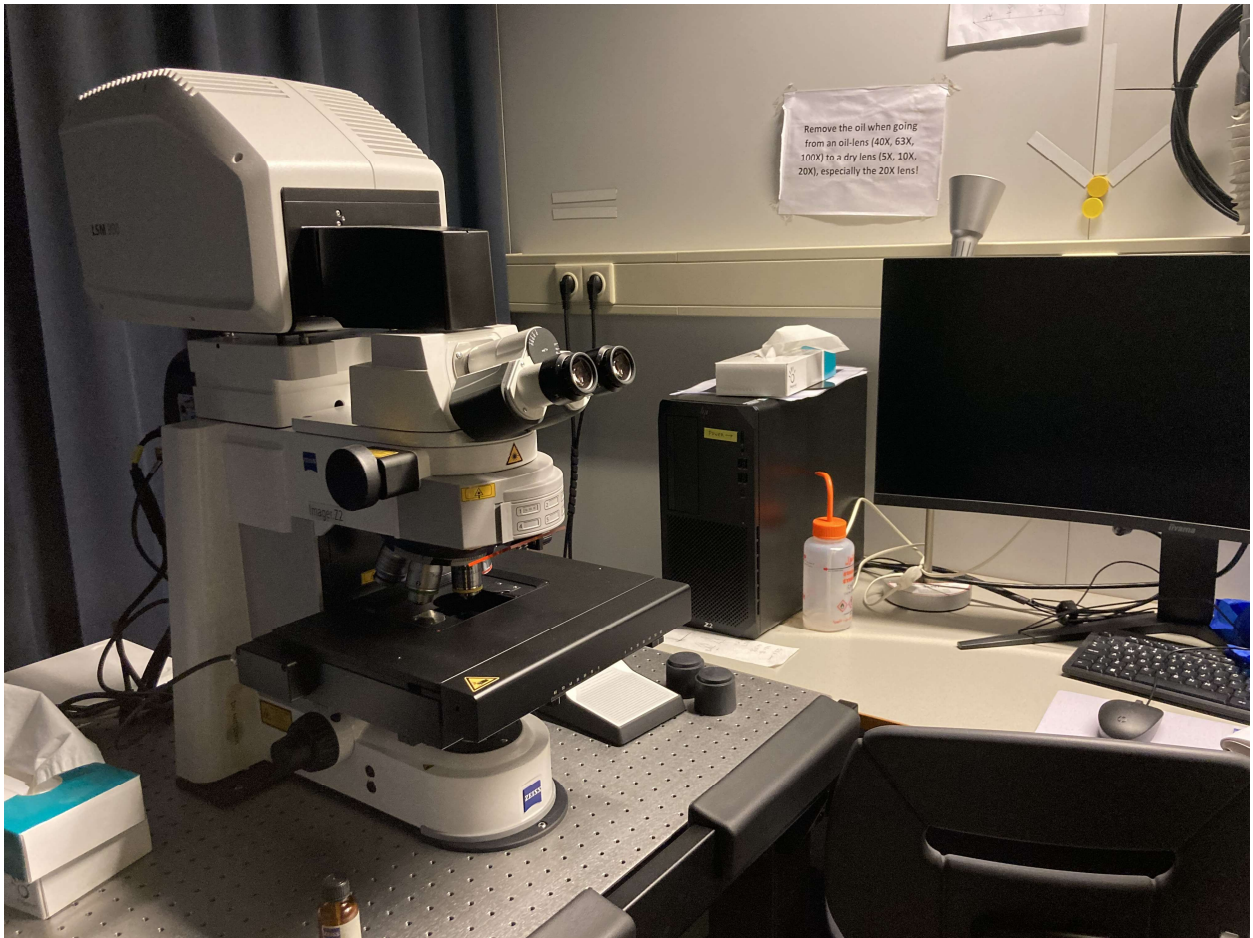


## Zeiss LSM900 Room Be311



## **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) after use with a tissue and ethanol**
- **Do not eat or drink behind the microscope**
- **Throw away your garbage**
- **Be careful with the microscope**

