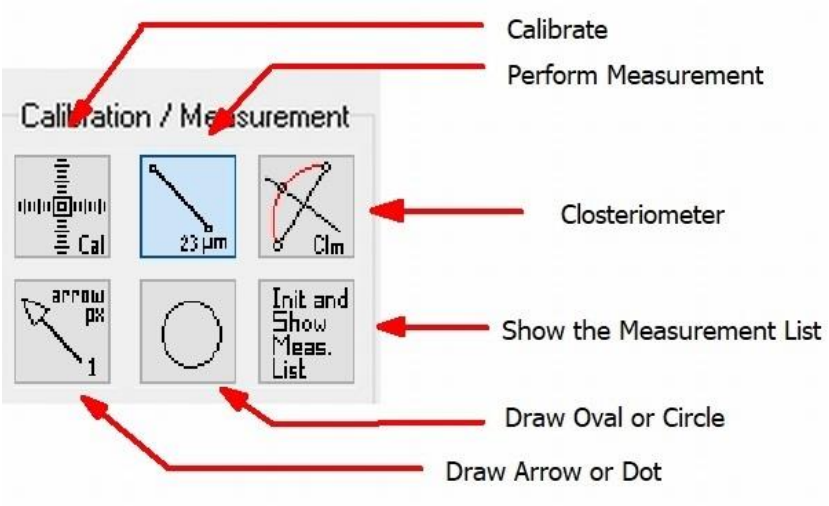

Figure 3.5. The processing tools

Calibrate
When this option is selected you can calibrate the current objective for the current selected MC-system as was discussed in Chapter 2.1.
The names in the objectives box are italic when they are not calibrated and bold face when they are calibrated. Once you have calibrated your system it is not necessary to do the calibration again, although it is good to check once a year if the calibration value is still correct.
During calibration MICAM stores the size of the image that is used for the calibration. Every new image (**with the same size**) is assumed to come from that camera and hence the stored calibration value will be used again.
The calibration values will be stored as the amount of µm per pixel in the MICAM-cal.ini file. In the preferences you can select the units you want to use for displaying the results of measurements. There are three options: micrometre (µm), millimetre (mm) or mil (1 mil = 0.001 inch= 25.4 µm).

Measurement
To perform a measurement you move the mouse to the place where you want to starting point. Press the left mouse button and with the mouse button pressed go to the stop point.
A line will be drawn and the length (in µm, mm or mil) will appear at the end of the line. This distance will also be displayed in the ***Action*** field. If you selected to display the units (in ***Preferences → Measuring and Tools***) they will be displayed as well (Figure 3.6).

In a publication you might want to present straight lines if you move horizontally or vertically. It is difficult to do that by hand, but there is a trick to make a straight line:
Try to draw a measurement line as horizontal (or vertical) as possible. Then hold down the CTRL-key and move the mouse a little bit. The line will become perfectly straight now.

Arrow or Dot
When you have selected (clicked) this button, you can cycle through the options of this button by additional clicks. Initially it shows the options to draw an Arrow, as is shown in figure 3.5. Click this button to use the ***Arrow px*** option. This operates in a similar way as the

Measurement tool: Place the cursor on the spot where the arrowhead has to appear and move to the place where you want to have your caption. By default the arrows are numbered starting with number 1 then 2 etc. But when you right-click this caption you can alter the text as is done in figure 3.8. Note that it is only possible to change this caption only direct after placement! The position of the arrow head (in pixels) will be stored. Clicking the button again you go to the ***Arrow µm*** mode. The operation is the same as the previous mod, except that now the arrow head position in µm (or mm, or mils) will be stored. The next possibility is the ***Dot px*** mode. A small dot will be displayed and the position of the mouse (in pixels) relative to the upper left corner will be stored. A number will be shown as a label as well. Another click is the ***Dot µm*** mode, but now the distance to the top left corner in µm will be stored. How useful that is will be explained later in this paragraph.
When you click the Arrow/ Dot button again you will go to the initial position and the counter will be reset to 1.

Oval and circle
When you click this button to select it you go to the ***Circle*** drawing state. This allows you to draw a circle. The centre of the circle will be where you start dragging with the mouse. When you release the left mouse button the final circle will be drawn and the diameter of the circle in the chosen units will be displayed in the centre of this circle.
Pressing the button again leads to the ***Oval*** drawing state. An oval will be drawn between the starting point and the point where you released the left mouse button. With this oval you can show an interesting area on the image.

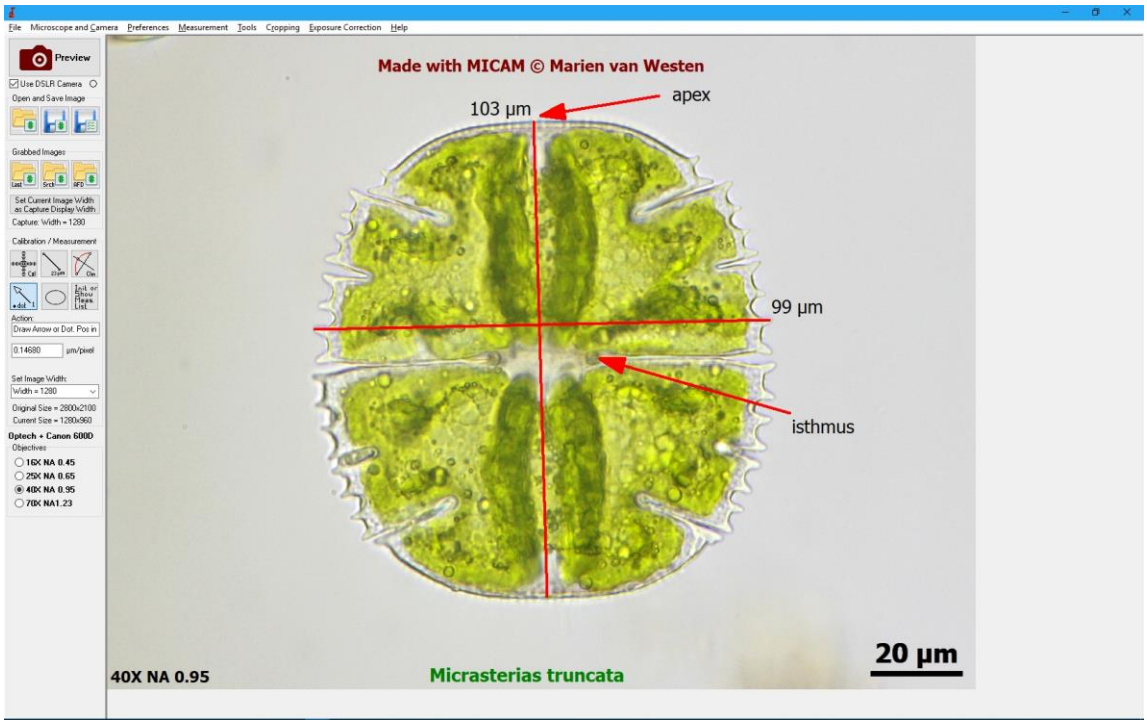

Figure 3.6. A demonstration of some of the capabilities of MICAM

Closteriometry
This function was implemented to measure the arc of the desmid species *Closterium*, but it can also be used to perform measurements on circular objects. You measure the arc in a few steps:
1. Move to the start point of the arc.
2. Press the left mouse button and move to the end of the arc.
A line between the two points will be drawn like in the case of a normal measurement. Also the length of the line will be displayed. An additional perpendicular line through the middle of this line will be displayed.
3. Move to the point where this perpendicular line intersects the arc of the *Closterium* and click the left mouse button. At the bottom left of the image the curvature (in degrees) and the radius of the circle will be shown (Fig. 3.7).

