L Berry April 2019

# Zeiss LSM 880 – Basic User Notes (in 10 pages)

# Airyscan (in 5 pages)

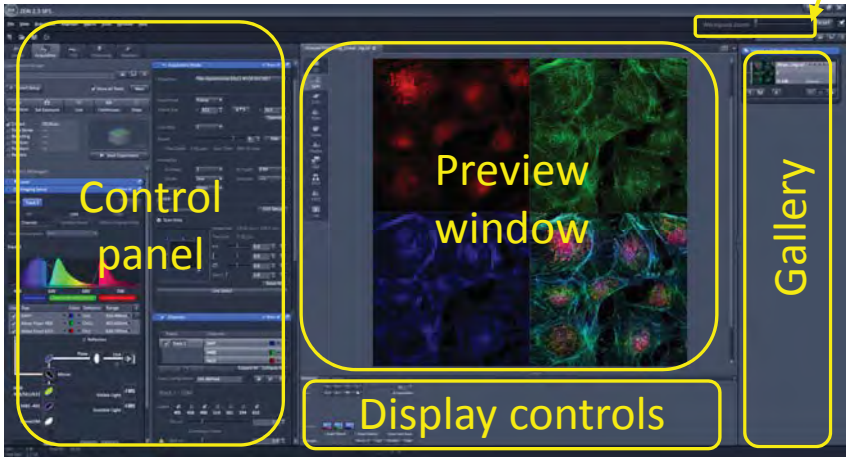
## 1. Turning on:

? Button = manual shortcut for next click

Switches on controller:
Main switch, Systems PC (if separate RTPC, wait for full boot up)
Turn PC on , and then Components
[System dependent: Fluorescent light source / For 710/780 Argon laser]

Open Zen software using desktop icon, and select Zen System for acquisition


Workspace Zoom




Near the top of the control panel, select **Show all tools**

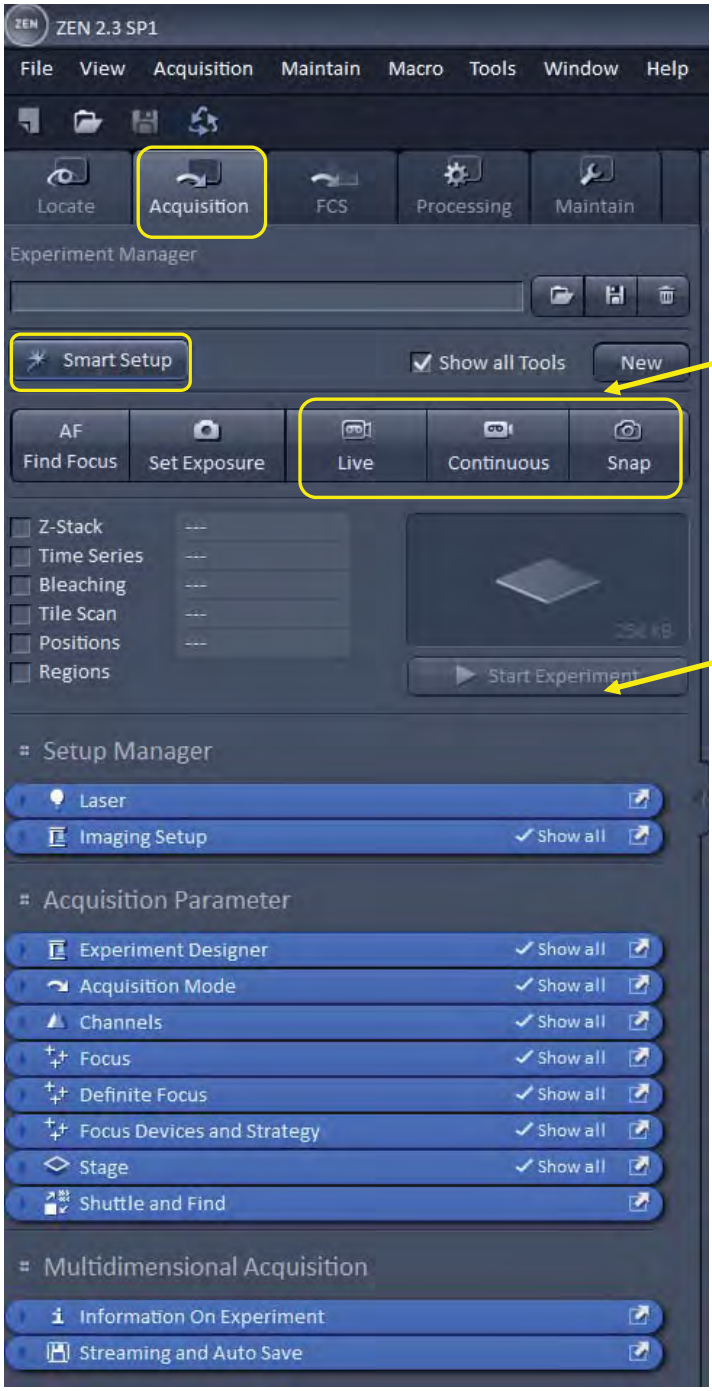
To show all functionality:
From the drop down menu, select **View > Show all (global).** Or **tick Show All** in each tab as needed

## 2. Locate tab: To view sample in microscope

Use pre-set configuration tabs to view sample in microscope


To create buttons:
**Microscope control** tab
Click on icons to choose light source and filter combinations
Save configuration
Assign – creates shortcut button

**3. Acquisition tab and turning lasers on:**

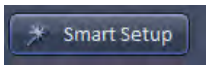



Imaging Mode:
**Live**: Fast 512x512 - no averaging
**Continuous**: As defined in Acquisition Mode tab
**Snap**: Takes one snapshot of all ticked tracks/channels as defined in Channels.

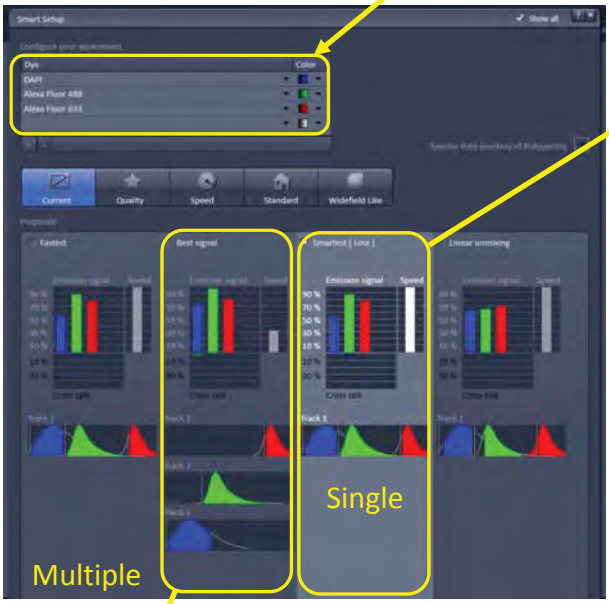
**Start Experiment**: All channels as defined for all selected parameters (Z, Time Series, bleaching, etc.)

3.1) **Lasers**
Turn on lasers
The multiline 458, 488, 514nm laser takes 5 mins to warm up.

## 4. **Smart Set up / 4.1** Imaging Set up

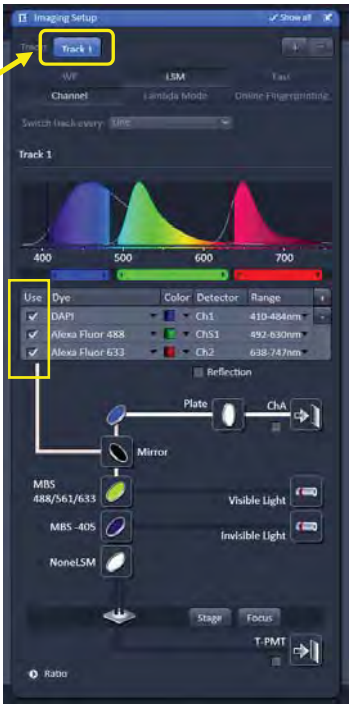


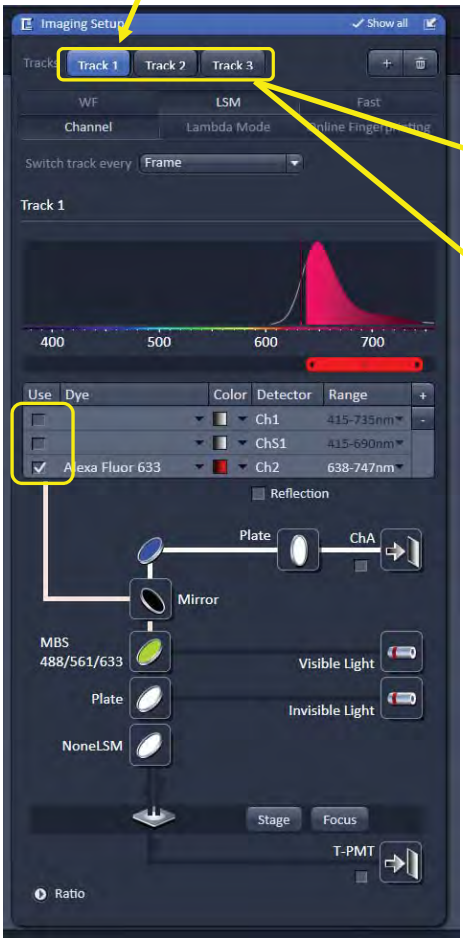

Use the drop down menus to select your dye combinations. Click OK to apply.




NOTE NUMBER OF TRACKS

**Single track** set up



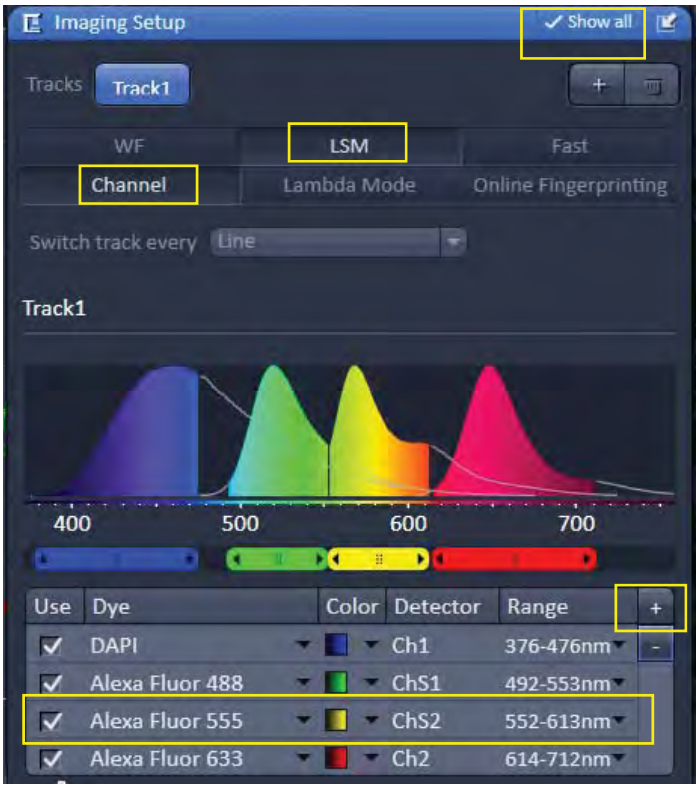




**Multiple tracks** = sequential (reducing crosstalk)
Allows changing hardware / specific filters between frames

Acquires longer wavelengths first


## 5. Setting up with Channels and Imaging Setup (without smart set up)

### Arrange detectors and filters



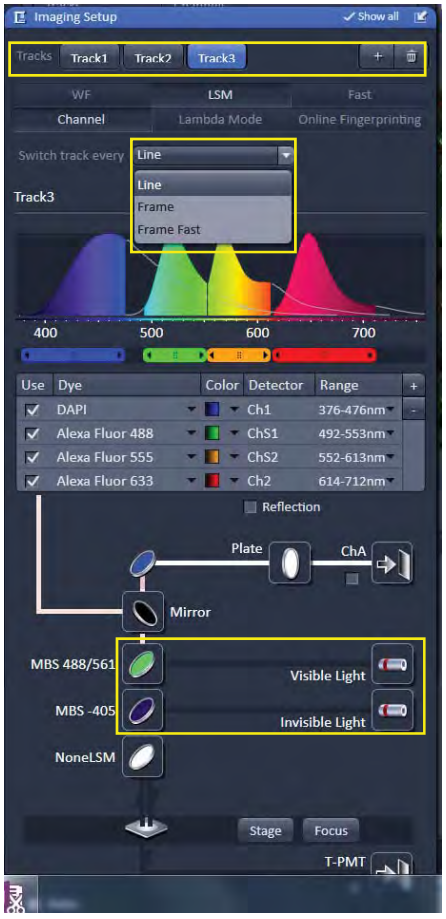

**Imaging Setup** tab:

Select: **Show all**, **LSM** and **Channel**

Tick your detectors, choose fluorophores and LUT colours from drop down menus.