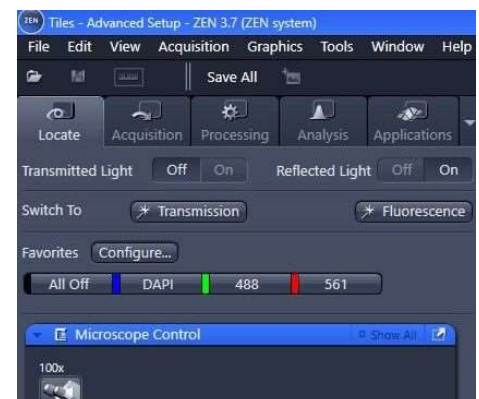
# Manual Zeiss LSM 900

## Turning on the system

1. Turn on the 'system'-switch behind the table, box above the box with the key. DO NOT TOUCH OTHER SWITCHES OR THE KEY!
2. Turn on the connector box on the microscope-table. DO NOT TURN ON OR OFF THE MICROSCOPE.
3. Turn on the computer
4. Double click 'Zen Blue'. If the whole system has been turned on click 'Yes' when asked to calibrate. The calibrate question will not appear if the system is on and only the software is started.

## Looking and searching through the microscope

1. **First use the 5X-, 10X- or 20X-lens!!**
    - a. Choose a lens by clicking on the right lens in the 'Microscope'-window on the right of the screen.
  2. In the Zen-program click 'locate'
  3. On the microscope touch screen touch 'load position'
  4. Put a slide under the microscope and touch 
  5. In the Zen-software click on one of the excitation wavelengths (DAPI, 488 561). This will automatically choose the correct settings and open the shutter.
  6. At 'Reflected light' you can close the shutter with 'Off'
  7. Push the slider on the microscope in (right side of the oculairs) and focus your slide
  8. If necessary change to the 40X- or 63X-lens: a message 'Change immersion' appears on the screen. Put a **little bit** oil on your slide, press 'done' and focus again
  9. 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'.
- BE SURE TO REMOVE THE OIL WHEN SWITCHING TO THE 20X-LENS!!**



# Manual Zeiss LSM 900

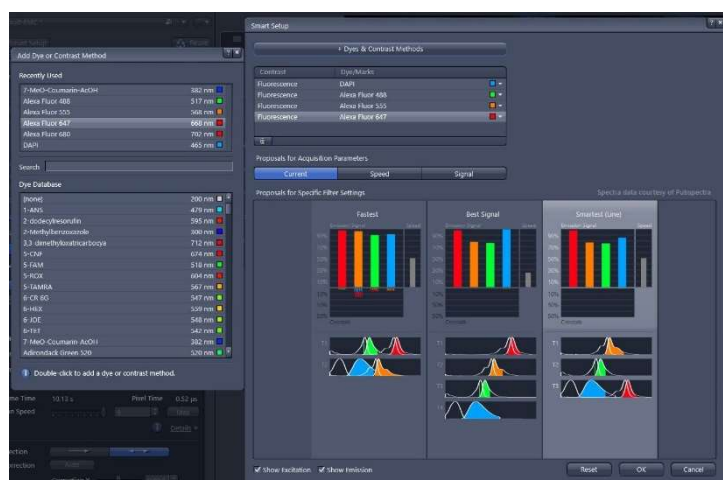
## Making a new configuration and a quick first confocal image

Tip: undock the channels window by clicking on the arrow pointing up to the right

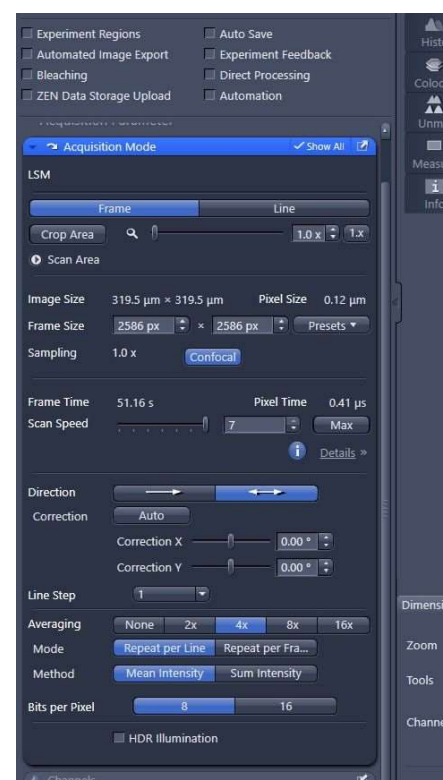
1. Click 'acquisition' and pull the slider on the microscope totally out (right side of the oculars on the microscope)
2. Click 'Smart setup'



