# Sneak Preview - ZEN Blue 3.1

# What's new in ZEN Blue?

## Sneak Preview

## ZEN 3.1 (blue edition)

## (Selected Features)

# New Features & Functions

**Take home messages & General Overview**

# Take Home Message:
# Highlights & Selected New Features ZEN 3.1 blue edition

**Highlights & Selected New features:**

- **ZEN Guided Acquisition module:** Allows increased experimental efficiency by combining OOI detection (previewing) and analysis to “guide” image data collection in a targeted manner. E.g. combine the speed and flexibility of wide field whilst scrutinizing detected objects with powerful LSM imaging techniques in a highly efficient manner!

- **ZEN Direct processing module**: Launched with ZEN 2.6, allows parallel processing **Airyscan or DCV** images during **acquisition.** In **ZEN 3.1** we have implemented a true serve like behavior for the processing PC. With discovery proxy. DCV can now be run with adjustable settings, and Airyscan settings can be adjusted per channel

- **ZEN Connect:** New module and product structure launched with 3.1. The new Connect allows S&F to be replaced. Viewing multi-resolution 3D data from projects in combination with 3dXL

# Take home message:
# Selected new features ZEN 3.1 blue edition

**Selected New features:**

- **ZEN Intellesis:** 3 new deep neural networks for segmentation are available in this release. Due to their size a direct download into ZEN blue Image Analysis as out-of-the-box functions is possible!

- **Image Analysis improvements:** As part of our process of continual improvements your now able to create heatmaps from multi-well data sets and create plots (Histo /scatter) for time series data

- **AxioScan.Z1**: Now supported with the latest ZEN version! Support of new light sources!

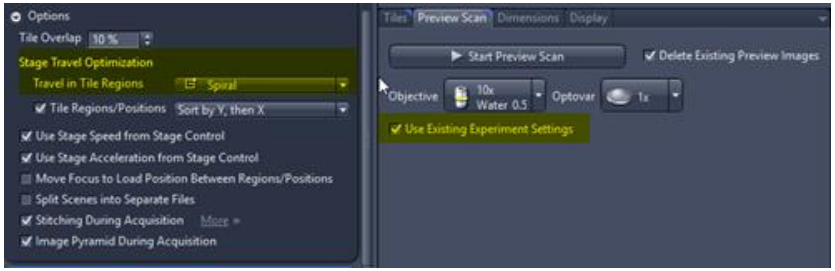
- **Celldiscoverer with LSM 900**: True and unique support of “Mixed-mode” channel imaging allows highly accurate on the fly combination of widefield and LSM 900 during imaging experiments.

- **ZEN Module Automated Photomanipulation**: Exclusive for the CD7 with LSM 900 – perform photomanipulation experiments at in multi-positions using a predefined IA setting to detect the OOIs!

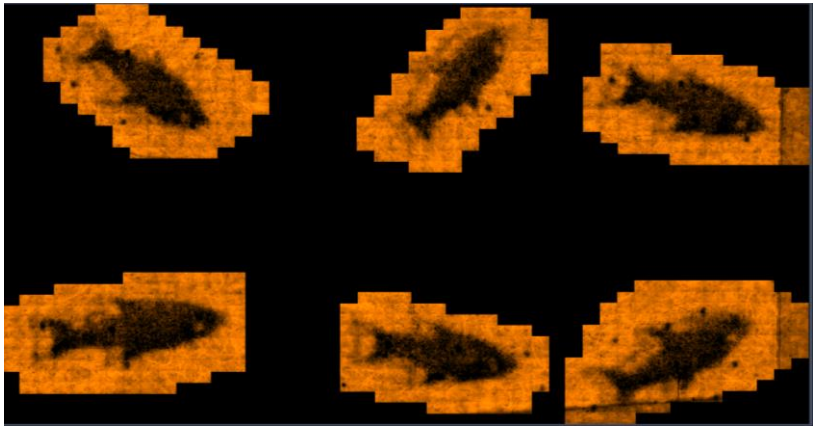
# Take home message:
# Selected new features ZEN 3.1 blue edition