Adjust detectors to collect appropriate wavelengths using sliders, or typing range. *Adjusting all detectors at this point will reduce movement between tracks.*

An additional detector can be created using the **Plus button** (by duplicating the central detector if the system has a 32 Ch GaAsP detector).




Create the number of sequential tracks needed with the plus button (Maximum 4 tracks).

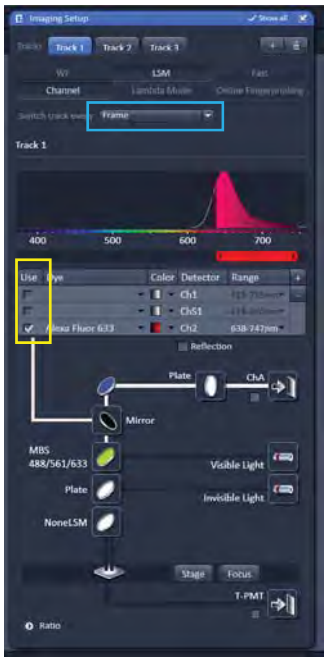
Specify: Line / Frame / Frame Fast
*(Line and Frame Fast require the same hardware between all tracks. I.e. same detector positions between all tracks, MBS)*

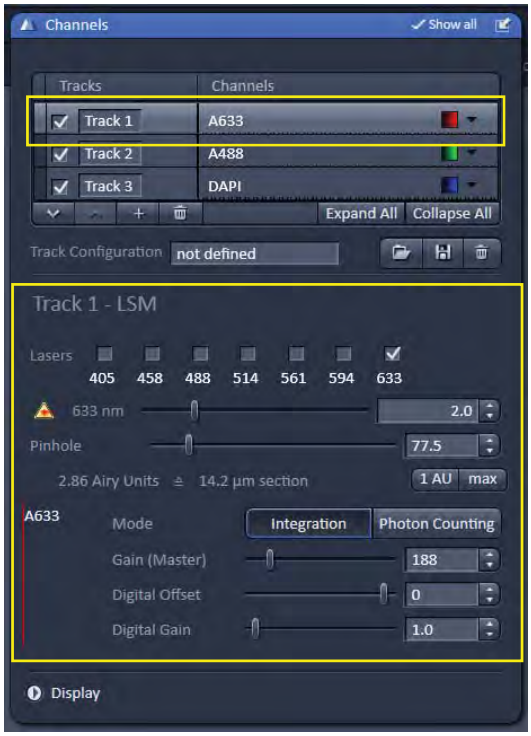
For each track button (at the top) select the appropriate detector(s), laser(s) and MBS combination for the Vis and InVis laser lines:


The T80/R20 can be used for multiple laser lines or reflection, but is not advised unless you have experience.

## 6. **Optimising acquisition:** Imaging Setup **and** Channels

In **Channels**, click on the track you want to adjust so it becomes highlighted. The chosen track will then be shown in the **Imaging Setup** tab. For ease, untick the other tracks. Click live or continuous and focus your sample. In Switch tracks every **Frame** mode, **Adjust each track** or channel one at a time (**Laser Power, Pinhole, Gain**), ensuring use of **full dynamic range without saturating signal** (see below).



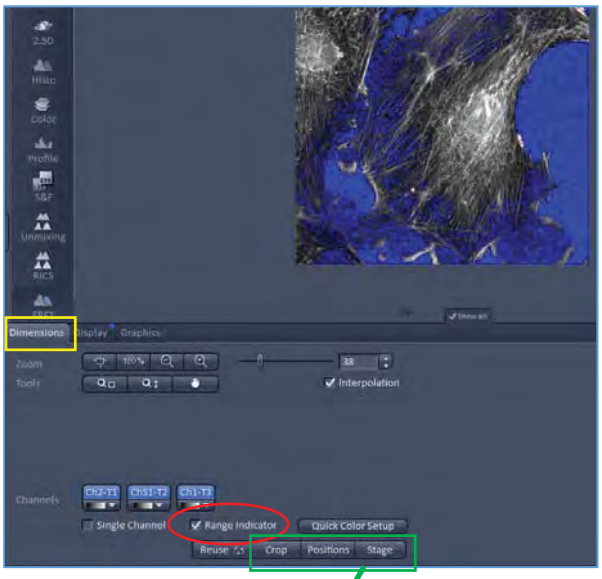


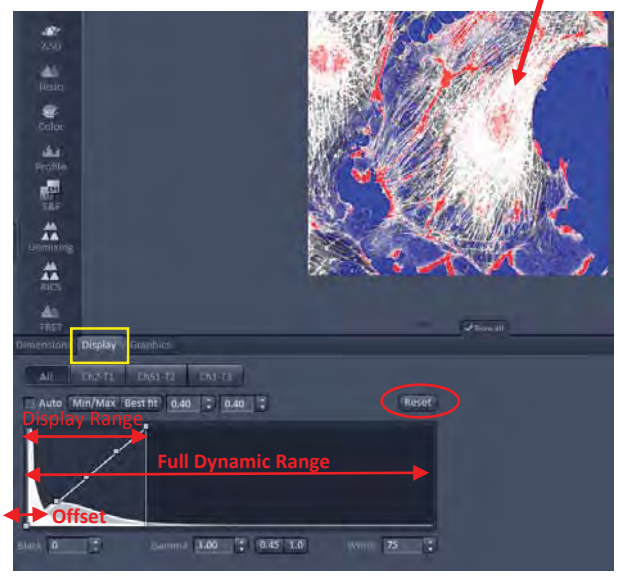

Note: In line-wise and frame fast-wise multitrack, you need the same pinhole, hardware, master gain if using a detector more than once

Adjust:

- ❑ **Laser Power**
- ❑ **Pinhole** (1AU for confocal / Section thickness)
- ❑ **Master gain** (600 – 800)

**Saturation**: Tick the **range indicator** and aim to fill enough of the dynamic range suitable for acquisition / analysis. Note – do this with display **RESET** or it will appear saturated when it isn't.




To centre and crop field of view:
Select **Stage** and click on image area of interest. Click **Snap** to see new field of view. This keeps the best optical axis for acquisition. You can then **Crop** a region of interest or **Zoom** in.

7. Define **Acquisition Mode**


← Check correct objective and immersion fluid.

Define frame size.
**Optimal** selects pixel size to satisfy Nyquist criteria. Increases acquisition time if large field of view. *Note: Frame size (Pixels) does not determine image size*

← Scan speed and pixel dwell time

Averaging – reduces noise
Can use line / frame

Bit depth - for additional grey values 8, 12 and 16 bit (256, 4096, 65536).

Uni / **Bidirectional** scanning *
Scans on return, halving scan time.
*(Might already be selected but hidden if Show all not ticked)*

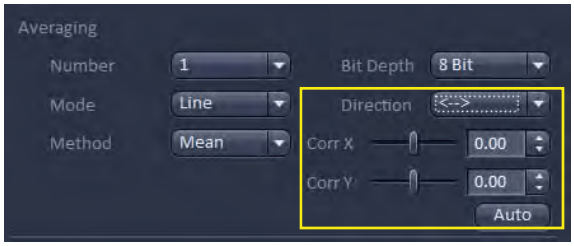
Scan area: *Note image size and pixel size*

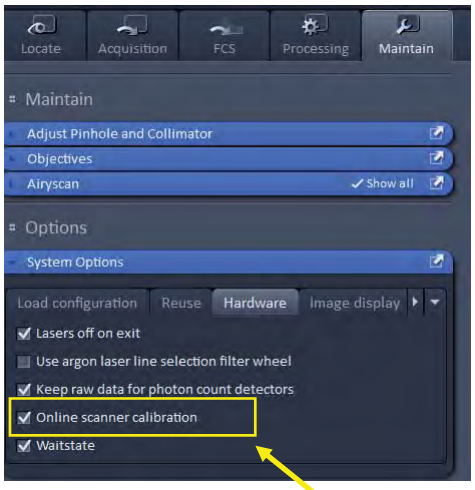
- Shift area in X and Y
- Rotate field
- **Zoom**. Zoom < 1 is possible but not recommended for quantitative imaging or tiling
- 1.0 = minimum zoon for confocal
- 1.8 = minimum zoom for Airyscan.

**Reset all** = quick reset button

**Bidirectional**

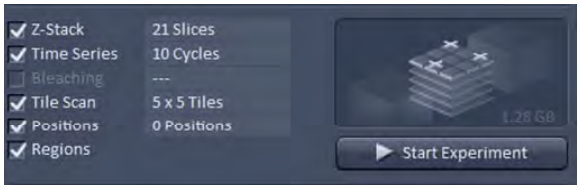
* Bidirectional may need aligning.
This becomes visible with Show All ticked.
Correct using the Auto button, or manually (X slider). Adjust on a single track image (not multitrack) and without rotation.




**Maintain tab, System Options, Hardware**
Check 'online scanner calibration' is ticked.
Correction is performed online in **Continuous** mode (not live).

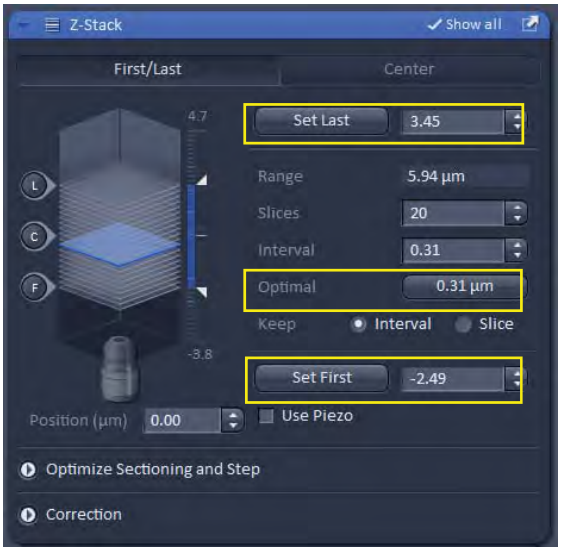
8. **Multidimensional acquisition.**

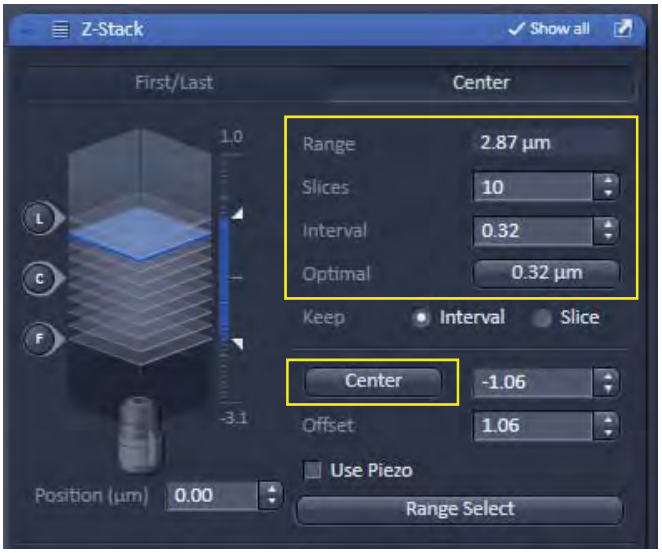



A combination of functionalities can be used to create a multidimensional experiment. Make sure there is enough space on the hard drive to save your experiments. Set up each tab, then **Start Experiment** to run acquisition.