3. Click on '+Dyes & Contrast Methods'
4. Double-click on a dye. The dye will appear in the Smart Setup
5. To set up your acquisition channels you have to select all the dyes that are in your sample.
6. When done, close the 'Add dye or contrast Method' window
7. If you want you can change the colors of the channels by clicking on the arrowhead next to the color
8. Choose one of the proposals (fastest, best signal, smartest) and click 'OK'. Best signal takes longer but gives better results (less crosstalk). Smartest (line-switching) is fast but also very accurate.



9. Go to the 'Acquisition Mode'-window, choose an 'Averaging number'. The higher the number, the less noise but also the longer it will take to make the image: quality vs. time, your decision.
10. Click on the button 'Confocal': sampling will be set to 1 and the frame size will be adjusted to get the best pixel size calculated from the numerical aperture and Nyquist rate. You can always change the frame size by clicking on 'Presets' and choose another setting.
11. Go to the channels window and turn one track on for focussing
12. Click 'Set exposure'
13. Click 'Live' and adjust the focussing
14. Turn on all tracks and click 'Set exposure'
15. Click 'Snap': a first confocal image will appear.



# Manual Zeiss LSM 900

## Zooming in

1. In the 'Acquisition Mode'-window click on 'Crop Area': a square will appear on screen
2. Click on the little cross, hold and move the square over the object of interest
3. The square is already zoom 2. You can increase the zoom by clicking on a corner, hold it and move the mouse so a smaller square will be made. Another way is using the slider or arrowheads next to 'Crop Area'
4. The frame size will be automatically adjusted, to get the best pixel size.
5. Click 'Live' to see if the image is OK or/and click Snap'.

## Adjusting tracks manually

*Set-exposure can result in too high signals for some specimens. You have to adjust the settings by hand*

1. Make a channel active in the 'Channels'- window. You can leave all tracks on!
2. Click live and check on the 'Range indicator'. A black and white image appears where blue has the grey value 0 and red has the grey value 255 (overexposed)
3. When necessary put the 'Digital Gain' back to 1
4. When necessary lower the 'Digital Offset' to a negative value to put the background to 0. Normally the current value (0) is OK. It is also possible to get rid of auto-fluorescence or a-specific staining by lowering the 'Digital Offset' more. But don't go too low or you will remove your positive signal!
5. Change the 'Master Gain' to a value that your positive signal has just a few red pixels.
6. The higher you put the 'Master Gain' the more noise will appear.
7. **Avoid the horizontal stripes that appear when the 'Master Gain' is set too high!** When this happens, raise the 'Digital Gain' to make weak signals visible
8. You can also raise the laser-power to get more signal, but don't go too high or you will bleach your slide. This differs per fluorochrome.
9. When done, activate another channel and do the adjustments again.
10. **Be aware:** when using line switching per track instead of frame-switching, the channels that are shown on the same detector (see the tracks in the 'Imaging Setup'-window) have the same 'Master Gain' setting. Set a common gain for both channels and do a fine adjustment with the laser power.
11. When all channels are set, click 'Snap'. When switching to a different lens, check the settings again. They will also change between lenses.