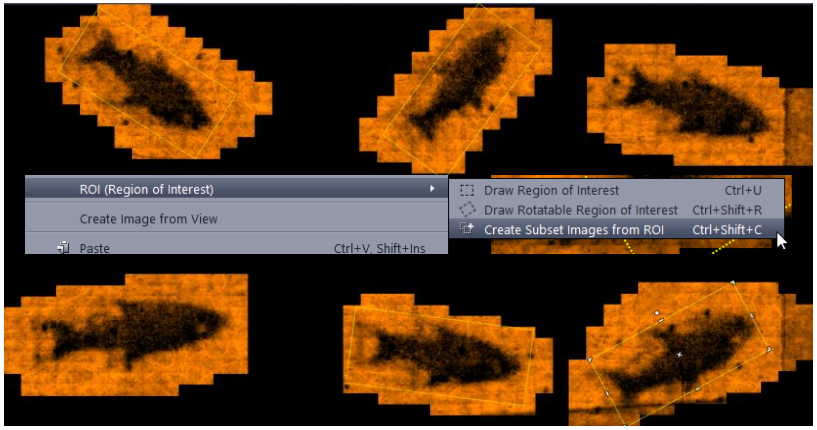
**Small bite size features**:  small, but not trivial for some:

- Create an image analysis setting from an analysed image („reproducibility“)
- Image analysis results: export charts (Scatterplot, Histogram, Heatmap)
- Batch processing : The IP function **Stitching** now allows you to assign a **reference image** for shading correction via a file path in the **Batch** interface!
- Workflow: Image rotation and cropping enhancements (**see next slide**)
- Tiles and Positions: Default stage motion for preview scans: The stage motion used for preview scans can be set in the Tiles tool options section. There is no mis-match between the preview and experiments or no more curious automatic selection for preview scans!



# Workflow: Image Rotation and crop enhancements



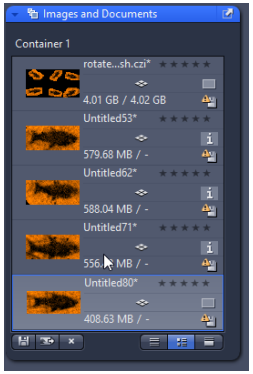


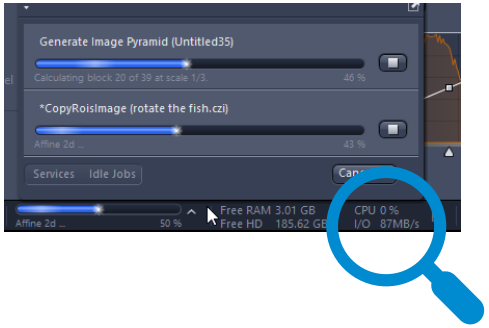

It is now possible to crop multiple areas from a multi-scene image with a new rotatable ROI.

This can be helpful if you need to align objects prior to export for creation of a figure.

Use multi-selection to crop all the desired ROIs in one go

Example here: 4 ROI @ 0.5 GB each on my Laptop
(Core i7 with 16GB RA, Win10) took ca. 2 min to process



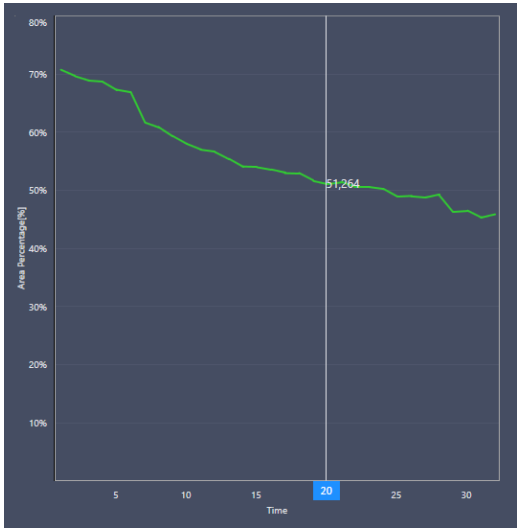




BTW – notice the new I/O indicator that gives you an indication of throughput on processor dependent tasks

