LSM700 Zen2012 manual

Turning on the system
- turn on the 2 connector sockets behind the microscope
- turn on the HPX120C unit and the PC
- log in with the LSM-user account (no password)
- double click 'Zen 2012' and click 'start system'

Looking and searching through the microscope (Figure1):
- in the Zen-program click 'locate' (1)
- **first use the 20X- or 10X-lens !!**
- on the microscope touch screen touch 'load position'
- put a slide under the microscope
- touch ⊠
- in the Zen-software next to 'Assign' (2) click on one of the fluorochromes (Alexa 488, Alexa 568, Alexa 488/546 or Dapi) or choose 'transmission' for normal light. At 'Reflected light' you can close the shutter with 'Off' (3)
- put the slider on the microscope in the eye-position (right side of the oculairs on the microscope, push in) and focus your slide
- if necessary change to the 43X- or 63X-lens by hand: a message 'Change immersion" appears on the microscope screen.
- Put a **little bit** oil on your slide, press 'done' and focus again
- Switching to other lenses from an oil-lens or from a dry lens to an oil-lens will result in a message 'Change immersion please apply immersion or clean objective/specimen'. After applying oil or removing oil press 'Done'

Making a quick first confocal image (Figure 2):
- click 'acquisition' (1) and put the slider on the microscope in LSM-position (right side of the oculairs on the microscope, pull totally out)
- click 'Smart setup' (2)
- click on the first ▼ and choose a fluorochrome
- click on the second ▼ and choose a colour
- for a second fluorochrome click the first ▼ on the second line etc.
- choose one of the proposals (fastest, best signal, best compromise). Best signal takes longer but gives better results (less crosstalk)
- click 'apply'
- go to the 'Acquisition Mode'-window (3), choose 'Averaging number' (6) 4 or 8 and choose a frame size by clicking on 'X*Y' (7). *Normally the standard values are good enough (frame, 512 X 512, speed 9, etc.).After making all the settings, use 'Live' (4) for searching a new position or 'Snap' (5) with an 'Averaging' 'number' of 4 or 8 (6) for a real recording. 'Averaging' is the times one line is scanned before the average result will be shown on the screen, and has effect on 'Snap' and 'Continous'. With 'Live' the averaging is always 1. By clicking on X*Y (7) you can choose a higher resolution. Beware: a 1024 X 1024 image takes 4 times as long as a 512 X 512 image. Also put the speed back to 9 when choosing a 1024*1024 image.*
- Go to the 'channels'-window (8)
- check on 1 track (9) (the one used for focussing by eye) and check off the other tracks. Activate the track that is on by clicking on its name (10).
- Choose a pinhole setting: 'Smart setup' puts the pinhole automatically at 2. See below at '**Pinhole settings**'.
- click 'Set-exposure' (11)
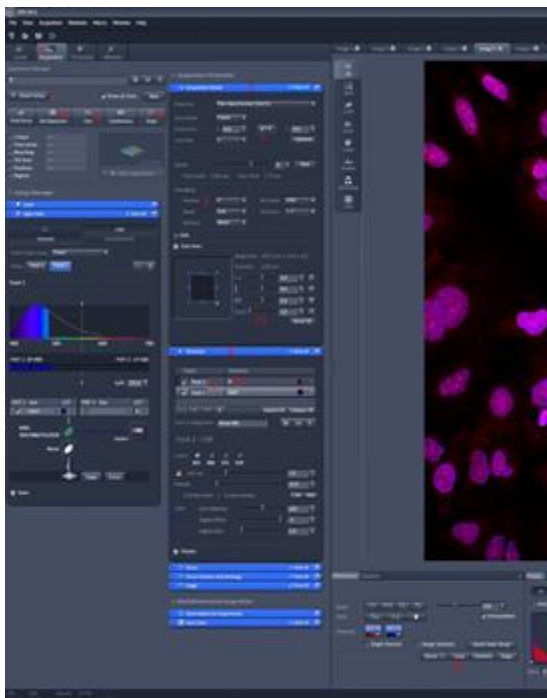- click 'Live'(4, changes in 'Stop') and focus again on the screen-image, click 'Stop'(4).