# Manual Zeiss LSM 900

## Saving the image

There are 2 ways of saving your image in .CZI format: uncompressed or compressed

Uncompressed:

1. Click 'File' and choose 'Save as CZI'
2. Go to the right network-folder, give your image a name and press 'Save'

Compressed:

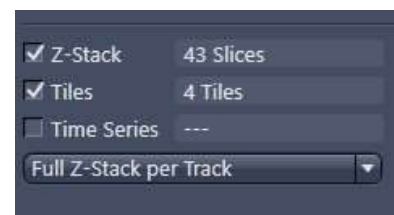
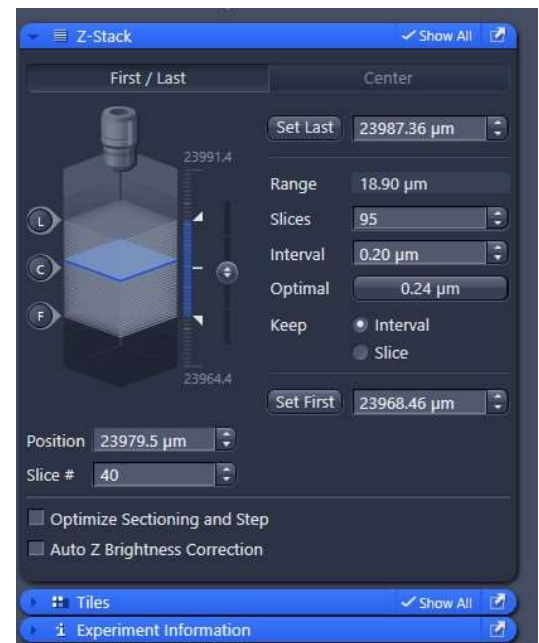
1. Go to 'File' and choose 'Save with options'
2. Go to the right network-folder, give your image a name
3. Choose a compression method at 'Compression' and press 'Save'

## Making images with old or the same settings

- Open a CZI-image that was made earlier; NOT a tif or jpg image!
- Go to the left top of the screen next to 'Smart setup' and press 'Reuse'.
- Activate a track, press 'Live' and if necessary adjust 'Master Gain', 'Digital Offset' and if necessary 'Digital Gain' per Track. Press 'Stop' and go to the next track for adjusting.
- Check on all tracks and press 'Snap'

## Making a Z-stack

1. Check in 'configuration' the 'Z-stack' on
2. Click on the 'Z-stack' bar: the settings appear
3. At 'Interval' set a value; this is the space between 2 consecutive slides. If you press 'Optimal' that value will be taken over in 'Interval' (half of the pinhole-setting)
4. Press 'Center', the other 'Center', press "First last"
5. Activate the track with the biggest volume
6. Click 'Live'
7. 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'.
8. Click 'Set last'
9. 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'.
10. Click 'Set first'; the number of slices will be automatically set
11. Move the fine coarse adjustment back to put the current level to the middle and click 'Stop'
12. Select "full Z-Stack per Track" to speed up the acquisition
13. Be sure all tracks are on, set 'Averaging' to 2 or 4 and click 'Start experiment'
14. A series of images will be taken with a distance of 'interval' between every slice. Save the series. On the left of the image a series of buttons appear (2d, Gallery, 3D etc.): try them out, see what happens. Nothing can go wrong.
15. In 'Dimensions' a slider appears at 'Z-position' for stepping through the series



# Manual Zeiss LSM 900

## Tile module

See **Picture A** next to microscope for yellow numbers

**Making an overview:** Decide if you want to make an overview. If not: go to **Tile-scan with high magnification**

1. Decide if you want to make an overview with the 5X-lens (mostly used to see where tissue or cells are with 1 color) or the 10X-lens (more detailed information and better if you want to see more than 1 color)
2. Change to the correct lens (make sure you remove any oil if necessary)
3. Click on the arrowhead next to 'Default-EMC' (1) and open either '5X-overview-diag-coupe' (whole slide, rectangle coverglass), '5X-overview-cells' (round coverglass) or '10X-overview-line-4colors'
4. Turn off the colours you don't want to use in 'Channels' (2)
5. When necessary check on 'Tiles' (3), open the 'Tiles' window and click on 'Show viewer' (4)
6. Zoom out until you see the contours of the slide; the blue cross is where the image will be made at this moment.
7. You can move the table by double clicking in the contour of the slide