# ZEN Module Image Analysis: Plotting

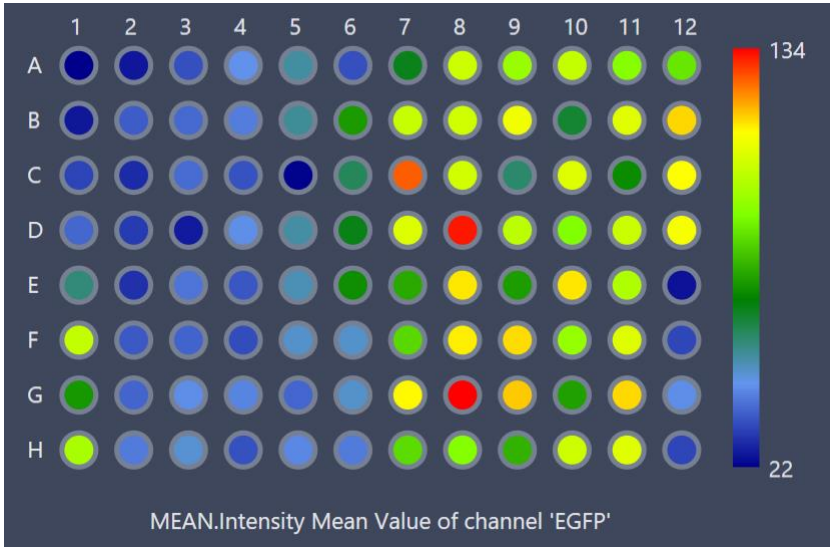
**What's new:**

ZEN blue now also supports plotting of time series and heatmaps so customers can directly visualize their image analysis results in ZEN.




**Nice & Easy:**

Show an image analysis of e.g. multi-well plates and plot the results as a heatmap. Hover over the single wells to show the respective measurement value.




**What you might notice:**

It is now also possible to export all charts, Including histogram, scatterplot, time series and heatmaps for use in publications and Powerpoints.
Furthermore, it is now possible to generate a scatterplot/histogram by selecting multiple wells on the sample carrier tab.

# ZEN Module “Guided Acquisition”

**What's new:**

Guided acquisition is now available as a separate ZEN module which provides significant time savings via automated and targeted acquisition of regions of interest. The sample can be imaged first with a low magnification objective, the relevant objects are then identified via automated image analysis and these objects are subsequently reimaged, e.g. with higher resolution. This module allows also for a combination of widefield overview images and LSM detailed scans.

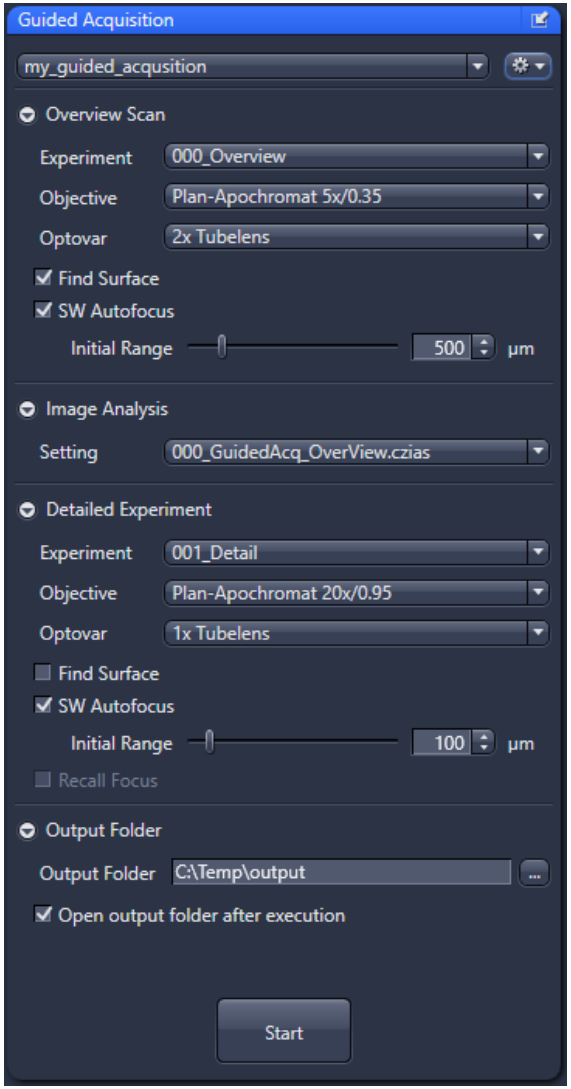
**Nice & Easy:**

Show how the guided acquisition workflow allows to first get a coarse overview image of the sample with low magnification and then detect objects of interest (e.g. mitotic cells) and image these objects at higher magnification.

**What you might notice:**

In order to show the complete guided acquisition workflow is necessary to set up a suitable image processing setting first using an image that was acquired with the experiment setting for the overview image.