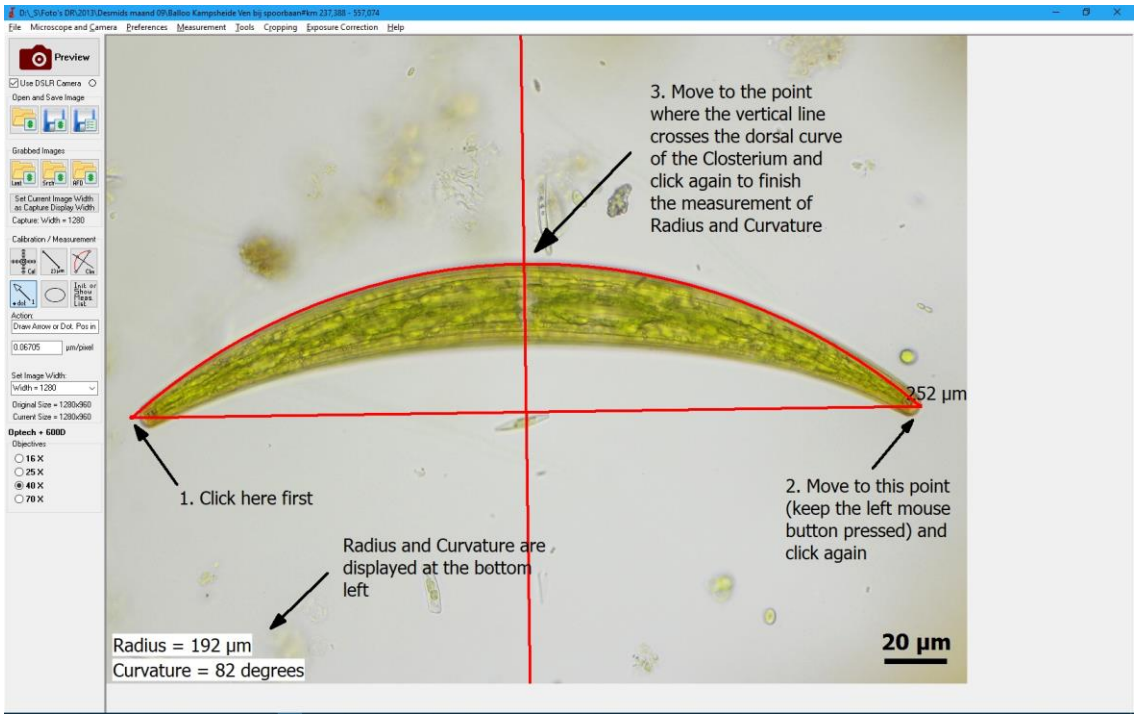

Figure 3.7. A demonstration of the measurement of the curvature of a Closterium

Initialize and show measurement list
This button initializes a table that will be shown on the right hand side of the MICAM window (Figure 3.8). Before you start select the right number of columns.
If you want to perform only length measurements, select one column. This way every measurement will have its own line in the table. If you want to measure length and breadth of several objects then it is more convenient to select two columns. When you measure length fist and then breadth you can create a table with in column 1 the length and column 2 the breath (Figure 3.8). Note that in the image not the measured length will be shown, but the number in the table. When you save the image after the measurements, you have a table that corresponds with the cells in the image.
It is possible to edit the values in the list by selecting a cell. By selecting the caption of a column, you can edit those values as well.

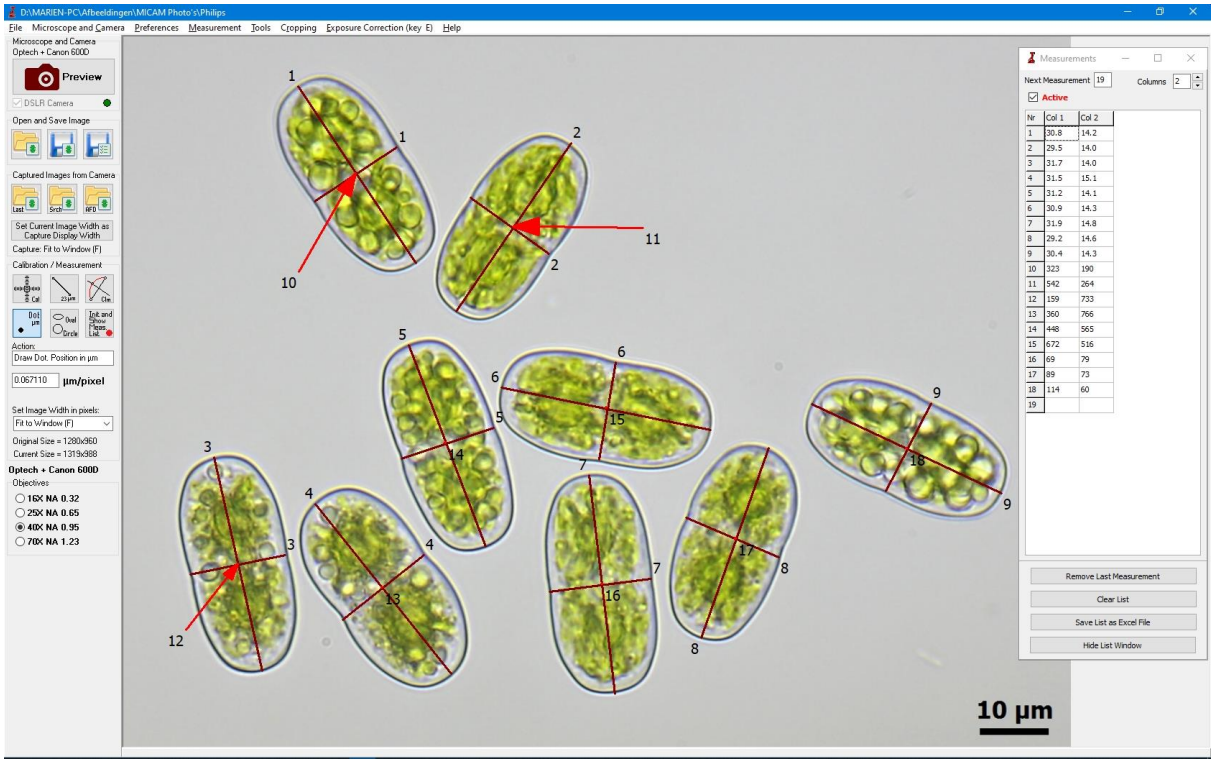

Figure 3.8. A demonstration of the use of a measurement list

When you draw an arrow now the starting point of the arrow will be entered in the table(measurement 10, 11 and 12 in the table). By default the numbers will be the amount of pixels in X and Y direction relative to the upper left corner (Figure 3.8). Measurements 13, 14 and 15 show the position in pixels of the dots in the centre of the cells. Finally for measurement 16, 17 and 18 the position of dots in µm was selected.

When you perform a measurement on a *Closterium* you need at least 3 columns. The first entry will be the length, the second the radius (in the used units) and the third the curvature in degrees. When you start with the measurement of the breadth of the cell and then perform the closteriometry, you need 4 columns (Figure 3.9). This way you can make a table with all important values of that cell (breadth (col 1), length (col 2), curvature (col 3) and radius (col 4)). When you load a new image, the list is not cleared, so you can collect measurements of many different cells in one table
Finally you can save the table in Excel format for further processing.