- If necessary zoom in: raise the zoom (12) by slider, arrowheads or typing. The zoom will be performed on the middle of the initial image. Another method is using the crop-function: click 'Crop' (13), a square appears. By dragging the middle of the square you can aim which point will be in the middle of the new image. By dragging a corner (NOT a side!) you can define the zoom-factor (12 will change). When you click 'Live' (4) or 'Snap' (5) the new image will appear.
- in 'channels' check on all tracks (9) and click 'Set-exposure' (11) again.
- Save the image by clicking on 'File' 'Save' or 'Save as' or by pressing CTRL-s
- Making a picture with the same settings: check on 1 track at 9, click 'Live' (4), find another interesting spot with the xy-table, click 'Stop'(4), turn on all tracks and click 'Snap'(5)

Figure 2

It is possible to adjust the tracks for fine tuning (Figure 3):

Set-exposure will result in too high signal for FISH-specimens. You have to adjust the 'Gain (master)' (8) by hand.

- In channels check on 1 track (1), check off the other tracks and make the track that is on active by clicking on the name (2).
- Click 'live' (3, changes in 'Stop') and click under the image in the 'Dimensions' window on the **"range indicator"** (4): the image changes in black, white, blue and red. Blue has a value of 0 (black), red has a value of 255 (white). **Beware:** if you click on 'Ch1' the channel will be turned off and NO image appears!!
- If necessary check on in 'Channels' 'show all' (5)
- For an optimal confocal image press '1 AU' (6); see below '**Pinhole-settings**'
- Optimal background: lower (for more blue) or raise (for less blue) the 'Digital Offset' with the slider or arrowheads (7). The lower (more blue) the value, the more signal will be set to blue (=0). Do not go too low, otherwise too many positive signal will be lost.
- Optimal signal: raise (more red) or lower (less red) the 'Gain(master)' (8) until just a few pixels are red in your positive signal.
- If necessary (weak signal) raise the 'Digital Gain' (9) and /or the laser power (10). Beware: too high laser power will result in bleaching of your signal!
- Click 'Stop' (3) and repeat this for another track.
- If all tracks are set: turn on all tracks, put 'Average' 'number' on 4 or 8 (11) and press 'Snap' (12)

Making images with old or the same settings (Figure 3):

- use the settings made with the first image or open an image that was made earlier.
- When using an old image, go to 'Dimensions' and press 'Reuse' (15).
- Activate one track, press 'Live' (3) and if necessary adjust 'Gain (master)' (8), 'Digital Offset' (7) and 'Digital Gain' (9) per Track. Press 'Stop' (3) and choose, if necessary, another track.
- Activate all tracks and press 'Snap' (12)

Pinhole-settings:

By adjusting the pinhole (13) you can decide how thick the image (slice) will be. By pressing '1 AU' the pinhole will be adjusted automatically to get a thin optimal illuminated sharp image, this depending on the used objective and laser. The higher the pinhole, the thicker the recorded slice and the more light will fall on the detector. By clicking on 'max' (14) a thick slice, almost comparable with normal fluorescence image, will be made.
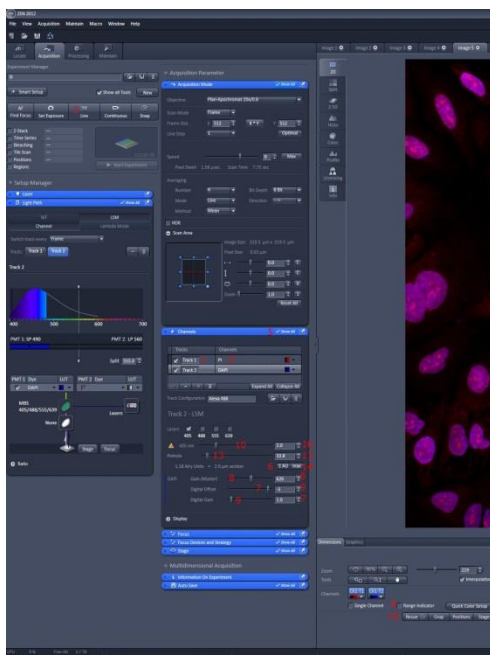
Figure 3