# Guided Acquisition – Find objects of interest

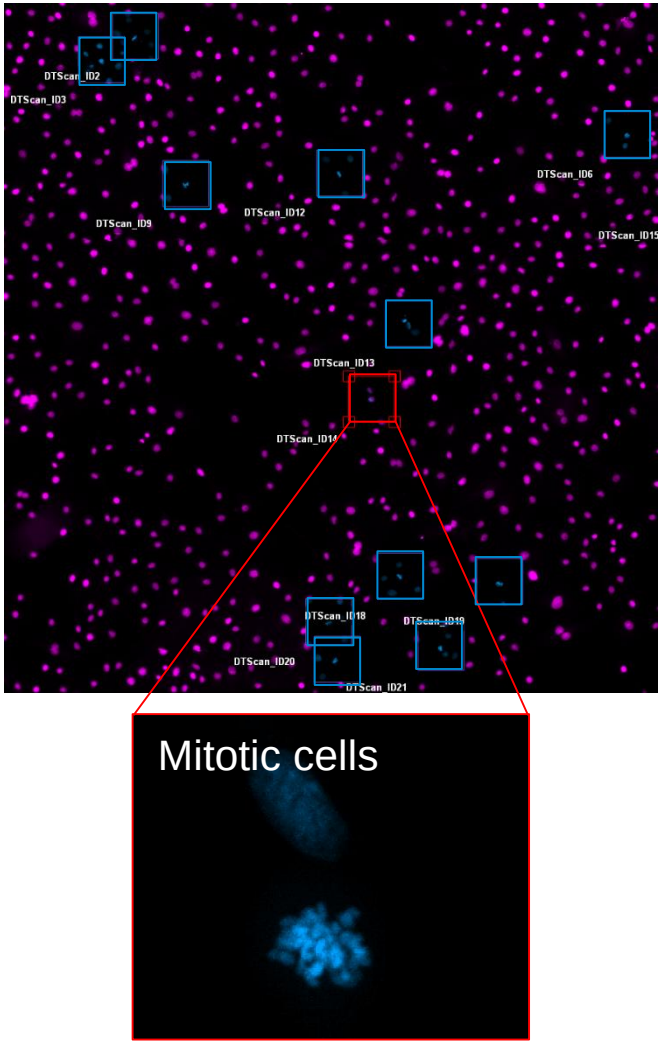



**Overview Scan**
(e.g. low-res widefield)

**Image Analysis**
(e.g. find mitotic cells)

**Detailed Scan**
(e.g. high-res confocal)

Overview



# ZEN Module “Direct Processing”

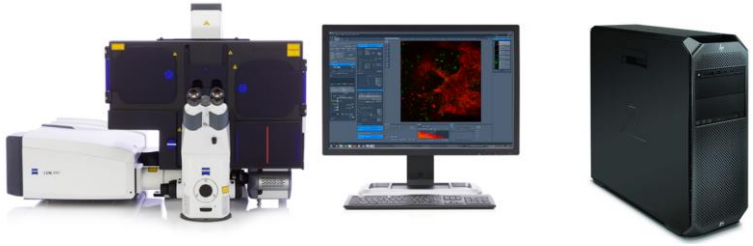
**What's new:**
Processing PC now can act as true server in a network which provides more robust communication and allows better integration into existing networks. Processing PC can now describe its features so that users can choose the best option for their needs.
Deconvolution (adjustable) is now supported in Direct Processing.
Airyscan processing now allows channel specific strength settings.

**Nice & Easy :**
Setting up the processing PC to act as server, add PC hardware information and letting it act as discovery proxy. Then on the acquisition PC using the Check connection function will show up with detailed information about the processing PC.
Perform direct processing with a setting for adjustable deconvolution, e.g. w/ spherical aberration correction.
Demonstrate how to do Airyscan processing via Direct Processing with channel specific strength settings.

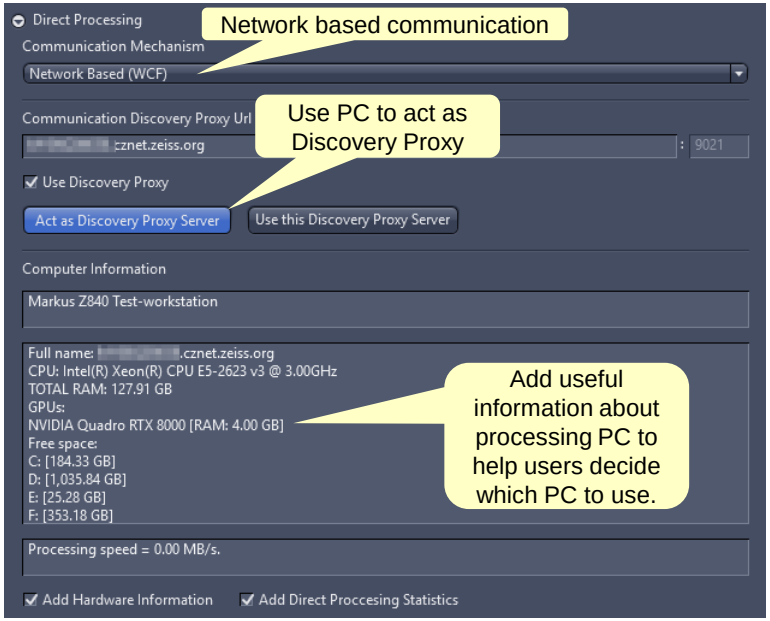
**What you might notice:**
While now truly powerful and modern, the networking setup can still be somewhat intimidating. Keep in mind, whom you demonstrate this to. System admins or facility managers will love you for this, normal users might get a bit square eyed.
Some deconvolution parameters such as spherical aberration correction are very image specific. Using one setting for all images might not in all cases get you the very best results, but still much better than not using SA correction at all.