On the ***Measurement List Button*** on the main screen a red light will appear to indicate that the measurement list is being populated. You can hide the measurement list if you want, but the red warns you it is still active. You can interrupt the process of adding data to the list by unchecking the ***Active*** box. Later on you can resume the process by checking this box.

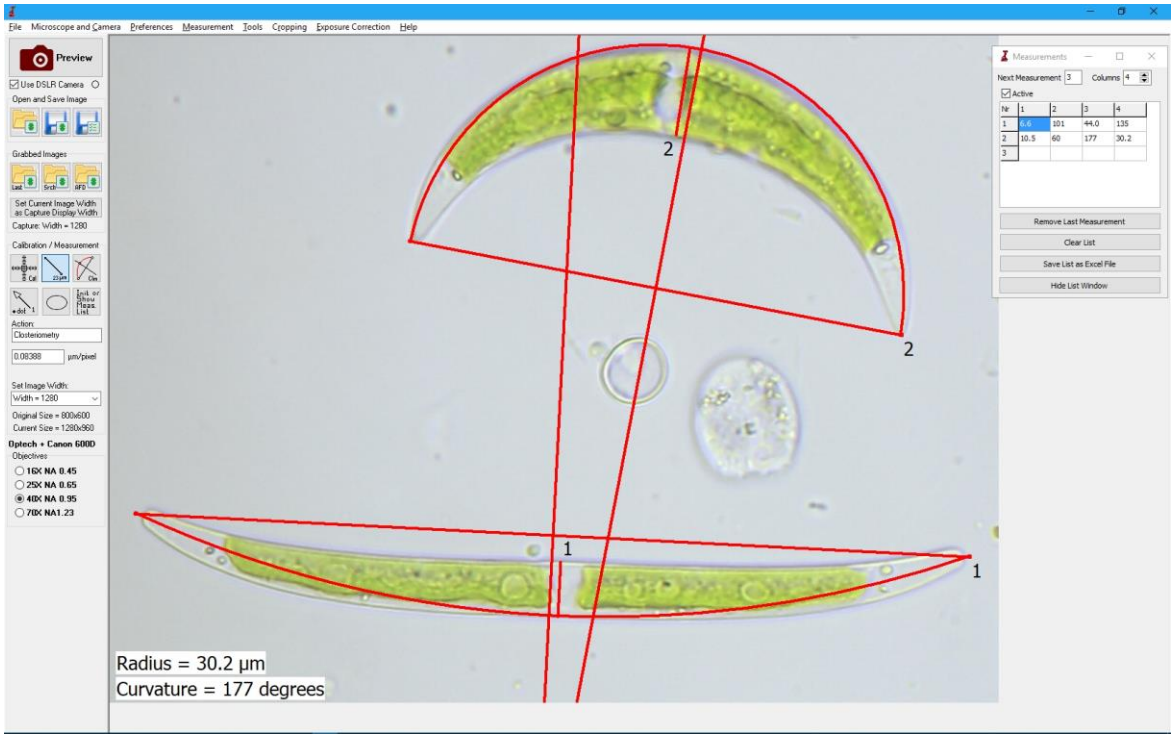

Figure 3.9. A demonstration of the use of a measurement list in measuring two Closterium cells

3.2.3 Placing Labels

You can place several labels in the image. They are called ***Label***, ***Copyright Label*** and ***Objective label***. The latter two contain the predefined text (see ***Preferences → Labels***), but as you can always change the content of a label directly after placement by placing the mouse cursor on the label and pressing the right mouse key, you have in practice three different kind of labels with a different font at your disposal. You can enter a longer text when entering the ***Label*** text and it will wrap after a predefined amount of characters (see ***Preferences → Labels***. See figure 3.7 for a demonstration of this feature. In that example the captions of the arrows are altered directly after placement. You can even copy past text from another source in the editing window of a label. By default only the Label text will be wrapped, but if you right click another label direct after placement, you can wrap those texts as well.

Labels can be placed by selecting the item in the Tools menu item (figure 3.10) or use the shortcuts: CTRL-L for Label, CTRL-M for Objective Label and CTRL-R for Copyright Label. Initially the labels will be placed at the default position as given in the Preferences, but direct after placement you can drag them (left mouse button) to any location you want. This operates in the same way as with the scale, including the centred placement. Figure 3.11 shows an example of the placement of the labels. For precise placement an indication of the position of the labels will be shown during dragging.

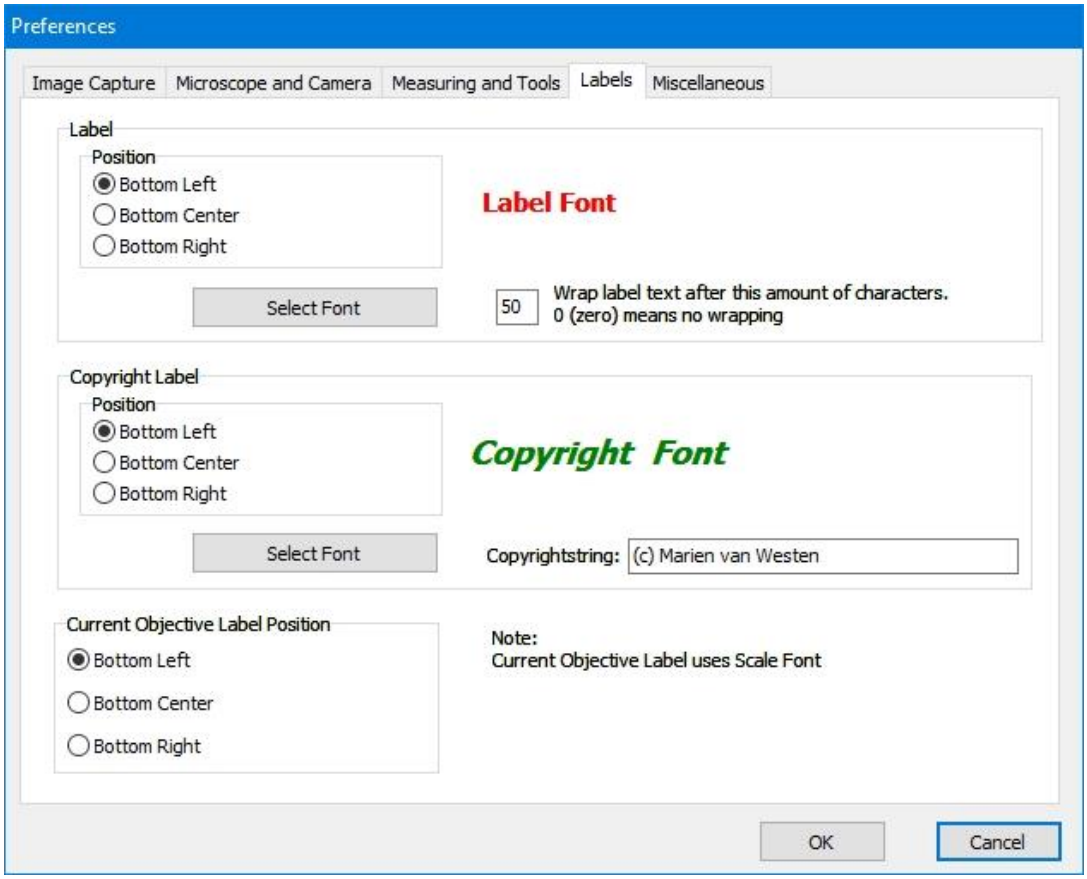

Figure 3.10. The Label tab.