9. **Z-Stack**

Tick the Z-stack option and expand the new **Z-Stack** tab that appears




Setting a Z stack: Use Live (for fast live view)

**FIRST / LAST:**

- Focus to the bottom of your required stack and click **Set Last**. Then find the top and **Set First**. Check the total range, slices and interval. Clicking **Optimal** will select appropriate Nyquist settings for 3D imaging. Start Experiment. Save your data (**autosave** or **stream** during acquisition)

**CENTRE**

- Focus your specimen. Click centre, then input range and adjust slices / interval /offset as needed.
- For **multiposition Z stacks using centre,** click **Centre** on the **first position only**

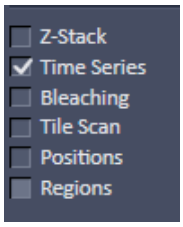
## Optimize Sectioning and Step in Z-Stack

- A 50% overlap is optimal for 2x Nyquist 3D visualisation. If you used **Optimal** for the interval, it is calculated for you and this step is redundant.

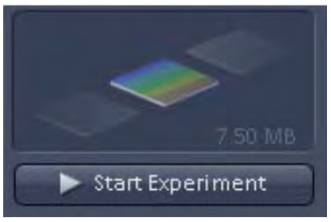
- If you have chosen a larger Z interval and have separate tracks, you need to adjust each channel to have equal optical section thickness and some degree of overlap.
- Match pinhole or optimal ensures overlap is calculated from the shortest wavelength and equalises section thickness for each channel. The pinhole and overlap are shown for each channel.

- X:Y:Z matches the X, Y and Z pixels, creating cubical pixels with same resolution in XYZ.

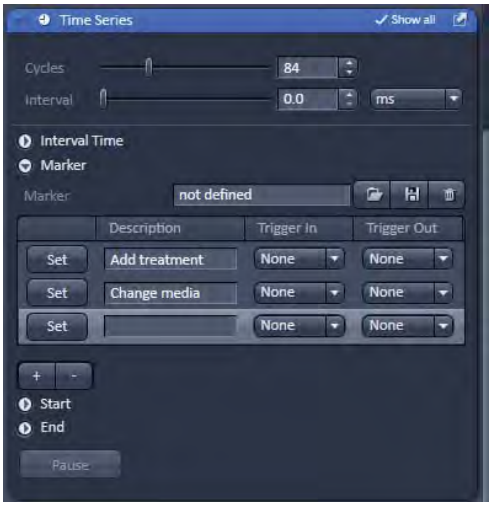
## 10. Time Series







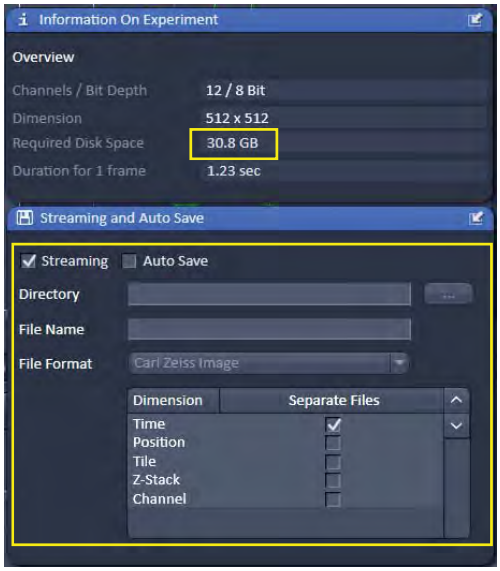

Tick time series in experiment window and expand the Time Series tab.
Set the number of Cycles and interval. Start experiment. Save your data (!)
(Ensure you check there is sufficient space on the hard drive to save your experiment).




Show all: You can add markers during your experiment, or change certain acquisition parameters.
These will appear as coloured square in the gallery format at the specific time points.

**Interval Time**: You can create a shortcut button to acquire at different time intervals. E.g., longer intervals to monitor sample, then click button for shorter intervals. Sets of intervals can be saved.
**Marker**: to label action.
Functionality can be set manually or set up using triggers. Sets of marker labels can be saved.

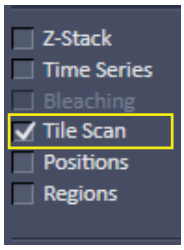
**7. Saving data**:
**File > Save as > .czi.** This can be viewed in Zen, and directly imported into 3D software.
You can also export as a tiff file. **File > Export** (choose tiff and appropriate options).

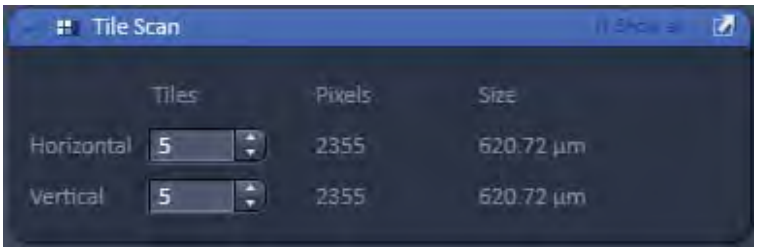


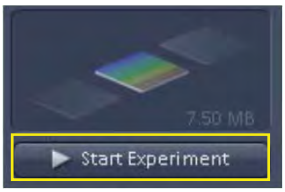

Check the **Information on Experiment** tab and ensure enough space is available on hard drive.
**Streaming** (recommended) will save directly to the hard drive as each image is acquired.
Files can be split for easier handling.
**AutoSaving** will save to the RAM during the experiment, and then to the specified hard drive at the end (not recommended).

Experimental configurations can be saved or you can click **REUSE** to apply settings from an image.

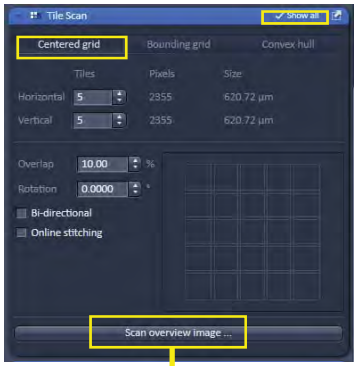
## 11. Tiles



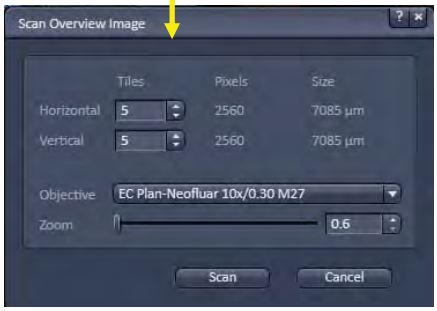




Tick Tiles in experiment window and expand the Tile Scan tab.
Set the number of tiles in XY. Start experiment. Save your data (use streaming !)
You will obtain a preview map that will need to be stitched in the Processing tab.



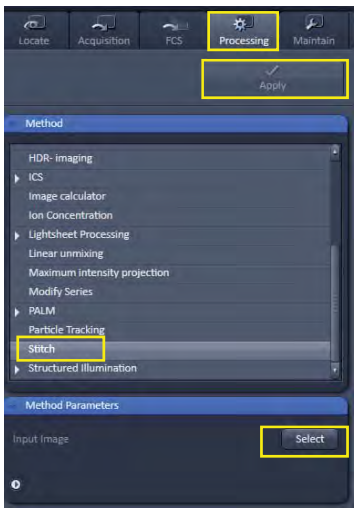

'Show all' mode has more functions to create your tiled area with.
**Centred grid** acquires a tiled scan around a central point.
**Bounding grid** will create a quadrangle around marked positions.
**Convex hull** will create a tiled region that encircles all points (like an elastic band around the added positions). This can save imaging blank corners of a circular sample.




**Scan Overview Image** allows you to choose a lower magnification objective to make a map of the sample. Once saved, you can click on the map to move the stage to that position with any objective.
Note:

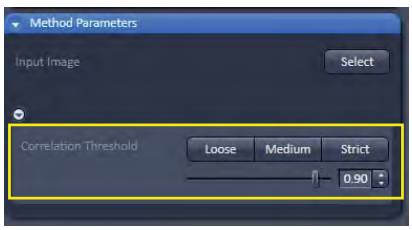
- Zoom 0.6 will have vignetting around each tile but it will function as a map (not for quantitative imaging).
- Save the map to avoid overwriting it.

### Stitching a tiled scan.



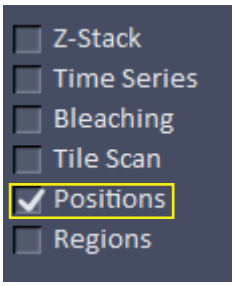

Go to the **Processing Tab**
In the **Method** tab list, select **Stitch.** Then click **Select** to select the data you want to stitch. Then **Apply.**
Save the stitched image – it will have the extension '**_stitch.czi**'

If stitching fails in 2D, you can choose the Correlation Threshold strength. This defines the accuracy the algorithm detects similarities in adjacent images.

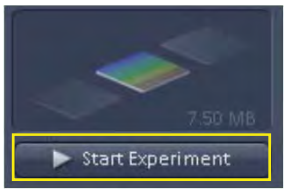



Zen Blue has more flexibility for stitching.
When stitching tiled Z stacks, if the sample is flat, **Ignore z-shift** can improve results. If tilted, it can improve results.

## 12. Positions








A number of positions can be added to your experiment. Tick **Positions** in the experiment and expand the **Positions** tab. There are various practical methods to add positions:

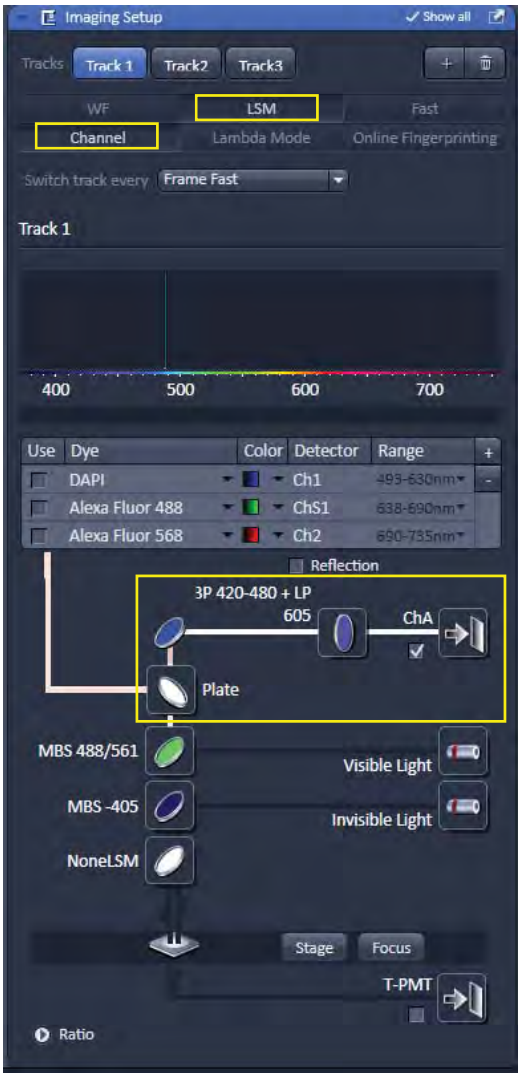
1. Click **Live** to view your sample, and click add each time you find a position to image. There are buttons to **Add** and **Remove** positions when they are highlighted. **Up** / **down** will adjust position order. **Move to** will go to the position highlighted.
2. View through the microscope and then add once centred in the binoculars.
3. Use **Scan overview image** to create a map and use the stage button to add positions from it.

Before you start your experiment, check all positions are in focus and click **Update.**
**Move to** (adjust focus / position as needed) **Update.**

**Note:** If doing multi-position Z-stacks, ensure stacks are added by their central position as this will define the centre of the Z stack specified in both **Centre** and **First and Last** Z stack options.
When **Show all** is ticked, it will give the option to lower objective between positions. This helps to preserve immersion fluid during multi-position time series experiments (Not on Examiner).
Activating '**Waitstate**' (**Maintain tab, Systems Options, hardware**) will add 350ms pause between stage movement and acquisition. This can reduce jitter.