3

Making a Z-stack (Figure 4)
- check in 'configuration' 'Z-stack' on (1)
- click on the 'Z-stack' bar (2): the settings appear
- At 'Interval' (3) set a value; this is the space between 2 consecutive slides. If you press 'Optimal' (4) that value will be taken over in 'Interval'
- Press 'Center' (5), the other 'Center' (6),press "First last' (7)
- Click 'Live' (8)
  next z-stack settings are in the yellow-bordered image
- Move the fine coarse adjustment knob of the microscope forwards until there is no more signal. The figure in 'Z-stack' shows a blue square going up towards 'L' (9).
- Click 'Set last' (10)
- Move the fine coarse adjustment knob of the microscope towards you until there is no more signal. The figure in 'Z-stack' shows a blue square going down towards 'F' (11).
- Click 'Set first' (12);the number of slices will be automatically set (13),click 'Stop'(8)
- Be sure all tracks are on, 'Averaging' is set to 4 or 8 (picture 2, 6) and click 'Start experiment' (14)
- A series of images will be taken with a distance of 'interval' between every slice. Save the series (CTRL-s). On the left of the image a series of buttons appear (15) (2d, Gallery, 3D etc.): try them out, see what happens. Nothing can go wrong.
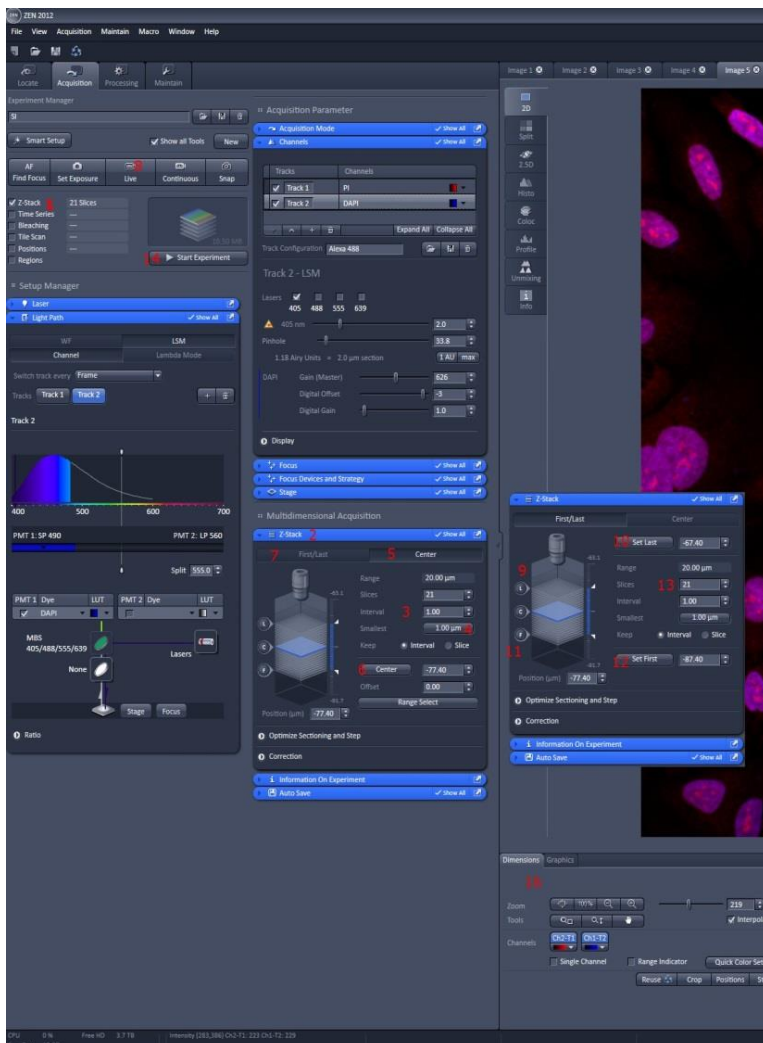- In "Dimensions" a slider appears at 'Z-position (16) for stepping through the series