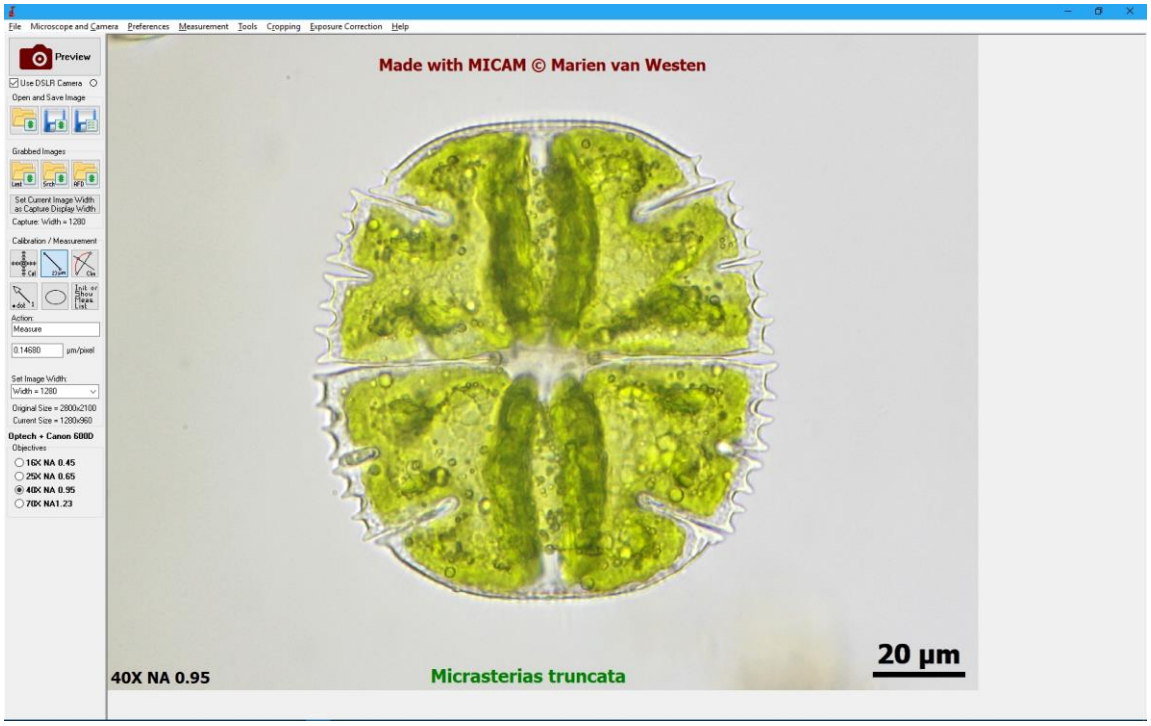

Figure 3.11. Placing a scale, objective label, copyright label and several labels

Cleaning up the image
When the image becomes to cluttered, there are several ways to clean up the image.
With CTRL-Z you can undo the last action (use Redo, CTRL-Y to apply the last action again). You can apply CTRL-Z 16 times at most, so you can go back several steps when necessary.
If you want to clear the whole image, use the C key.
If you want to undo Exposure correction and cropping, use CTRL-U.
As a last resort you can reopen the original image (see chapter 3.3).

3.3. Saving and opening image files

A apart from the image processing tools, one of the most often used elements of MICAM is the saving and opening of image files. As there are many options and ways to open image files, a thorough explanation of the purpose of the options will be given here. The first point to start is the File menu item (see figure 3.12).

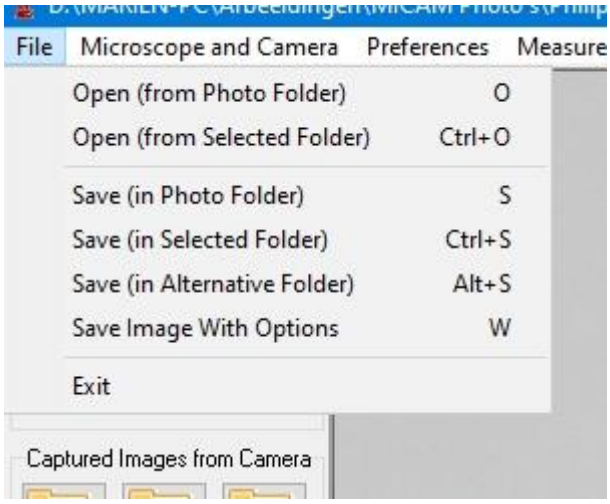

Figure 3.12. The File Menu item

The main purpose of MICAM is to get images from the camera and store them after processing on your hard disk. As stated earlier in MICAM you can choose from four different file formats to save the image:
BMP uses no compression and has no losses, but the file size is rather big.
PNG uses some compression but has no losses, so the files will be a bit smaller.
JPG uses compression, but is in principle not lossless. This gives the smallest images. MICAM uses a compression factor with as less loss as possible.
TIF (or **TIFF**) is also a lossless format.

MICAM uses the so called ***Photo*** folder for that. Open and Save to that Folder can be done with the ***Open (from Photo Folder)*** item and the ***Save (in Photo Folder)*** item. This folder can be chosen in the ***Preferences*** menu item. Shortcuts are keys O and S. As those functions are used so often, you can also find a quick access button for those functions on the tool panel (figure 3.13). During your work you can easily get access to earlier stored images to do an additional measurement, do some colour enhancement, or insert as scale. MICAM stores the calibration information inside the image. When you open an image later on, MICAM recognises this information in order to do additional operations in a later stage.

In my work I study desmids in water samples. For every sample I create a new folder to store the images. This way it is easy to find the images belonging to a particular sample later on.

Working at the microscope you will probably use the Photo Folder, but when you want to look at images taken from another sample you want to go to that folder without changing your working folder. Here you can use CTRL-O for opening that folder or CTRL-S for saving to that folder. But it is also possible to open an image from that Selected folder with CTRL-O but save it to another folder after processing with ALT-S.
Pressing CTRL or ALT and clicking on one of the Tool Panel buttons is also possible. Of course it is possible to select the appropriate function from the ***File*** menu item, but the buttons on the tool panel will be more convenient.