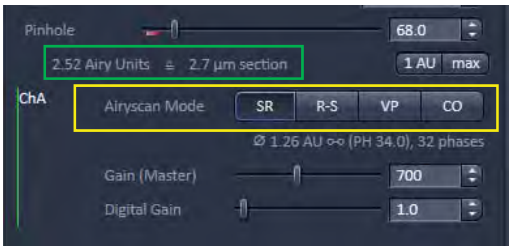
**Adding positions from a scan overview:**
You can create a tiled / scan overview, then use this map to add positions, moving to each position with the **Stage** button.
You can then verify the focus of each added position before you start your multiposition acquisition.
You can also use this technique to create a map for bounding grid or convex hull, then acquire better image quality / multichannel on refined area. And to quickly add multiple positions.

## 13. Airyscan



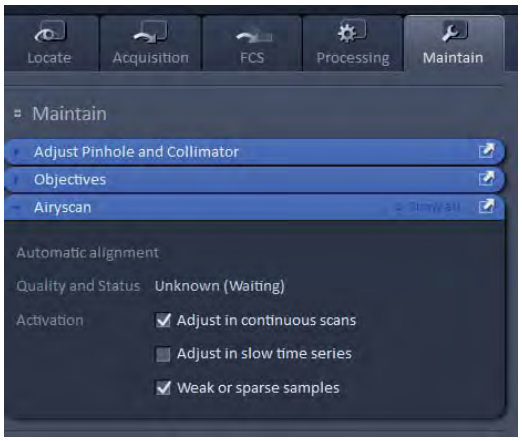

Create your acquisition set up in **LSM Channel** mode as **single channel tracks**. Tick the Airyscan detector **ChA**, appropriate laser and select the correct **MBS** and emission **Filter** before the **ChA**. Repeat if using Airyscan for in other Tracks for multiple channels. Keep hardware and filters the same if using **Switch track every Line** or **Frame Fast.**

In the **Channels** tab, choose Airyscan mode:
**SR**: Increased resolution and S:N ratio. Pinhole default = 0.2AU, total detection area 1.25AU.
**R-S**: Increased speed and sensitivity at expense of resolution. Pinhole 0.2AU, total detection area 2AU.
**VP**: Virtual pinhole. A slider will allow signal shown from element rings with appearance of changing pinhole post-acquisition. Default pinhole 6
**CO**: All Airyscan detector elements are used as one confocal detector and you can choose your pinhole.



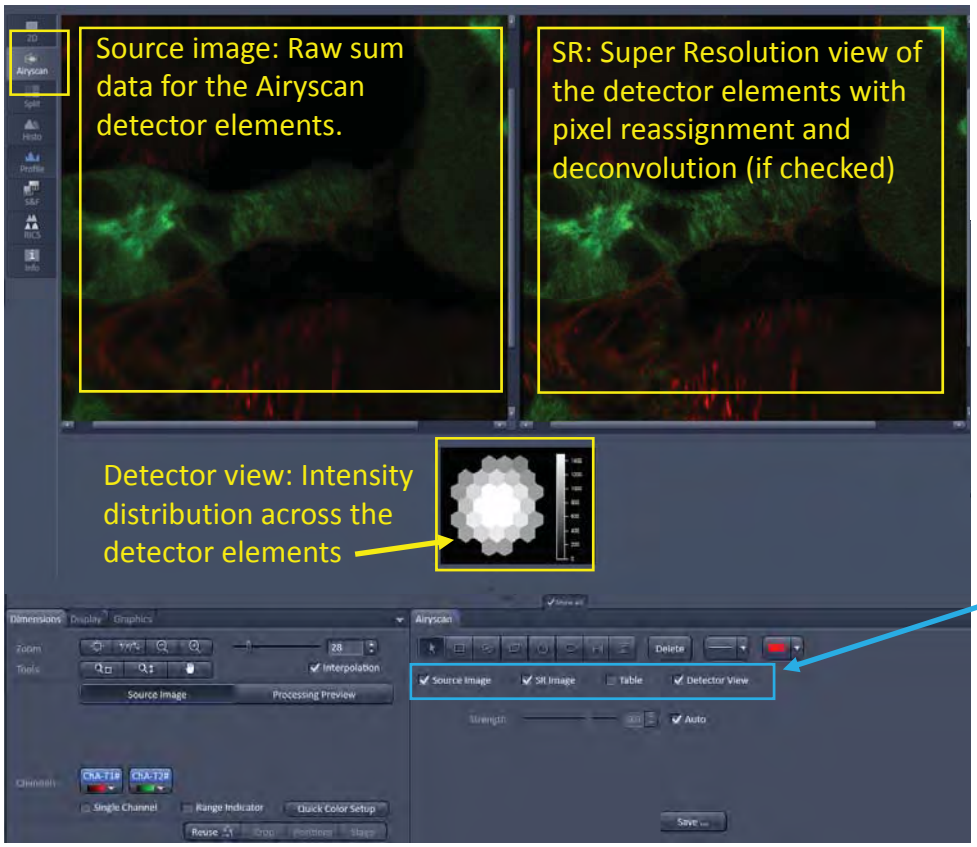

**Note:** Confocal pinhole set by software to remove stray-light and allow for the Airyscan detector alignment. Value specific to SR or RS.

Open the Maintain Tab and undock the **Airyscan** tab (place under Gallery area on monitor)
Tick '**Adjust in continuous scans**' and '**Weak or sparse samples**'
You may also need the **Adjust Pinhole and collimator** tab



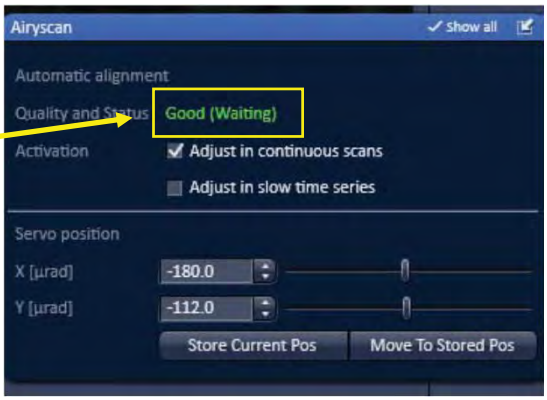

14. **Airyscan: Detector Alignment – Single Track**

To check Airyscan detector aligns:
Set frame to 512 x 512 pixels, max speed, no averaging, 0.5 - 2% laser, gain 600 – 800 and increase as necessary.
Ensure preview is not saturated when display is reset.
Click **Continuous** to view sample and select the **Airyscan tab** to the left in the image window.
The **source image**, **preview image** and **detector view** can be viewed when ticked (click show all if not available)..




The **source image**, **preview image** and **detector view** can be viewed when ticked (click show all if not available).

**Single track / channel**:
When scanning in continuous mode, the detector view should align so the brightest elements are in the first ring or central element. The quality status will show **Good (Waiting)** when OK. **Bad** when not.




If the detector view is not responding or aligning, try:

- Close / open detector view (check numbers active or numbers jigging at the side)
- Move/ refocus your sample so the signal is central
- Increase gain – don't worry about saturation until detector aligns, then reduce before acquisition.
- Increase laser (avoid bleaching sample)
- Try aligning in RS mode, or on another slide if really weak, then untick adjust in continuous mode. (Only one VIS line needs to be aligned).

The detector may look aligned but Good (Waiting) may not appear. You can still acquire your image.

*Go to the 2D tab to start an image. Sometimes the Live / Continuous / Snap do not work in the Airyscan view tab. Go to the 2D tab to start an image, or create a **new** document.*

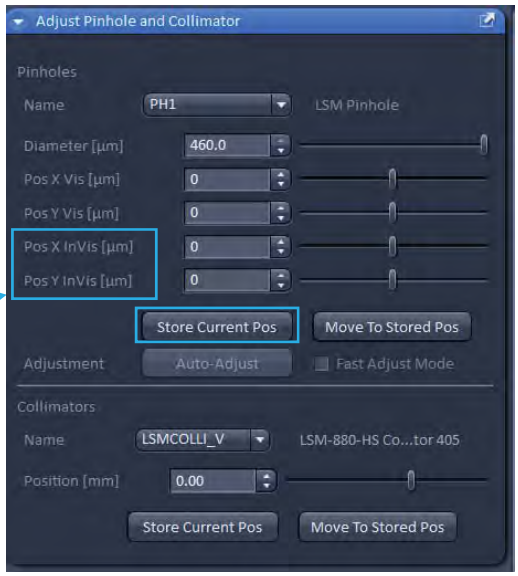
## 15. Airyscan: Detector Alignment – Multi-Track

**Multitrack Airyscan**:
As for LSM, multiple Airyscan tracks can be acquired in **Frame** mode (if different hardware) or **Line** and **Frame Fast** modes (if hardware and Master gain are the same for every track).
In Continuous mode, all channel detector alignments will be super-imposed onto the one detector view.
The Vis and InVis laser lines will need to be aligned to each other. Turning off each laser in turn, while still in continuous mode, will allow viewing of each channel alignment:

**Aligning a multitrack acquisition**:
To CHECK alignment, for multi-track with Vis and InVis laser lines. Scan in continuous mode and untick the InVis laser on that track to see the AS alignment for the Vis track only. Untick **Adjust in continuous mode**. Turn 405 back on and untick the Vis laser to see the InVis alignment on the detector view.
The system aligns the Airyscan using the Vis track, and the InVis steering mirror for the InVis track.

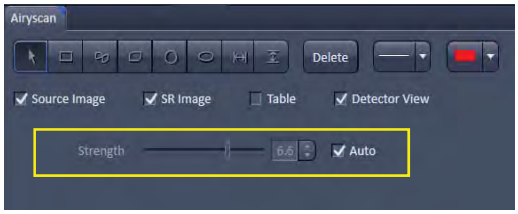
**Manual Alignment of InVis line**:
If auto-alignment fails and the 405 is not aligned to the Vis line, you may have to adjust **Pos X InVis** and **Pos Y InVis** so that the **InVis** signal is centred on the detector view. Use arrows, not sliders (in **Maintain t**ab, "**Adjust Pinhole and Collimator**").

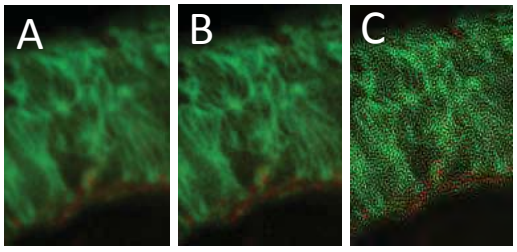
Ensure **Adjust in continuous mode** is off while aligning 405. Once aligned, click **Store current position directly underneath**, then tick **Adjust in Continuous Mode** again and restore Vis laser.




## 16. Airyscan: Pixel Re-assignment and Deconvolution:

The SR image represents the pixel reassigned image + Deconvolution.



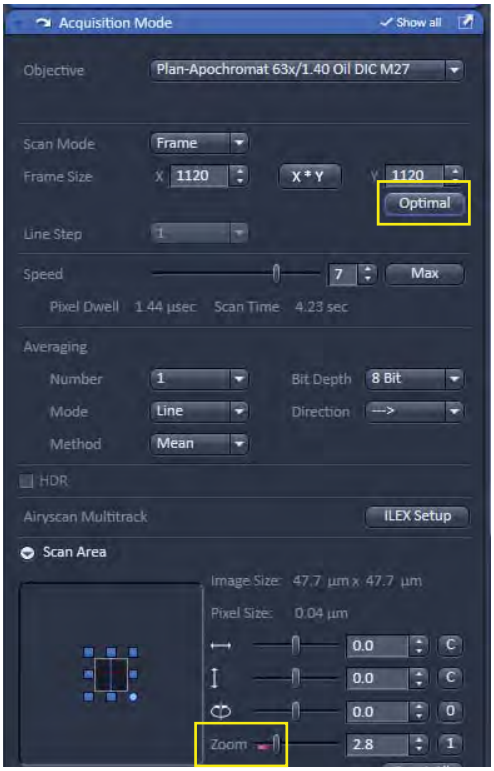

You can adjust the strength of the filter by deselecting auto, and adjusting slider.
A) If the filter strength of the deconvolution step is set to 1, the image will be pixel reassignment only. (1.4x LSM resolution).
B) Clicking Auto will add a safe deconvolution, as calculated by the software (up to 1.7x LSM resolution).
C) Over-deconvolved creates 'structured noise'.
Remember the new value when processing image for export. Individual values can be processed for each channel in Zen Blue software.