Figure 4

Multi-positions with z-stack and autofocus (Figure 5):
- adjust the image of the microscope at 20X and pinhole 1AU (1)
- open the pinhole to 'max' (2) for every channel that will be used with the overlay-image and lower the Gain (master) (3)
- turn on 'Positions' (4), click 'Show all' (5) and click 'scan overview image' (6)
- in the 'Scan Overview Image'-window choose the 20X-objective (7), determine how many tiles there has to be scanned (8) at zoom 0.5 and click 'scan' (9)
- click under the image on 'positions' (10) and click in the image on cells or regions that have to be imaged with the 63X-lens
- export this image as Tagged Image File with the option (data) 'Full resolution image window': the image with all the crosses will be saved.
- You can go to another area, make a new overview image and click on more positions
- Click in the 'Positions'-window on 'save' and store the positions (11)
- Click on 'New' (new image) (12)
- Go to the 63-lens and put oil on your slide
- Click in the position list on position 1 and click 'Move to' (13)
- Adjust the microscope and the settings for every channel (Gain, zoom, pinhole etc.); with these settings every position will be recorded. You can reuse an image; if the positions disappear or change, the right positions can be loaded again in the 'Positions'-window with 'load'(14).
- If necessary, turn on 'Z-stack' (15), click on the upper 'Center' (16) and adjust the number of 'Slices' and 'Interval' (17)
- Click in the 'Positions'-window on position 1 and click 'Move to' (13)
- Click on 'Focus devices and strategy'(18), choose at 'Autofocus mode' fluorescence (19), check on 'Autofocus every n Position' and choose 1 (20)
- Click in the Z-stack window on the lower 'Center'
- Choose the right 'Frame size', 'Speed' and 'Averaging' (Mode Line)
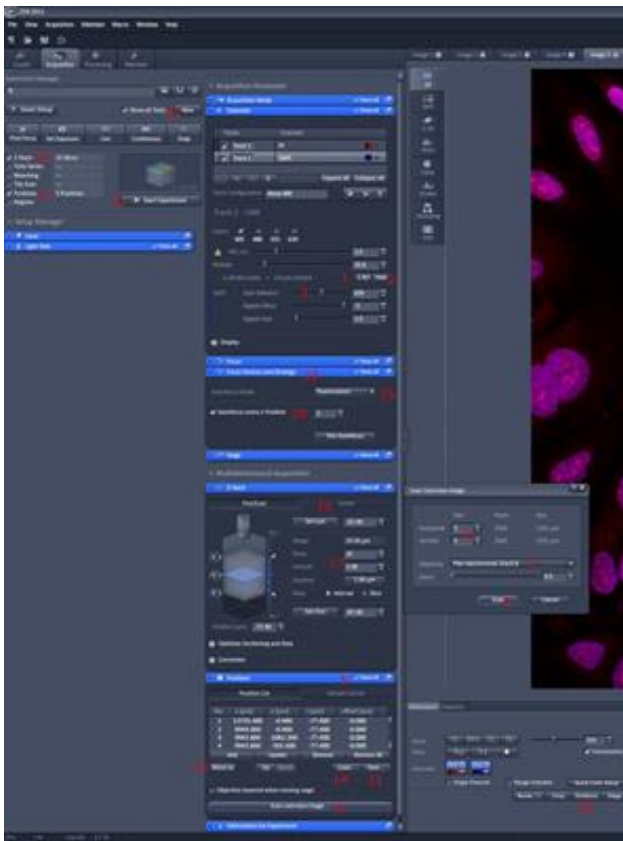- Click 'Start experiment' (21) and save the result.