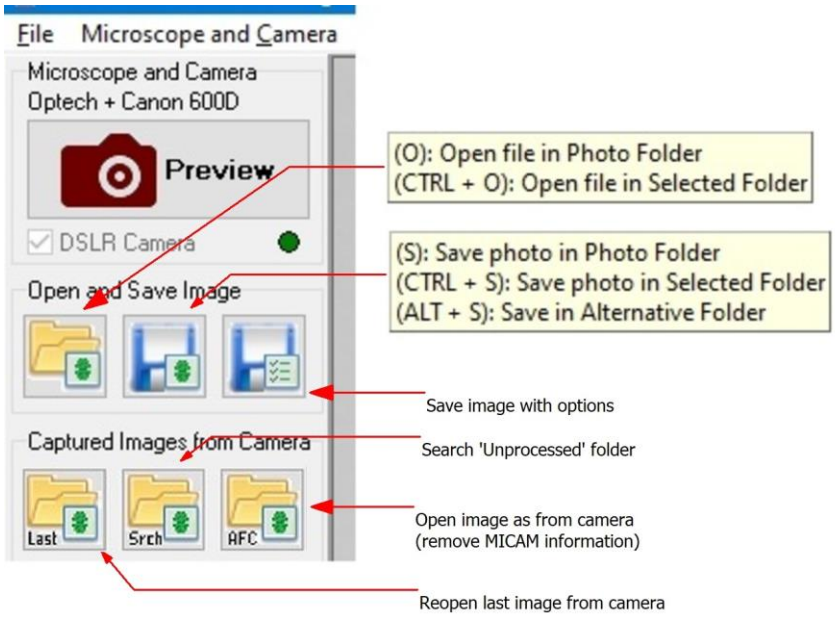

Figure 3.13. The Open and Save buttons on the Toolpanel

Save Image with Options
MICAM will create a default name for every image that is captured from an imaging device (camera). This name in general won't be very informative, hence the ***Save Image with Options*** menu item (or button). Saving the image with a name selected from a database is now possible. You can also press 'W' on the keyboard to perform this action.
When you press this button a window will open where you can change the name of every image you want to save in a convenient way with as less typing as possible (Figure 3.14).

When you open this window the cursor is in a field where you can select the name from a database. Just type a few letters of the name and in the box at the top-right you will see all the names that match the text you entered.
In figure 3.14 the selection was restricted to all names that match 'co con'. Note the space between co and con! The database is only a list of names in a text file. Take a look at the databases supplied with Notepad, to get an idea of the structure of those databases. If you want to use your own database you can easily create your own as it is just a text file with each name on a separate line. The list automatically will be sorted alphabetically.
In the file name you can also choose to add a date and objective (Magnification). The program chooses a unique number to add to the filename. So every filename in that folder will be unique, even when you select the same name several times.

You can search for a name in the database of your choice. If this name isn't there, you can decide to use the name you typed in the search field as the name of the image.
A list of recently used names is available, so you can pick a recent name without using the search facility.

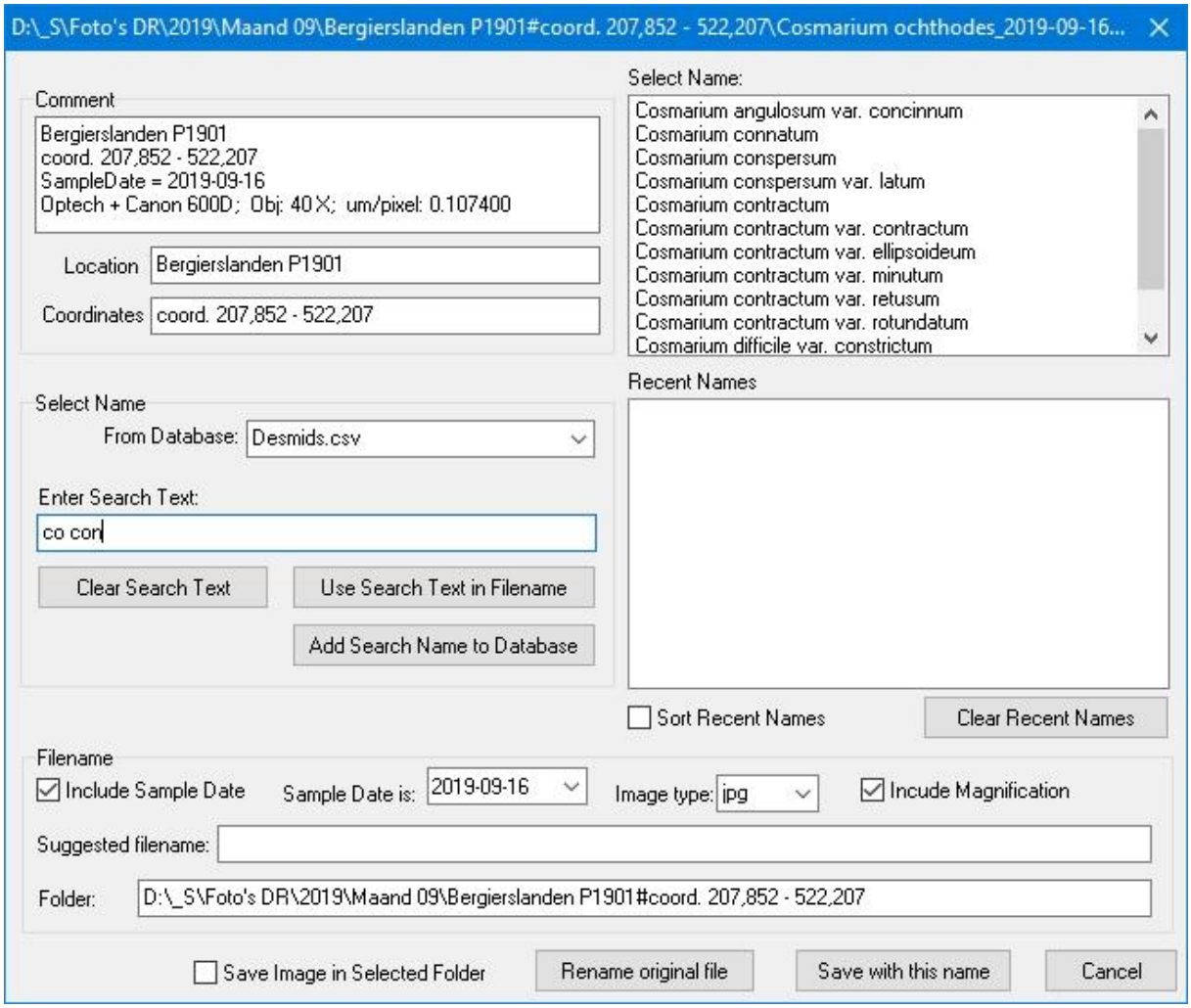

Figure 3.14. The Save With Options window.

The use of the Save Options dialog is made as easy as possible. At first use you might have to set some options, but later on you only have to find the right name. When you hit the ***'Save with Options'*** button, the dialog opens with the cursor in the ***'Enter Search Text'*** field. Enter some characters of the name and all possible matches will be visible in the list in the upper right of the dialog window (the ***Select Name*** list). When you want to find a name like '*Cosmarium hornavanense*', it is sufficient to enter 'co hor' (note the space between 'co' and 'hor') to get a very limited list to choose from. When you see the name you were searching you can hit the TAB-key. The cursor goes to the list and with the cursor keys you can select the desired one. Then hit the Enter key and the proposed filename will be shown almost at the bottom of the dialog. With another hit on the enter key you can save the image.
This name will also appear in the ***Recent Names*** list. When you want to use a ***Recently Used Name*** you only have to hit the TAB-key twice: use the cursor keys to select the right name and hit Enter.
If you want to add names to the present list of species, you can add them with the ***'Add Search Name to Database'*** button.
Note: You cannot edit the suggested filename. However, you can only edit a name in the

Enter Search Text field. Note that when you select a name in de Recently Used Names list, this name will appear in the Enter Text Search field. This can make editing a name much easier!

If you opened the image from file you also have the opportunity to rename the original file instead or to create a new file. Here (bottom left of this form) you can also select if you want to save the Image in the Photo Folder or in the Selected Folder (file opened with CTRL-O).

By default the program will save the Location (Name and Coordinates) and Sample Date in the comment. The program stores the microscope and camera information also in this comment, but normally this information is invisible for the user. With ***Tools → Show System Comment*** (or Y-key) you can make this information visible.