## 17. Airyscan: **Acquisition and saving raw data**

Check list before acquiring Airyscan data:

- SR – R-S, VP, CO selection
- Data is not saturated
- Field of view has been stage-centred
- Frame speed chosen
- Zoom is at least 1.8 (or more)
- Then select **Optimal** !
- …Start Experiment.

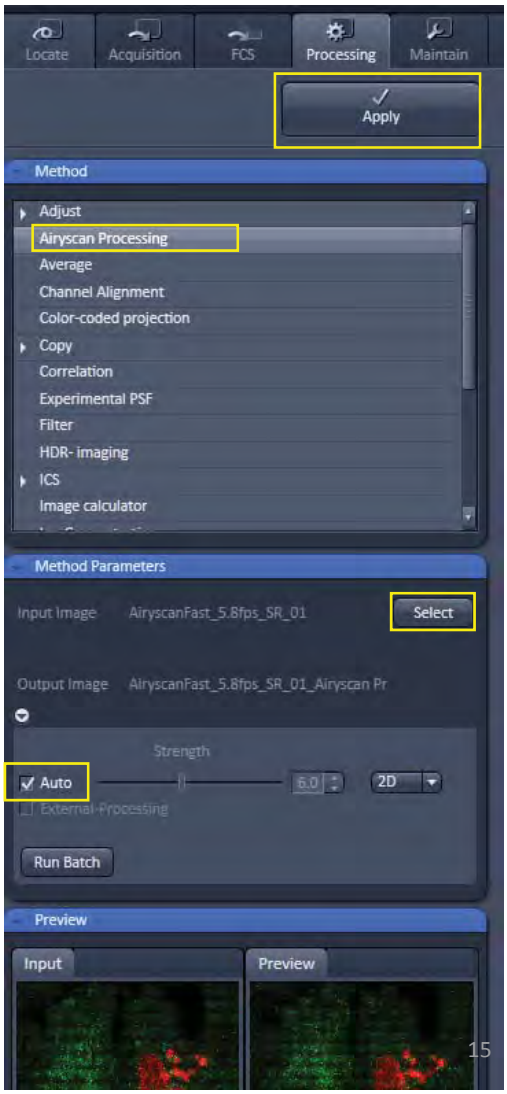
File > Save as
Or
Save icon below gallery view.
This will only save your raw data (and preview if opened in Zen. You will need to process the raw data to export.




## 18. Airyscan: **Processing for export**

To process, Go to Processing tab.
In **Methods**, select Airyscan Processing.
In **Method Parameters**, **Select** the raw image so it appears in the thumbnails in the preview.
Check Weiner filter strength or use tick Auto (Auto recommended as starting point).
If a Z stack, click **3D**. If single plane click **2D**.
Then **Apply**
A new image tab will appear with the post-fix **_Airyscan_processing.czi**
Select display – min / max to view.

This can be exported as normal .czi files.
Data size much reduced.

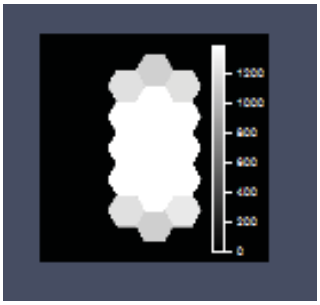



Notes:

1. Airyscan-process images before stitching, creating MIPS or unmixing etc.
2. 2D-SR is available in Zen Blue for single planes. Enhanced deconvolution for greater resolution (120nm).
3. Zen blue Airyscan processing will also allow different weiner filter strengths to be chosen for each track.

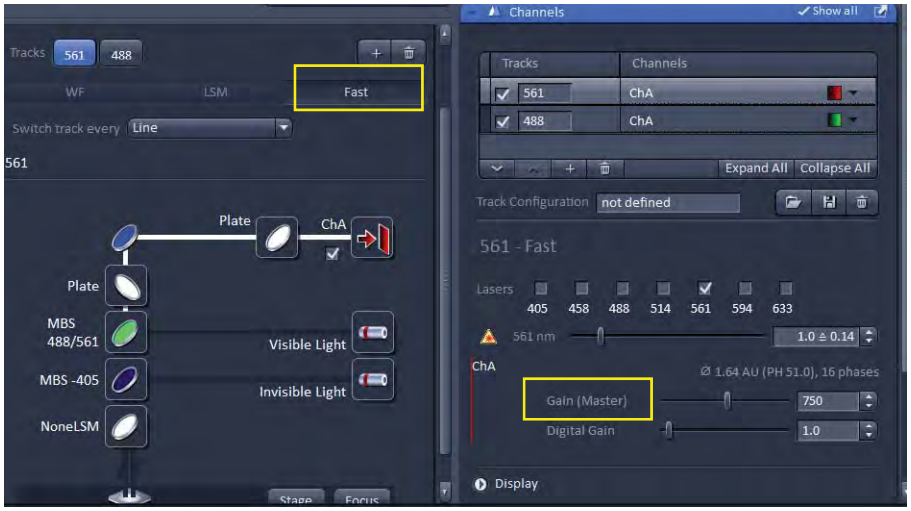
19. **Airyscan: Fast**

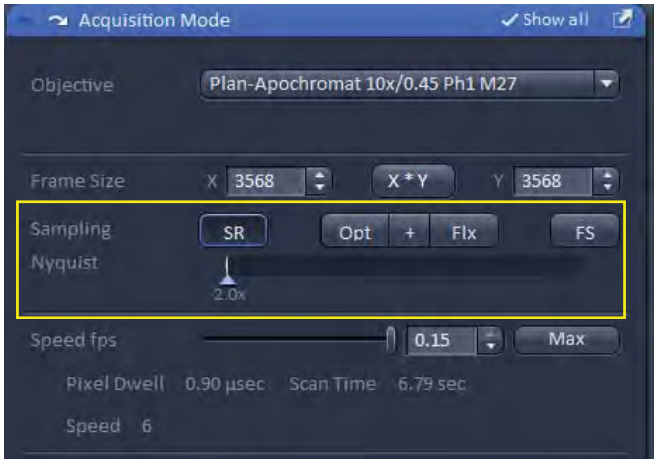
Similar to acquire, align and process as for Airyscan.



Differences to note :
Beam now shaped to acquire 4 pixels, so imaging is 4x faster
Resolution is up to 1.5 x better than LSM

Set up as for Airyscan and select the **Fast** tab.
For speed, set up in Line mode (same hardware and master gain).




Airyscan Fast modes:
**SR**: 2x Nyquist at 4x the speed of the Airyscan detector.
**Opt**: 1x Nyquist
**Flx**: 0.7x Nyquist
**FS**: 0.5x Nyquist with fastest imaging speeds (or you can decrease speed slightly and use it for higher sensitivity in AS Fast mode)
**+ button**: you can choose any Nyquist value by moving the slider

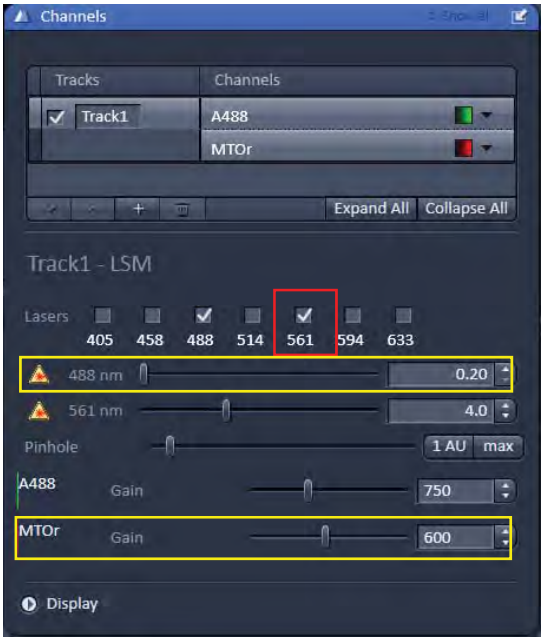
Notes:
Once selected, frame size is automatic according to Nyquist – no optimum button for pixels.
Speed (now frames per second) will affect pixel dwell time.
Increasing pixel dwell time recommended over averaging to improve image.
Zooming in and reducing the Y axis will allow faster frame rates
27 frames per second at Frame size 480x480
You cannot rotate Airyscan Fast field of view.

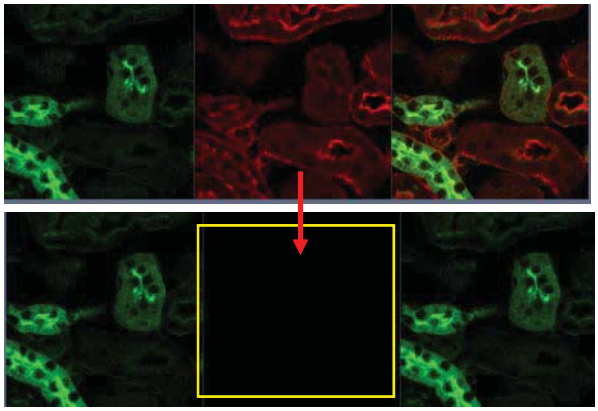
## i. Experimental Notes

**a. Optimise dynamic range** on your brightest treatment / time-point to avoid saturation in part of your data set. (Remember to put your strongest fluorophore on your weakest signal).

### b. Checking and reducing Cross talk in tracks with multiple channels:

Deselect the laser with the longest wavelength - all signal should disappear in that channel. Remaining signal may be cross talk.

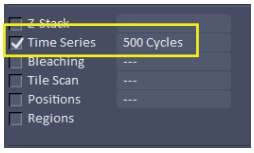


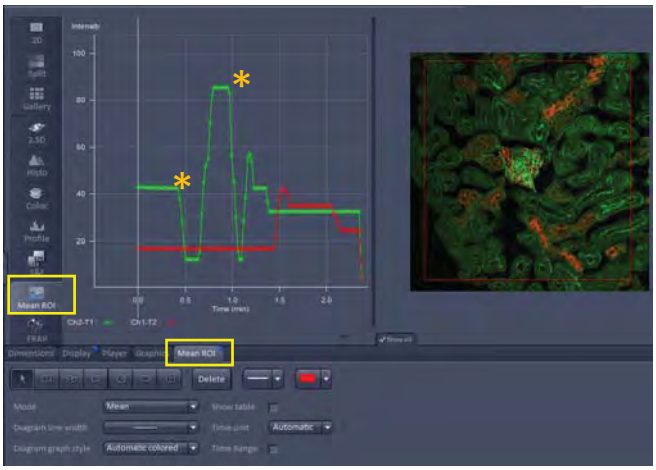
Reduce either the laser power from the first channel, **AND / OR** reduce the gain of the second channel. Do not adjust these values when adding the second channel laser back in.

### c. Checking for Photobleaching:

Select **Time Series**
Select e.g. 500 cycles at 100ms interval (avoids dark state). Use c.600 gain.
Click **Start Experiment**



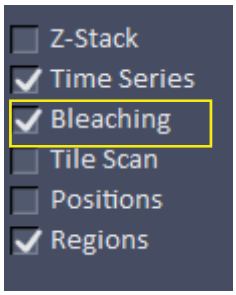


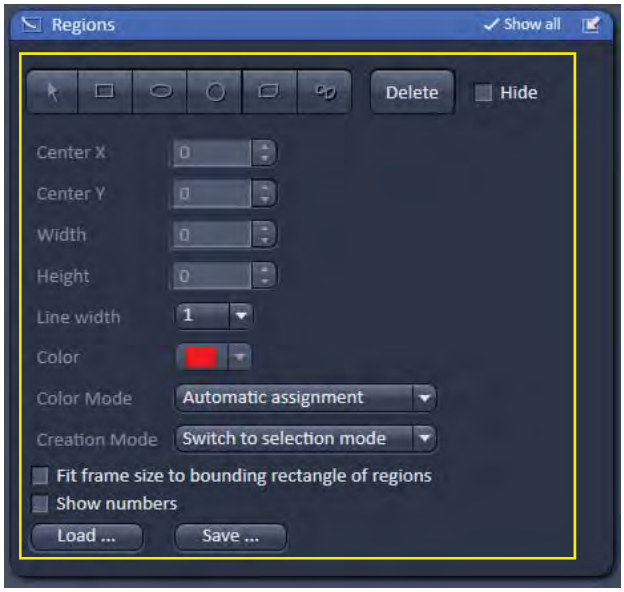


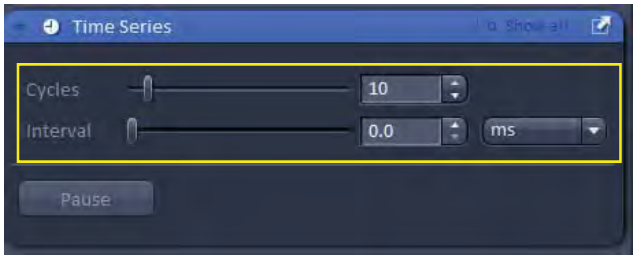

In image display, select **ROI tab**
In the **ROI tab** under image, create an ROI region in the image
The ROI Intensity vs Time plot will appear for each channel.
Check for decrease in intensity with time.
If a decrease (bleaching) is evident, decrease the laser power (*) until the signal levels, use gain to fill dynamic range.
If no bleaching, you could increase your laser / lower your gain for a better signal.