Figure 5

Making a normal fluorescence image (Figure 6, only for LSM700-311):
- In 'Lightpath' click on 'WF' (1)
- in 'Channels' make track 1 active (2)
- click on the open folder (3) and choose a fluorochrome
- if necessary click on the +-sign (4) to add a track, make that track active and open another fluorochrome. You can choose up to 4 tracks.
- In the 'Acquisition Mode'- window put the averaging on 1 (5). A higher average does not make the image better. You can not change the frame size. Zooming has no effect: you will only make a smaller image. If you want to zoom in change the objective to a higher magnification.
- At 'Bit Depth' you can click the arrowhead (6) and change it to the desired value.
- In 'Channels' turn on 1 track (7), activate it, set the 'Exposure time' (8) on 1 ms, press 'Live' (9) or 'Continuous' (10), adjust the 'Exposure time' and focus the slide with the coarse-knob on the microscope. If you turn on the 'Range Indicator' (11) you can see if the image is overexposed.
- Do this for all tracks separately
- Turn on all the tracks and click 'Snap' (12)
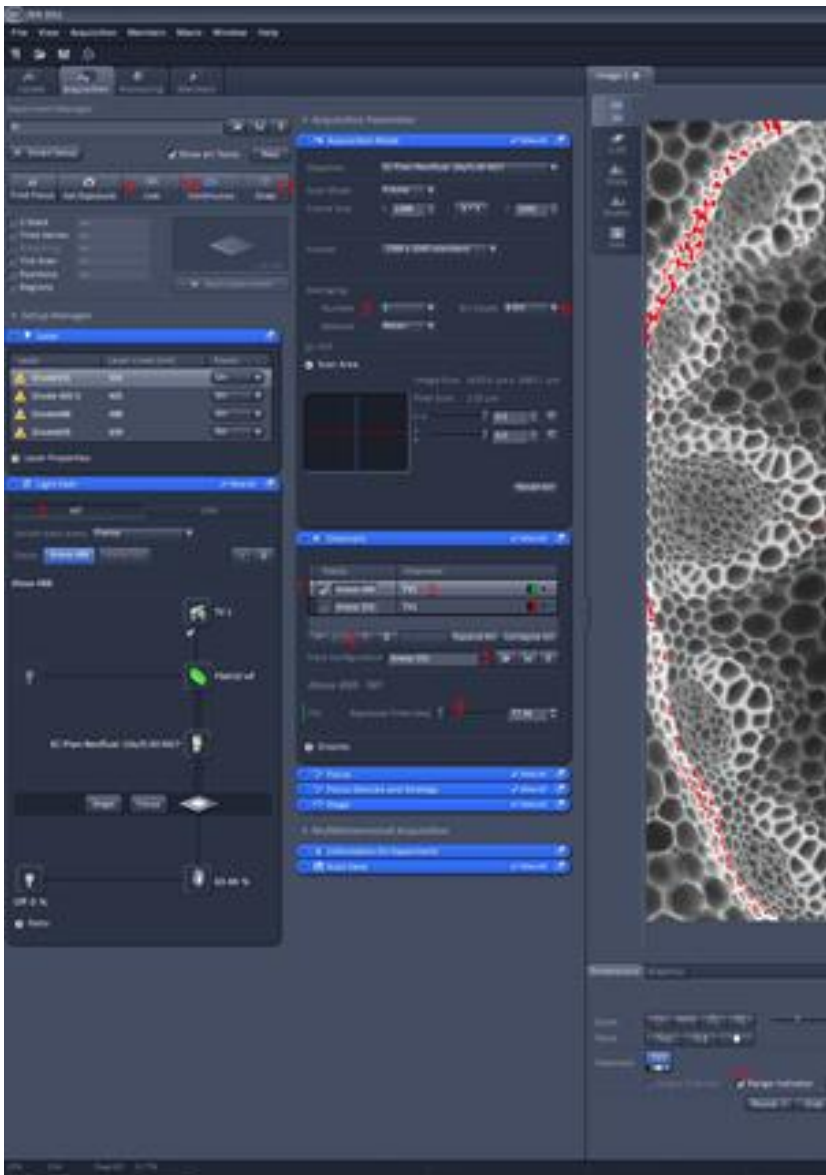- Save the image

Figure 6

Shutting down the system:
- press 'load position' on the touch-screen and remove your specimen
- if necessary clean the oil-lenses with a tissue with some ethanol
- move to the 20X-lens and press ⊿
- Save your images and close the Zen-program.
- Shut down the computer
- Power off the HPX120C unit
- Switch off the 2 connector boxes behind the microscope
- put the dustcover over the microscope
- remove any garbage

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A few points of attention:

- **Do not use too much oil**
- **Do not use oil on the dry-lenses**
- **Clean the oil-lenses (40X, 63X, 100X) after use with a tissue and ethanol**
- **Do not eat or drink behind the microscope**
- **Wear a lab-coat (be-311)**
- **Throw away your garbage**
- **Be careful with the microscope**