Grabbed Images
As stated in paragraph 2.1, the images from the camera can be stored in a folder called ***'Unprocessed'***. This is a subfolder of the current Photo folder. This only works if you enabled this option in ***Preferences → Image Capture***. You can retrieve the last 'grabbed' image from this folder, or you can search this folder for earlier grabbed images.
See the ***Open Last*** and ***Open Srch*** buttons in the main window (Figure. 3.13).

Additional ways to open and save images
It is also possible to open an already calibrated image as if from file (Open button with text AFD meaning As From Device, see figure 3.2). As MICAM normally uses the information stored in the image to set the calibration value, with this button you can override that action in case you made a mistake when applying the calibration on that image.

MICAM supports Copy (CTRL-C) and Paste (CTRL-V) options as is known for many programs. This way you can make interaction with other software much easier. Note that MICAM does not know where those images came from, so saving to the original folder is not possible.

Dragging a file to the image surface is another option to open files. In this situation MICAM will set the folder where the file came from as the ***Selected*** folder. In this way you can easily save the image after processing to the original folder.

Using O, CTRL-O, ALT-O, dragging or 'Edit with MICAM' an image file can be opened and MICAM knows the folder where this image came from. The next image in that folder can then be opened by pressing the **Space**-bar. The previous image can be opened with the **Backspace**-key. This way you can easily scan through that folder.

Make all images the same size
Sometimes you have images from different sources that should be set to the same size. That can be useful if dimensions of the objects have to be compared visually.
I assume calibration information is available. The first step is to determine the size of the images. For instance you want to set all images to a size of 80 x 60 µm. After opening of the image you crop them to this size. Probably many images end up with different sizes in pixels. Now set the size of the image to for example 800 x 600 pixels.
Save this image. Repeat this operation for all images.

4. Miscellaneous

This chapter describes some additional features of MICAM.

When MICAM is open, you can also drag images from the windows explorer (or Picasa) to the measurement window of MICAM and drop it there.

To demonstrate the use of Databases in MICAM I have added some databases:
Diatoms, Desmids and. Some of the databases only contain the scientific names, but others the Dutch common names of ‘Paddenstoelen’ en ‘Mossen’ (in MICAM referred to as 'Local names'). You can use MICAM for other subjects by creating your own databases. I hope the information you can get from the supplied ones will be sufficient for that purpose
Note: When you place your own databases in the Databases folder of MICAM, MICAM will recognize them and let you use them.

You can use shortcut keys to start the most used functions in MICAM. The following shortcut functions are implemented:

- A Select an Area you can resize (use the mouse to move this area)
  ……..Use the mouse to change the area size in pixels
  ……..Use ALT + ←, ↑, →, ↓ keys to increase/decrease the area size in
  ……..10 µm (1 mm of mil) steps
- C Clear the image from all scales, measurements, labels etc.
- D Reload image from the ***MICAM-Temp*** folder (if enabled)
- E Open the ***Exposure Correction*** dialog
- F Fit image to window
- G Set Image size to original size
- K Toggle the ***Crop Size Label*** colour between White and Black
- L Rotate image left (90°)
- O Open image file
- P Go into Preview Mode
- Q Interchange width and height of the cut area
- R Rotate image right (90°)
- S Save image as (in the ***Default Image Folder***)
  Use CTRL-S to save the image in the ***Open image file*** folder
- T Open the ***Measuring and Tools*** tab in ***Preferences***
- W Save with options
- Y Show the comment that is installed in an image by MICAM
- F1 Insert a 5 µm (0.5 mm/mil) scale
- F2 Insert a 10 µm (1 mm/mil) scale
- F4 Insert a 20 µm (2 mm/mil) scale
- F5 Insert a 50 µm (5 mm/mil) scale
- F5 Insert a 100 µm (10 mm/mil) scale
- F6 Insert a 200 µm (20 mm/mil) scale
- F7 Insert a 500 µm (50 mm/mil) scale
- 3 Set Image Width to 320 pixels
- 4 Set Image Width to 400 pixels
- 5 Set Image Width to 480 pixels
- 6 Set Image Width to 600 pixels
- 7 Set Image Width to 640 pixels

- 8 Set Image Width to 800 pixels
- 9 Set Image Width to 1024 pixels
- 0 Set Image Width to 1280 pixels
- 1 Select primary resample method
- 2 Select secondary resample method
- Esc Cancel the current Select and Cut action

- CTRL-C Copy Image to Clipboard
- CTRL-V Paste Image from Clipboard
- CTRL-Z Undo last action
- CTRL-Y Redo last action
- CTRL-L Insert Label
- CTRL-M Insert Labels showing objective used
- CTRL-R Insert Copyright message

Most short cut keys will be clear from this list. Keys 1 and 2 need some explanation. The primary resample method will give you the best results when you resize an image. Sometimes however, when the old size and new size have a certain relation to each other, a kind of Moiré pattern will be visible in the image. Selecting the secondary resample method will give a better result in that situation.

The Miscellaneous tab in Preferences

(Figure 4.1 shows this tab.

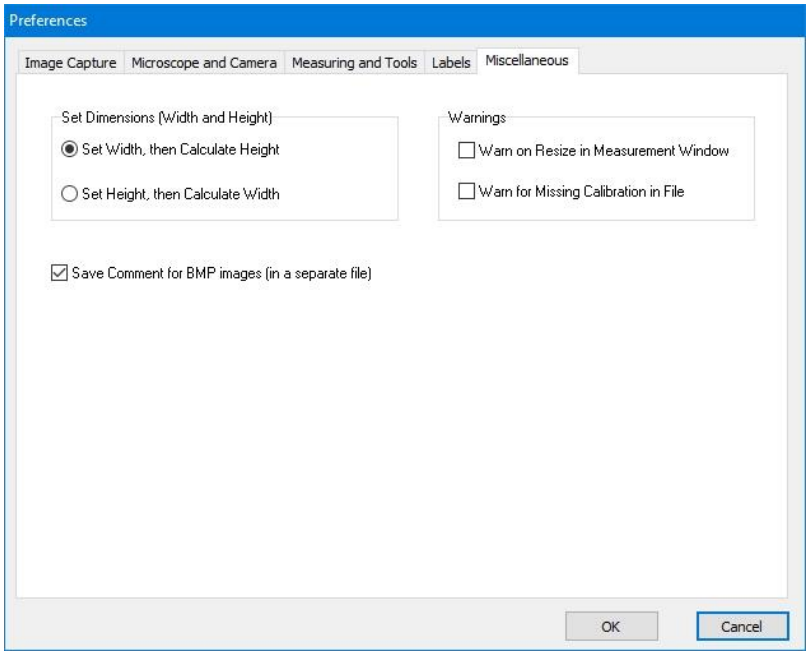

Figure 4.1. The Miscellaneous tab in Preferences.

Set Dimensions (Width and Height)
In the cropping menu you can select the preferred width of the cropped image. In some situations you might want to set the preferred height. Change those setting here.

Save comment for BMP images (in separate file)
The BMP files cannot store the comment inside the file, so a separate file is needed. If you don't like this option, you can uncheck this box.

Warnings

Warn on Resize in Measurement Window
When you resize an image in the measurement window ALL measurements, labels and the scale will be gone. Furthermore when you save the image it will be saved in the current format.