## ii. Time Series, Bleaching and Regions (FRAP)

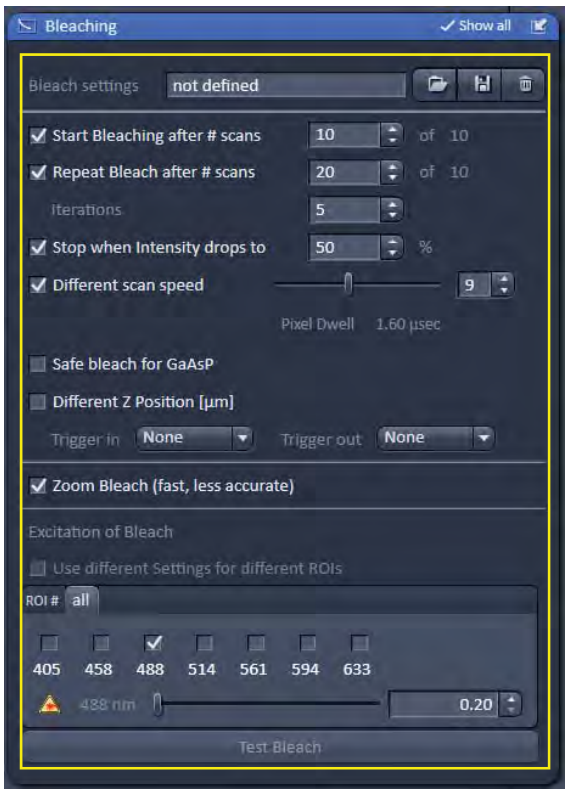
FRAP (Fluorescence Recovery After Photobleaching) can be monitored using **Bleaching**, which automatically combines **Time** and **Regions**. Intensity profiles for each ROI can be viewed once you have started the experiment.



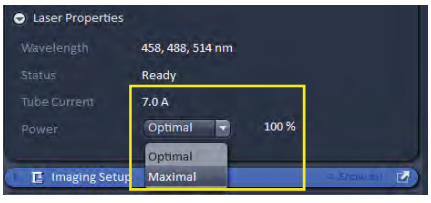

Create regions across your sample in the **Regions** tab. Reference and Background regions can be added before or after (if added before, ensure bleaching is unticked for these control regions). Create a continuous scanning mode in the Time Series tab. E.g. 10000 cycles, 0.0 interval.




**Bleaching**.

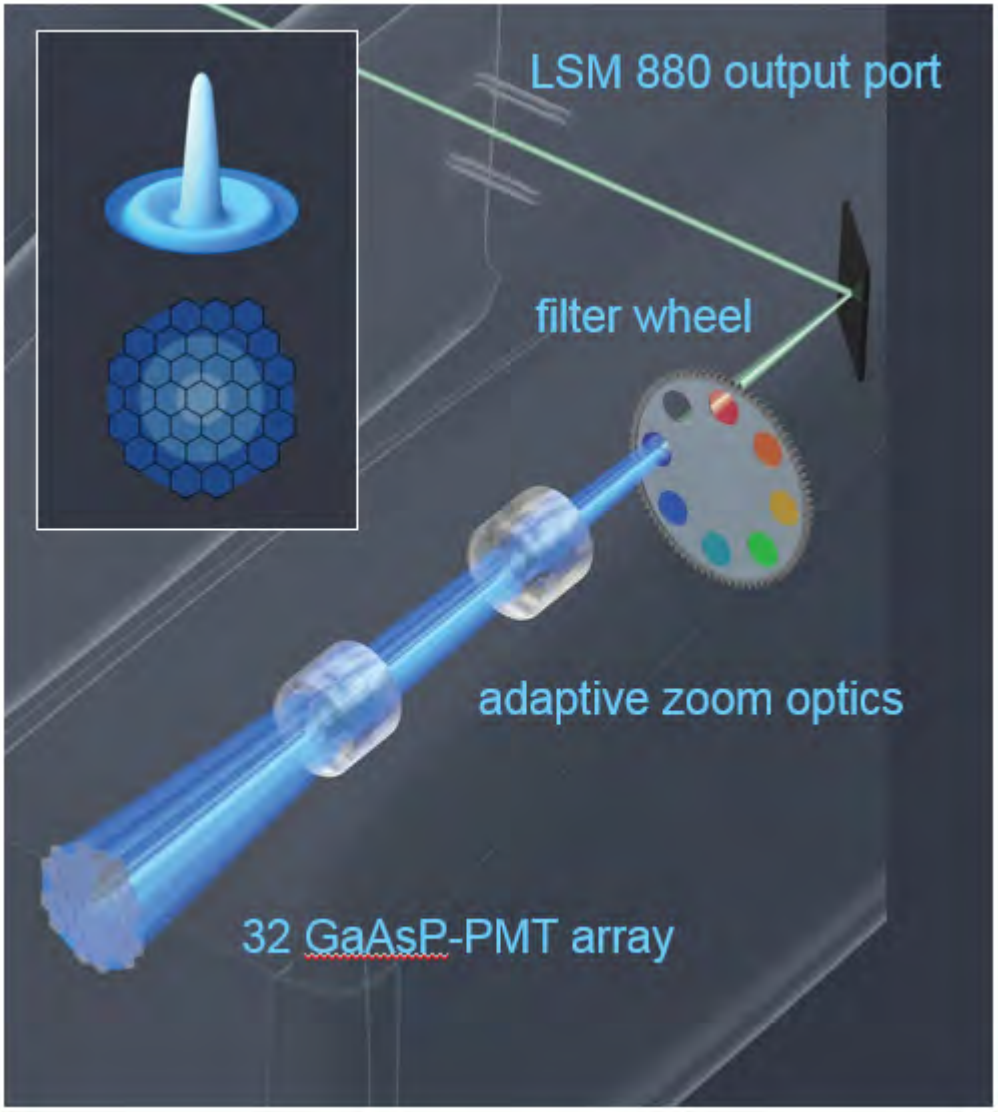
- **Start bleaching after**… : Creates baseline
- **Repeat Bleach**: Sets interval between bleaching
- **Iterations**: Total number of scans performed for bleaching of selected region (Frame)
- **Stop when intensity**…: in repeat bleaching, bleaching will stop at the specified intensity
- **Different scan speed**: determines pixel dwell time during bleaching event. Bleaching efficiency is increased with increased dwell time. To reduce irreversible photodamage, this can be combined with Iterations.
- **Safe bleach for GaAsp**: protects detector. Adds 100ms to each cycle. Try first without, if detector shuts off due to overexposure, add to experiment.
- **Zoom Bleach**: Restricts scanning and bleaching to ROIs only.
- **ROIs:** define control / bleaching parameters.
- **Different bleaching settings** can be set for ROI's..
- Configurations can be saved / reloaded.

**Note:** If bleaching efficiency is not obtained after increasing pixel dwell time or iterations, the 488nm laser can be run at maximum / twice the power (Visible when **Show All** is ticked).

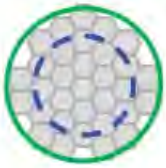



**Experimental Note**: To speed bleaching, create ROIs within a small Y range. In normal mode (not zoom bleach) the scanners scan the whole width (X) but start at the topmost ROI and stop at the bottom-most ROI.

Airyscan:



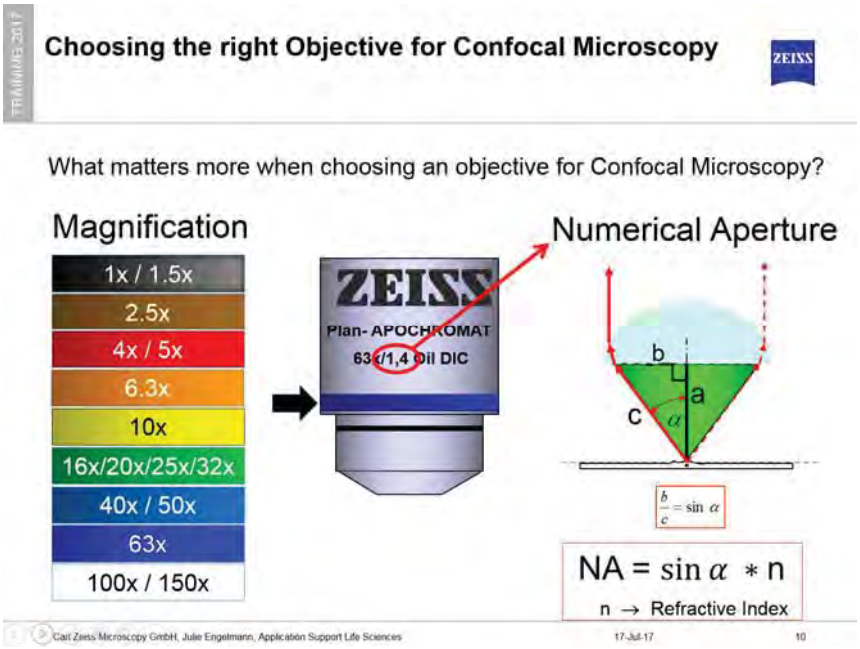

**Superresolution Mode**
Resolution 1.7x increased

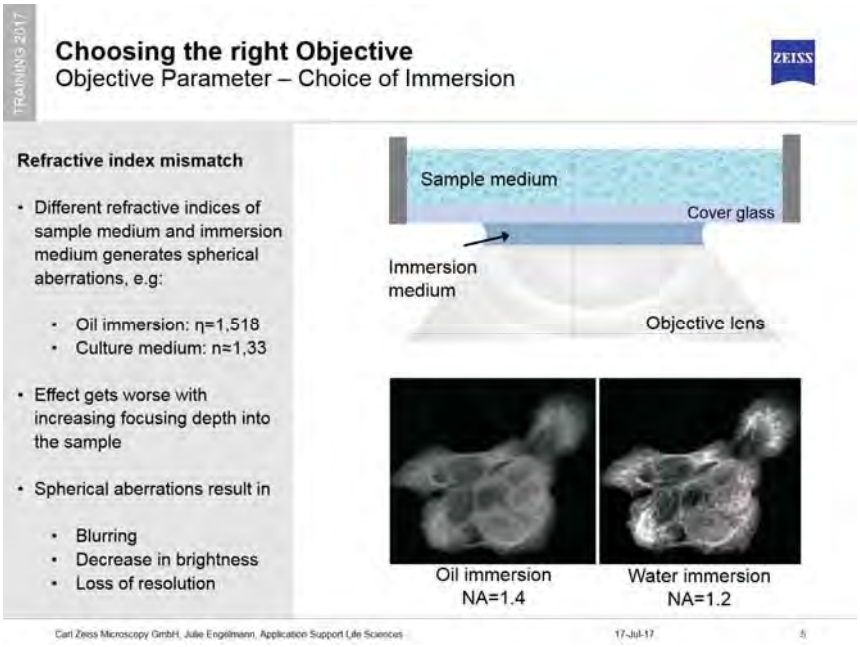


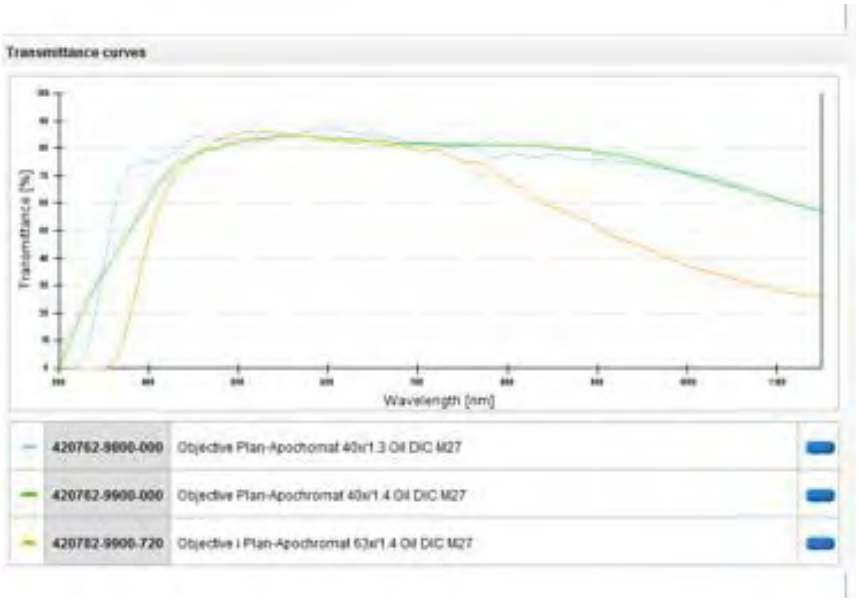
**Sensitivity Mode**
Resolution ~1.2-1.6x increased