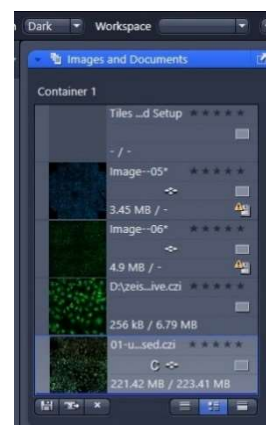
### 5X-overview:

1. When necessary click on the tile and adjust the size with the white edges (5). You can move the tile by clicking on the cross (6), hold it and move it.
2. In 'Channels' (2) activate a track you want to record, press 'Live', focus and adjust the settings. Do this for all tracks you want to record.
3. In the 'Tiles'-window right-click on the tile (TR1) (7) and click 'Set current Z for selected tile regions'.
4. In the Tile-viewer click 'Preview' (8), check off 'Use experiment settings' (9) and choose if you want to 'Delete Existing Preview Images' (10)
5. Turn off the channels (11) you don't want to record by clicking on the name and click 'Start' (12). You can stop the scanning whenever you want.

### 10x-overview:

6. In 'Channels' (2) activate the channel you want to record, press 'Live', focus and adjust the settings.
7. Make a new tile by doing one of these options:
8. Click on the square with the plus (13) and define a tile by numbers at x and y, click 'Add tile region'
9. Click on the square with the plus (13), click on the circle and define a tile by either number of tiles or diameter in um, click 'Add Tile Region'
10. Click on the square without the plus (14), choose rectangle, circle or freehand and draw a region
11. *You can define more than 1 region*
12. In the tile-window at 'tile-regions' (4) a new tile appears. When the tile is checked off the tile will not be visible in the tile-viewer and will not be recorded.
13. In the 'Tiles'-window right-click on the tile (TR+nr, 7) and click 'Set current Z for selected tile regions'. When you have more regions/tiles you can click "verify" and check the Z-settings for every region or tile.
14. In the Tile-viewer click 'Preview' (8), check off 'Use experiment settings' (9) and choose if you want to 'Delete Existing Preview Images' (10)
15. Turn off the channels (11) you do not want to record by clicking on the name and click 'Start'.

A preview will be made and showing on the screen. The preview image can be saved by right-clicking on the image and choose 'Save preview images': the image will appear in the main window and can be saved with 'Save as CZI'. When more preview images are made with 'Delete Existing Preview Images' checked off, they will be saved as different scenes. Saved overview images can be reloaded by right-clicking in the Tile-viewer and choose 'Import Preview Image' or by dragging an image from 'Images and Documents' into the Tile Viewer.

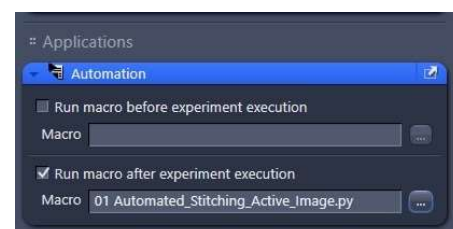


When you check off the tile in the Tiles-window (7) you will only see the preview image. When you double click on an area in that image the stage will go to that area.

If you want to make a tile-scan with a higher magnification:

## Tile-scan with high magnification

1. Change the lens
2. Make a new configuration (smart setup) or load an image and press 'Reuse' (right below 1)
3. If necessary change the image settings in the 'Acquisition Mode' window
4. Check on 'Tiles' (3) and click 'Show viewer' (4) in the Tiles-window
5. If necessary right-click in the viewer, choose 'Import preview image...' and load your previously saved preview image
6. Double-click on an area you want to image or go to an area by eye (locate)
7. Click 'Live' and adjust the focus and settings (laser, 'Master Gain' etc.) of all channels you want to record
8. Make a new tile by doing one of these options:
9. Click on the square with the plus (13) and define a tile by numbers at x and y, click 'Add tile region'
  - a. Click on the square with the plus (13), click on the circle and define a tile by either number of tiles or diameter in mm, click 'Add Tile Region'
  - b. Click on the square without the plus (14), choose rectangle, circle or freehand and draw a region; right-click for ending freehand drawing
  - c. In the 'Tiles'-window right-click on the tile (TR+nr, 7) and click 'Set current Z for selected tile regions'. When you have more regions/tiles you can click "verify" and check the Z-settings for every region or tile. When you have very big images which are not completely straight, you can add multiple focus points (see below).
10. You can define more than 1 region
11. Check on 'Automation' (15)
12. In the automation-window check on 'Run macro after experiment execution', click on the dots and choose '01 Automated\_Stitching\_Active-Image.py'
13. Start the imaging by clicking 'Start Experiment' (16).
14. Save the result as CZI