Warn for Missing Calibration File
When you open an image file made with MICAM the program tries to find information about the used Microscope-Camera combination and objective used. When this is set, you will get a warning when the program cannot find a suitable MC-system.

Help menu

Help
Displays this Help-file. Users of MICAM who want to translate this file in their own language can contact me. I can provide them with the manual in Word-format to facilitate translating.

About (menu item in Help)
Shows version information and how you can reach me. Clicking on the link in this window opens my website in your browser to see if there is an update.

Extension of the right click menu in Windows Explorer
A very useful extension of Windows would be the ability to open an image file with MICAM (Figure 4.2).

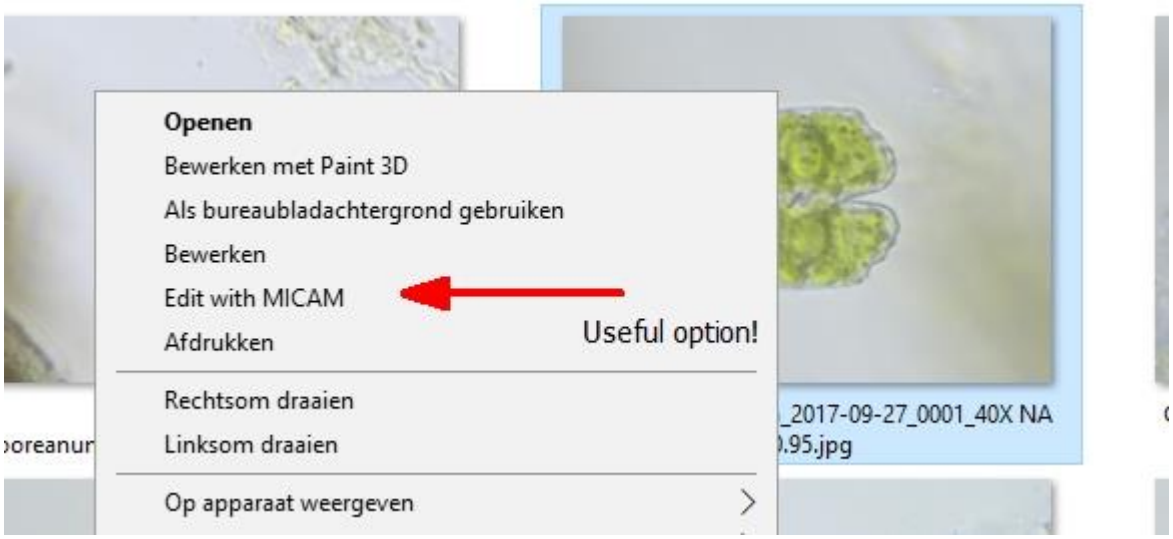

Figure 4.2. The right click menu in Windows Explorer.

To realise this you need a small script to add this option to the registry. This script is included in the installation folder of MICAM. MICAM creates this file automatically.
Double click the file named: ***Add "Edit with MICAM" to Windows context menu.reg*** and allow Windows to execute the file. Now you can open an image file with a right mouse click.

5. The Preview Window

For a DSLR the preview is determined by the preview that you got from the manufacturer of your camera like LiveView for the Canon EOS cameras.
For USB Cameras like the Philips ToUCam, the Tucsen and many others a dedicated preview window is available (Fig. 5.1).

Figure 5.1. The Preview Window.

5.1 The Menu Bar

Only a few items are available here.

5.1.1 Camera

As pointed out in chapter 2.3.1 you have to select your camera using menu item ***Device.*** Here you also can ***Disconnect Camera***. If you have several cameras then selecting another camera will disconnect the current one.

5.1.2 Camera Options

Select the right video size, colour space, exposure time, etc. with ***Camera Options***.

5.1.3 Settings

Image Capture
The only option you have is to set the name of the captured frame. So when you press the ***Snapshot*** button (or F1) the image will be saved under that name in the Photo Folder.
When you press the ***To Clipboard*** button (or F2) the image will be copied to the clipboard.

When you press the ***Measure*** button (or F3) the original image will go to the ***Unprocessed*** subfolder in the current Photo folder. At least when this option is enabled
Note that the number will be incremented automatically so you cannot accidentally overwrite a file.

Miscellaneous
Enable sound on Snapshot and To Clipboard action
When you take a snapshot of the preview window you can hear a camera sound. It is disabled by default. Note: the name of the sound-file used is ***Camera_Shutter.wav***. If you want to use your own WAV-file then you have to rename it.
Show Panel on Right-hand side
If you prefer to have the panel on the right-hand side you can change that here.

Remote
Hardware triggered Snapshot
If you want to use external hardware to trigger a snapshot, you can use the serial port. When your computer doesn't have one you can buy a USB to serial cable and use that. Find out which COM-port you use and determine what input you want to use. The current level on this input will be considered as the non-active level. If necessary you can use two outputs (DTR or RTS) to generate a reference level.
If you don't know what you are doing, don't change these settings!

TCP/IP Server
This version of MICAM has a built-in TCP/IP server. With a client program you can make contact with MICAM and trigger a snapshot. This can even be done from another computer! When you start the server, you will see the server name and its IP address. You need that to make contact with MICAM. You can also select the port you want to use for the contact with MICAM. To give you a small bit of security you have to send the text in the 'Command that triggers a Snapshot box' to trigger the Snapshot. MICAM returns with an error message (when something went wrong), or with the filename used to store the snapshot.
Again: If you don't know what you are doing, don't change these settings!

5.1.4 WindowSize

Maximize
When you want to see the live image as large as possible you can hide the panels. This is useful when you give a presentation for an audience and want to have the largest display possible.
When you select ***'WindowSize'*** again you see ***'Restore Size'*** to get back to the original format. You still can take snapshots and send images to the measurement screen by using the Function keys (F1, F2 or F3).

5.2 The Tool Panel

This panel only contains a few buttons:

Show Measurement Window
This brings you back to the Main Window
Snapshot (F1)
Take a snapshot and store the image in the Default Image Folder.
To Clipboard (F2)
Copy the captured video frame to the clipboard to open it in another program.
Measure (F3)
Store the image in the ***Unprocessed*** subfolder of the current Photo folder and also transfer it to the Main Window. The program automatically switches back to the main window.
Fit to Window
If the resolution of the camera is larger than that of you monitor you can fit the video size to the size of the window to see everything. For focussing purposes it might be better to switc Fit to Window off.
Noise Reduction
A simple but very effective noise reduction feature is to average several images. When you enable this option you can set the number of images that will be averaged. The program then will take that many images when you take a snapshot, copy the image to the clipboard or to the main window.
AVI and Frame Capture
A new window will open and allows you to make settings and capture an AVI or capture frames with a selected interval and create time-lapse AVI-movies.

5.2.1. AVI and Frame Capture

When you click the Start button the AVI Settings window will open. This only functions when you have a live video stream on screen. You can select Normal Video (Fig. 5.2) or Time Lapse Video (Fig. 5.3).