**Speed Mode**
Resolution up to 1.5x increased
4x faster image acquisition

# Choosing the right objective:



# Confocal Microscopy:

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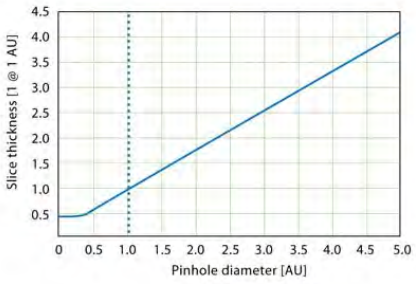
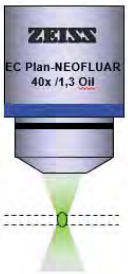
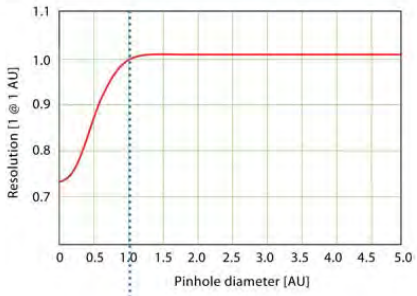
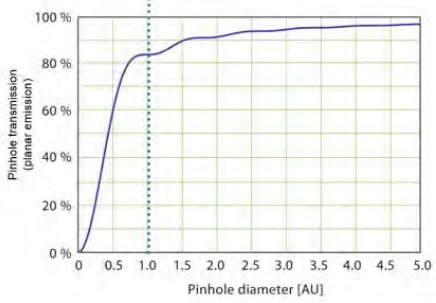
## The Confocal Pinhole

*Pinhole Size Dictates Slice Thickness, Resolution, & Signal*



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## Confocal Laser Scanning Microscopy

*Emission filters*

- **Short Pass**

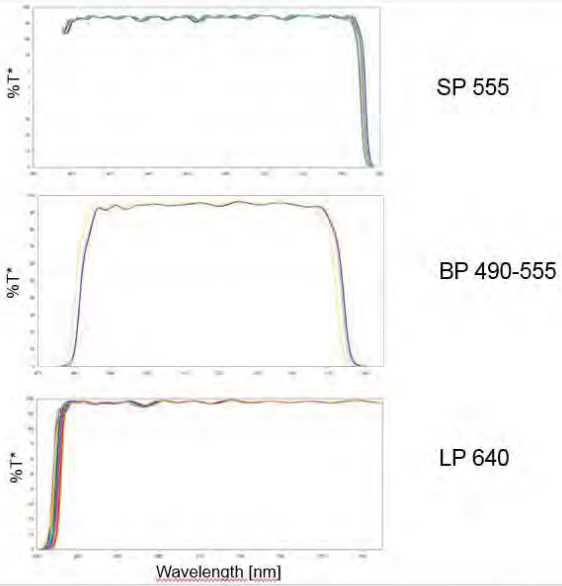
transmit below the indicated wavelength (e.g. SP or KP 555). "K" stands for the German word "kurz" (short). The KP 685 is used in NLO Systems as a IR blocking

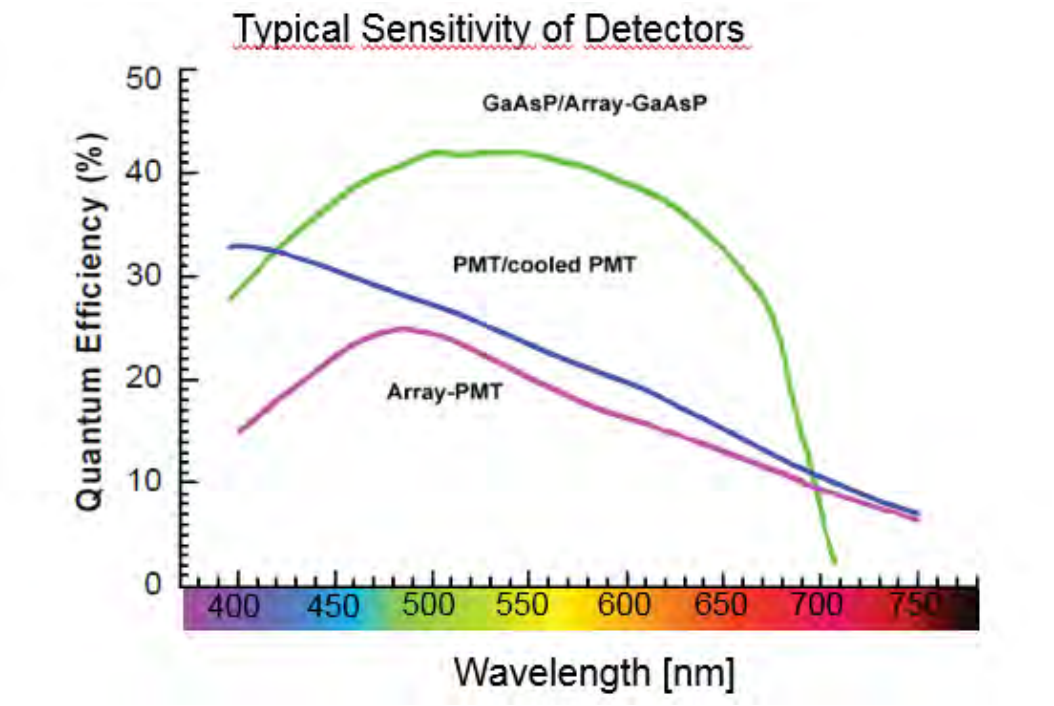
- **Band Pass**

allow a defined spectral band to pass through (e.g. BP 490-555)

- **Long Pass**

allow transmission starting at the indicated wavelength (e.g. LP 640).



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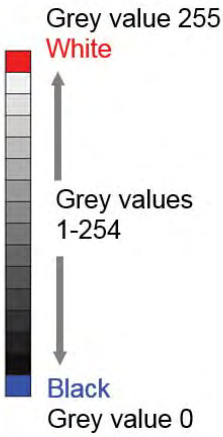
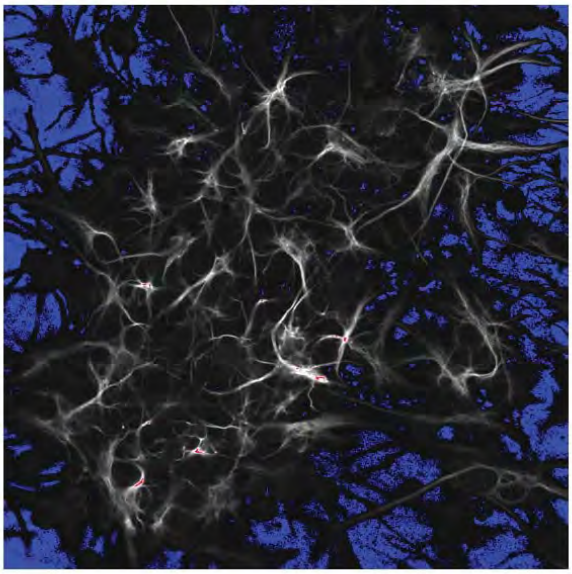
## Range Indicator

*How to evaluate the dynamic range*

**Look-up table**
**Range Indicator (8bit)**



# Resolution: Signal and Pinhole size

*Fig. 15 As shown in Part 1, small pinhole diameters lead to improved resolution (smaller FWHM, deeper dip – see normalized graph on the left).*
*The graph on the right shows, however, that constricting the pinhole is connected with a drastic reduction in signal level. The drop in intensity is significant from PH <1 AU to PH = 0.05.*

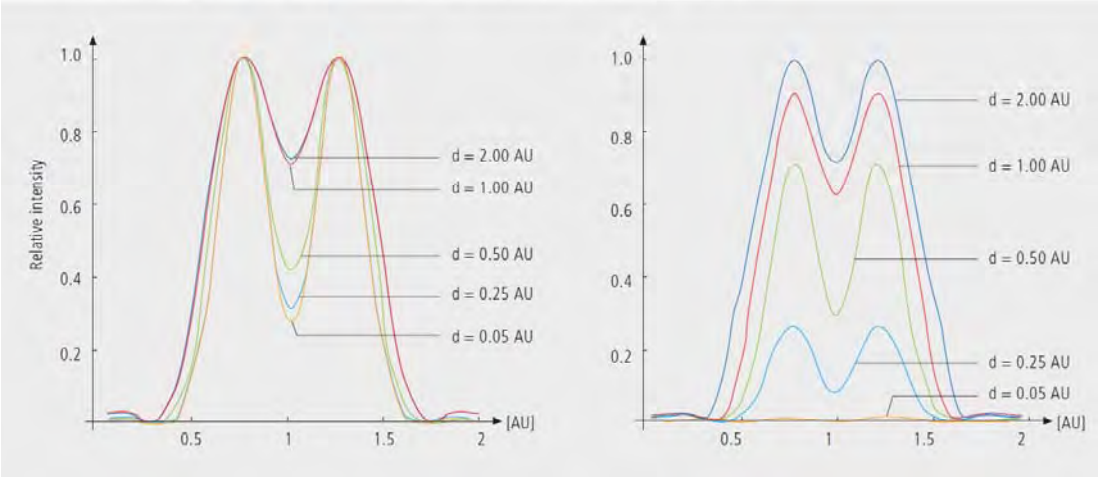
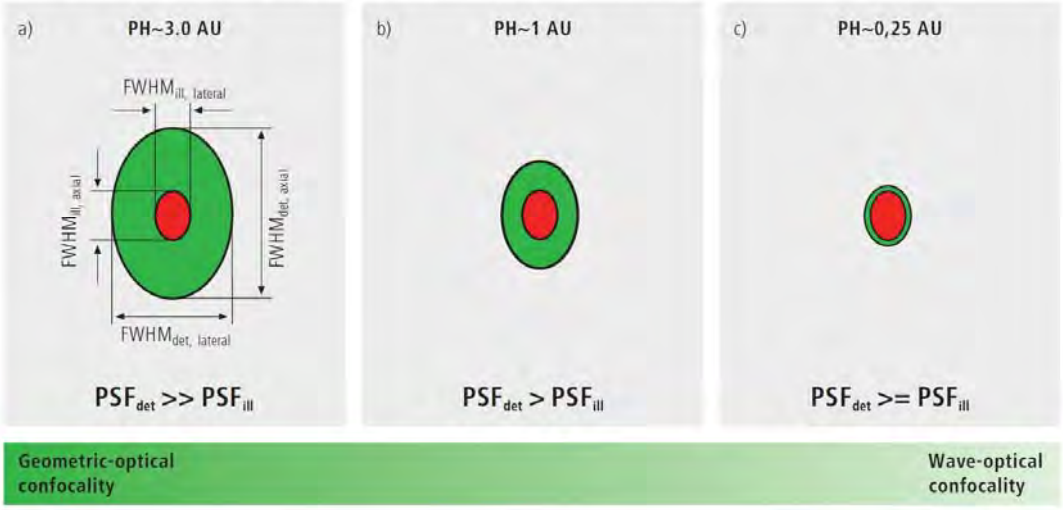
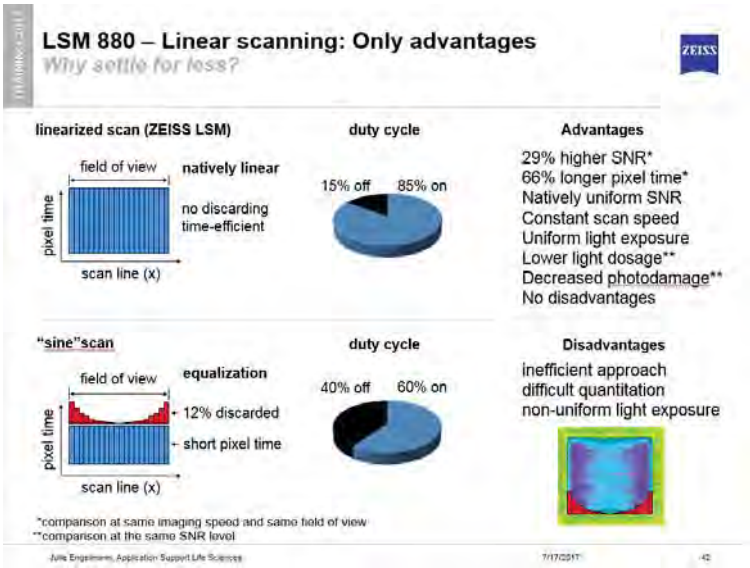
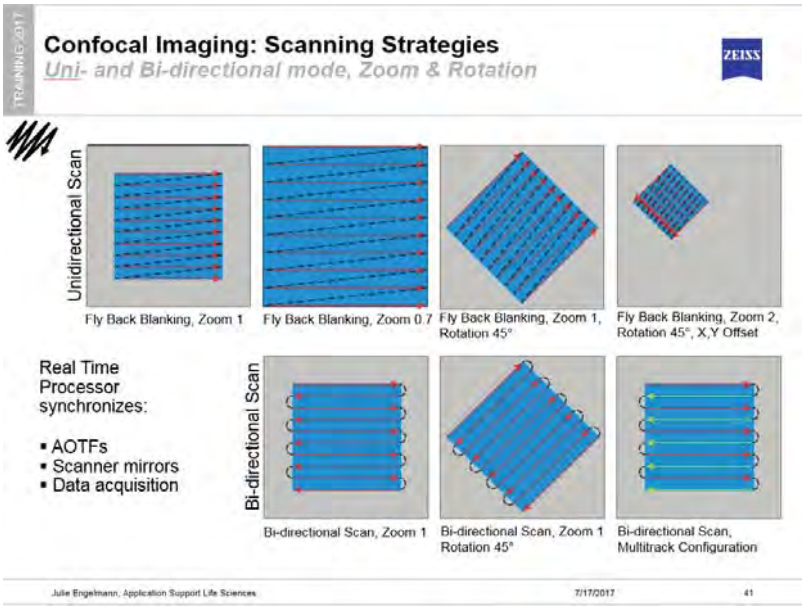