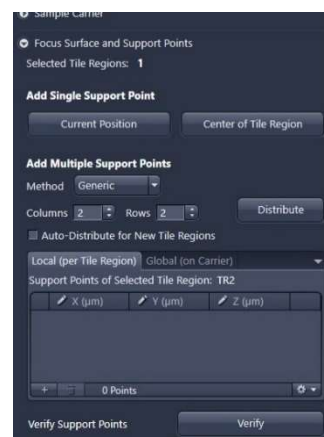


The result images can also be reloaded in the tile-viewer in a later stage, even on another day.  
Be sure to put the right slide in the same way under the microscope!

Images with more scenes can be split into separate images at 'Processing'- 'Utilities'- 'Split Scenes', also possible with the freeware Zen-software you can install on your own computer.

## Adding multiple focus points

1. In the tile module click "Focus Surface and Support Points"
2. Make the tile where you want to add support points active by clicking on the name
3. Scroll down to "Add Multiple Support Points"
4. There are 2 ways of adding support points:
  - a. Generic: choose the number of columns and rows and press 'Distribute'
  - b. Onion skin: choose density and margins and press 'Distribute'.
5. When you are not happy with the distribution you can press , choose 'Delete all support points from selected tile regions' and try again.
6. In the Tile small circles with dots appear that indicate the focus points.
7. You can verify the support points or do an autofocus before scanning



### Verify

8. Click "Verify" and click "Live" to see the live image.
9. Click "Move to Current Point" and set the correct focus. When the focus of this point is correct, press "Set Z & Move to Next". The system will go through all the points and remember the Z-position.
10. When all points have been verified, click close and press Stop.

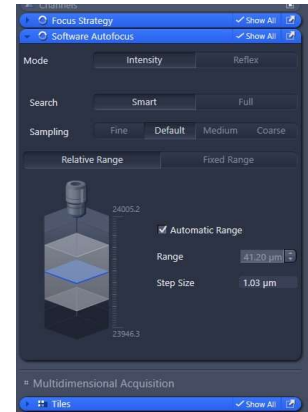
### Autofocus:

11. Open the 'Focus strategy' window
12. At the first arrowhead choose 'Use Z values/Focus surface defined in Tiles setup'
13. At the second arrowhead choose 'By software autofocus'



# Manual Zeiss LSM 900

14. Open the 'Software autofocus' window
15. Activate 'Intensity', 'Smart', 'Default', 'Relative Range' and check on 'Automatic Range'




Now you can image as before:

16. Check on 'Automation' (15)
17. In the automation-window check on 'Run macro after experiment execution', click on the dots and choose '01 Automated\_Stitching\_Active-Image.py'
18. Start the imaging by clicking 'Start Experiment' (16).
19. Save the result as CZI



It is possible to combine a tile-scan with z-stacks! Be aware of the time this will take.

## Shutting down the system

1. On the microscope touchscreen press 'Load position'
2. Remove your slide and press  Always end with the table in the up (= working) position!
3. If necessary clean the oil lenses
4. Save the images you want to save
5. Close the Zen software
6. Have a look in the agenda and see if there will be someone after you
7. Someone after you:
8. Leave the system as it is
9. Last user of the day:
10. Shut down the computer
11. Turn off the connector box on the microscope table
12. Turn off the 'system'-switch behind the table, box above the box with the key. DO NOT TOUCH OTHER SWITCHES OR THE KEY!