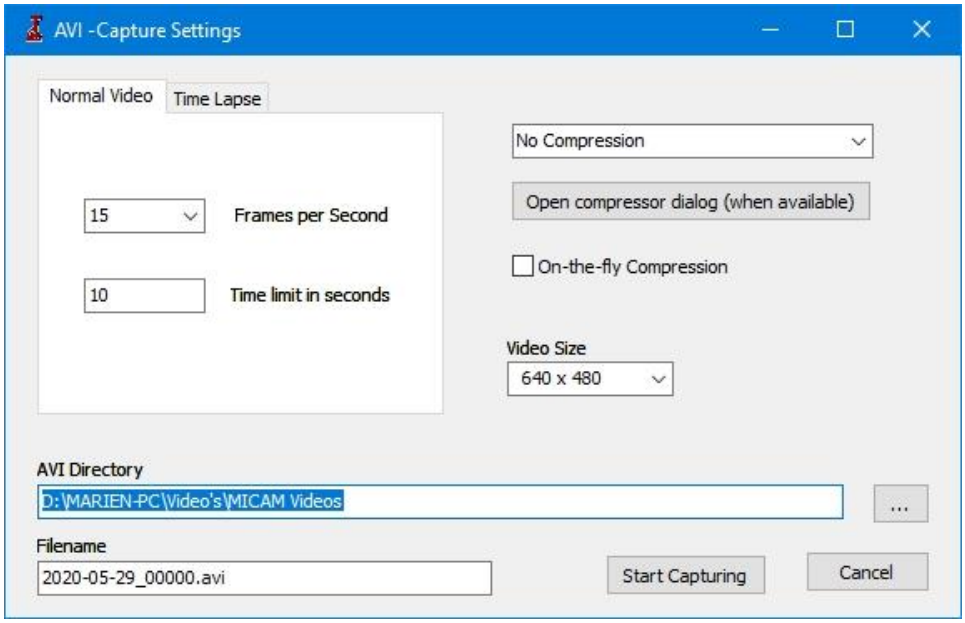

Figure 5.2. Settings for Normal Video AVI

Normal Video
For a normal video you can select the amount of frames per second and a time limit. Although you can always stop recording once you started the capturing. As videos will get very huge

when you don’t apply compression you can select a suitable compressor from the drop-down list. On slow computers it is maybe better not to use on-the-fly compression as the computer might not be fast enough.
The captured frames will be stored in a subfolder (Temp) of the AVI Folder and after capturing all the frames the compressed video will be generated.
Another way to reduce the size of the AVI-movie is to reduce the video size.

You can use the captured frames in the Temp subfolder also to do some stacking if you scanned the object during capturing. Be aware that the temp folder will be cleaned up if you capture another video.

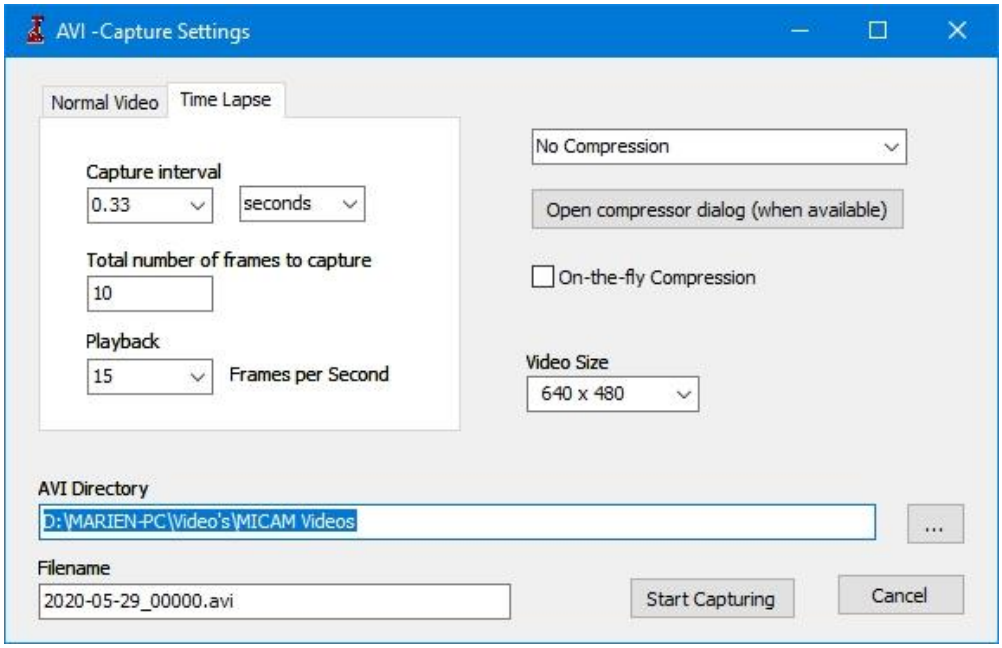

Figure 5.3. Settings for Time Lapse Video AVI

Time-Lapse Video
For a time lapse video you need to make other settings (see Fig. 5.3). First you have to set the capture interval. Then enter the total number of frames you want to capture. Finally select the playback frame rate. All other settings are the same as for normal video.

6. An example of the use of MICAM

Here an example of how MICAM can be used:
In the study of desmids in the northern part of the Netherlands, water samples are collected and the desmids seen under the microscope are brought to name. In many cases a photograph is made of what is seen, so others can verify the determination in a later stage. For this example let us take a water sample from a garden pond at location (square kilometre area in the Dutch national grid) km 236 - 559. A few days later the investigation of the sample under the microscope is started.

First the Live View program is started and all settings to grab images from the camera are set and checked. Then MICAM is started and the MC-system that will be used is selected. In this stage it is possible to change other settings like the right ***MICAM-Temp*** folder, but as those settings are already made earlier, the program uses the earlier settings. The camera in use is the default one so all settings for the microscope and camera are ready now (Go to ***Preferences → Microscope*** to set the right microscope and camera settings if you haven't done that yet).

Now open Preferences and move to the folder where photographs of desmids will be saved. Or, for example, create a new folder with the name "Garden Pond #km236 - 559". Note that because of the #-sign the Location and Coordinates strings at the bottom are set in the proper way! In the box ***Default File Options*** the sample date (pull-down box) is selected and as default filename only the sample date and unique number (option number two) are selected. Finally set JPG in the ***'Save Image As'*** box. Then click OK. All preparations have been made now. Normally it will take 10 to 20 seconds to make all preparations.

Then start searching the sample for interesting things, the real work!
When using a USB camera it is possible to press the snapshot button to make an image and store the image with a generated name. No questions are asked. With a DLSR unfortunately the image will be stored in the ***Unprocessed*** folder and the MICAM main window will be opened with this image, forcing you to do something with this image.
The live view on the computer screen makes it very easy to focus.
When something under the microscope is recognized, make a photo and the image is shown in the measurement window. After adding a scale, labels etc. use the 'Save with Options' button to save the image. As the right database (Desmids) is already selected, a few characters of the name (the box at the upper right shows all names that contain the selected characters) is sufficient to select the right name. So when a ‘Tetmemorus *brebissonii var. brebissonii*’ is seen, only 'tet' has to be typed in the Search field and only a few matching names are left. Select the right one in the list with matching names and a proper filename will be shown in the Suggested Name box. Note that all other settings are taken from the preferences you made earlier, but if you want to change them you can do that. Clicking the 'Save with this name' button saves the photograph with the suggested name.

All images on my website http://desmids.science4all.nl/?Desmid_pictures were made using MICAM.

7. Registration of the software

This software is shareware. You do not have to register this software, but if you continue to use this program after a trial period of one month then you have to pay an amount of 20 euro via PayPal (to mvanwesten@home.nl).
Click on the ***'Check for updates'*** button on the right. Once you are on my website you can select the ***'Donate'*** menu item.

I hope you enjoy working with MICAM as much as I do.

Marien van Westen,

June 2020