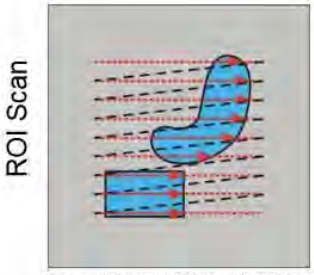

*Fig. 7 Geometric-optical (a) and wave-optical confocality (c) [XZ view].*
*The pinhole diameter decreases from (a) to (c).*
*Accordingly, $PSF_{det}$ (green) shrinks until it approaches the order of magnitude of $PSF_{ill}$ (red).*



# Scanning system and scanning strategies:




Selective excitation, bleaching, activation , uncaging , and data acquisition from user defined ROIs

With a motorized scanning stage, single XY frames can be tiled together for an overview image that exceeds a single field of view

# More scanning strategies:

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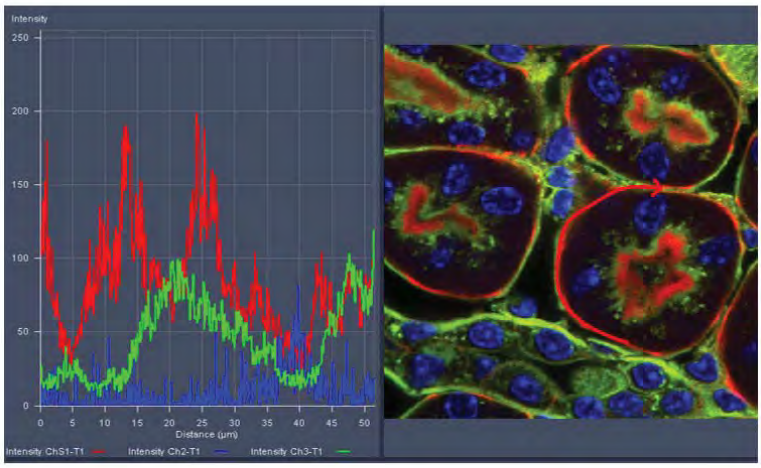
## Confocal Imaging: Scanning Strategies

*Regions of Interest (ROI), Line Scan, Spline Scan, Tile Scan*




Spline Scan



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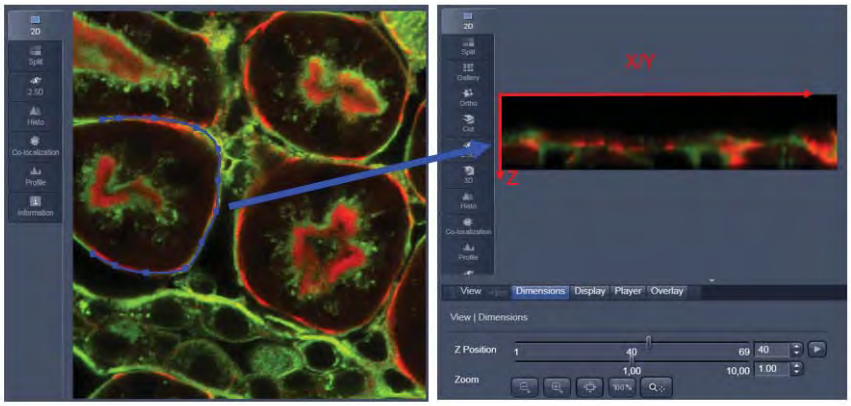
Confocal Imaging: Scanning Strategies

*Regions of Interest (ROI), Line Scan, Spline Scan, Tile Scan*




Spline Scan in Z

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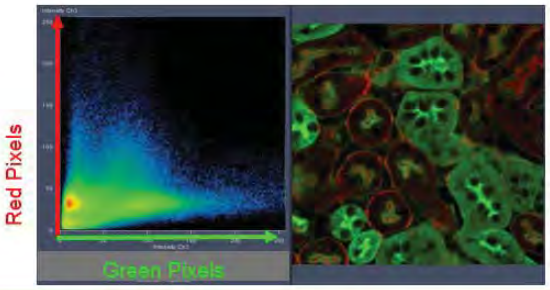
## Bleedthrough or Colocalization?

*Scatterplots as versatile tools*

Image of a red, green double labeled specimen (mouse kidney), acquired in Sequential Mode

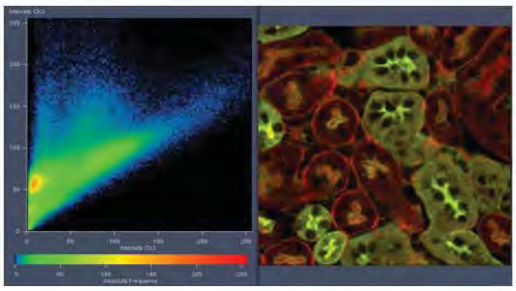
- Besides the background, three pixel populations can be seen
  - Only green,
  - Only red
  - Double labeled.




Same specimen, acquired in Simultaneous Mode

Notice the strong, diagonally shifted "green cloud" implying strong colocalization of green and red labeled structures.

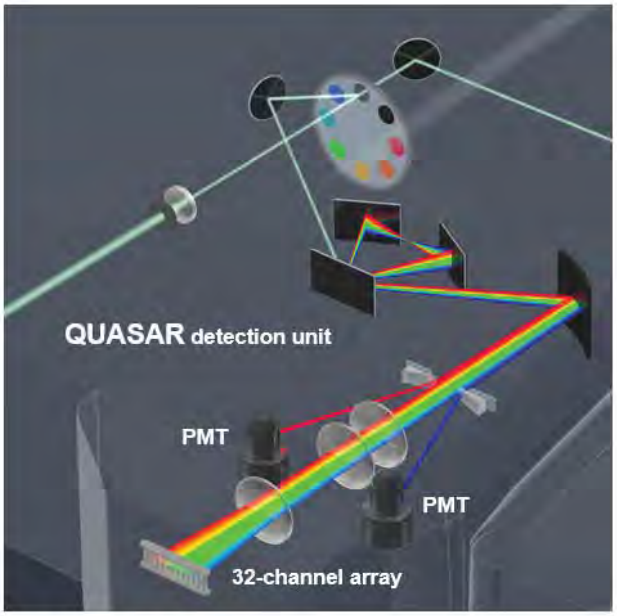
Top image reveals this apparent colocalization is **bleed-through** of green photons into the red detection channel.



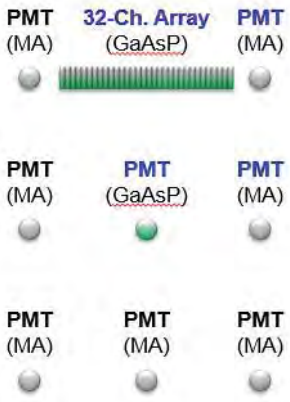


TRAINING 2017

## LSM 880 QUASAR Detector Configurations



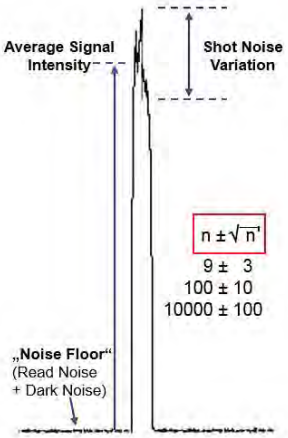

**Configurations of the QUASAR detection unit:**




Blue = Cooled

TRAINING 2017

## Scanning Strategies
*Speed and Averaging*




**Shot noise = √Signal**

→ Shot noise will increase with signal intensity,
BUT Signal / Shot noise ratio will also improve

$$SNR = \frac{Signal}{\sqrt{Signal}}$$

→ say 9 photons fall within a pixel during a given pixel dwell time; from pixel to pixel the Shot noise uncertainty is 3

$$SNR = \frac{9}{\sqrt{9}} = 3$$

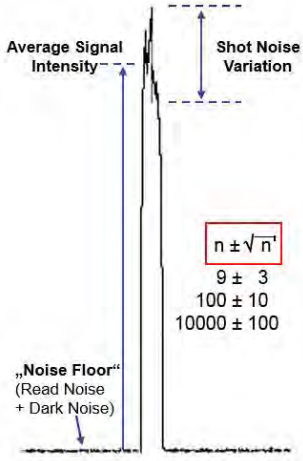
→ significant improvement of SNR if 100 photons fall within a pixel

$$SNR = \frac{100}{\sqrt{100}} = 10$$



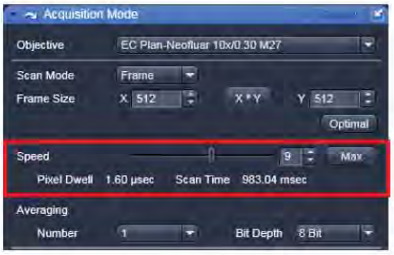
TRAINING 2017

## Scanning Strategies
*Speed and Averaging*




**Scan Speed influences SNR**

- The lower the pixel dwell time (the faster the scan), the less photons that can be collected.
- The Image becomes noisy due to **shot noise.**




- Detector Read Out: **40 MHz** (40 events / µsec) = Oversampling