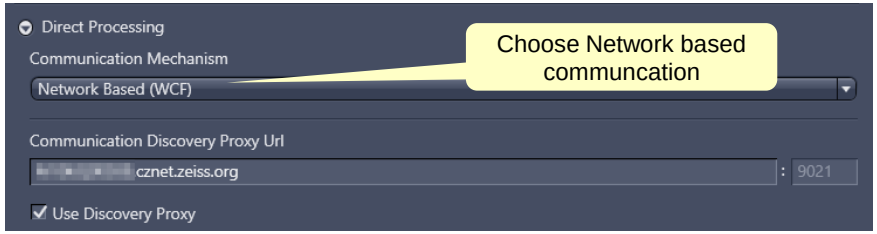
# Direct Processing - Check connection function

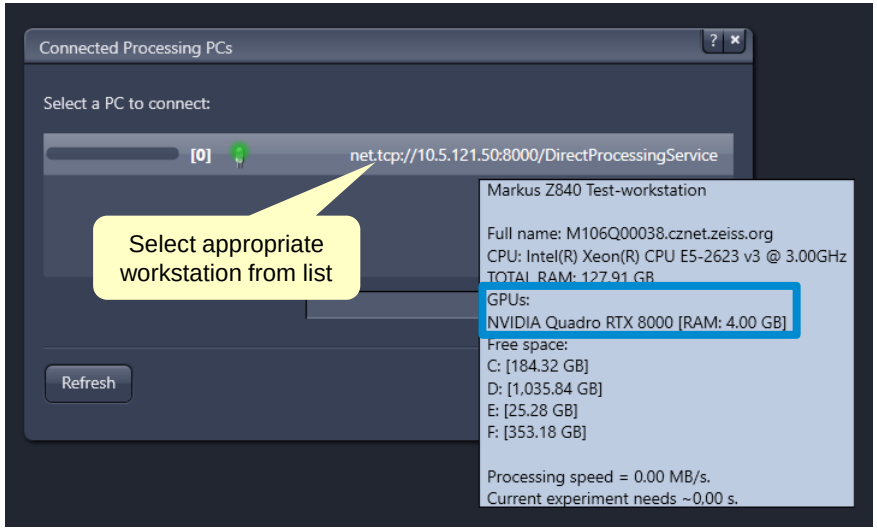
## Acquisition Workstation



## Processing Workstation

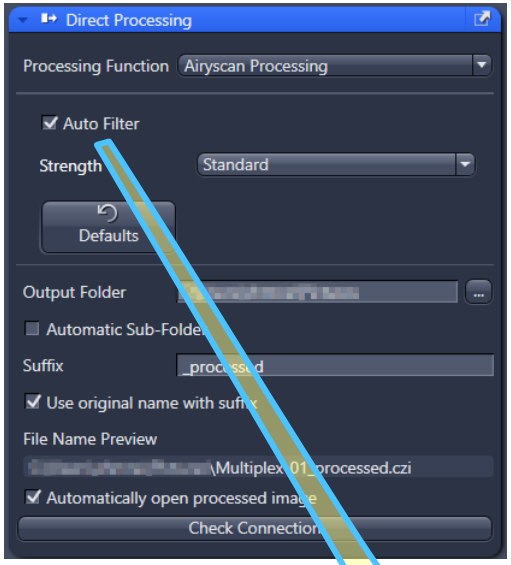


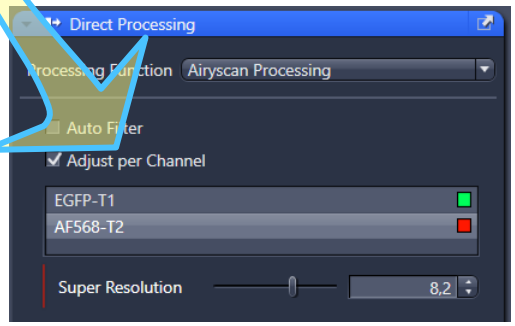



# Direct Processing – Extended Functionality

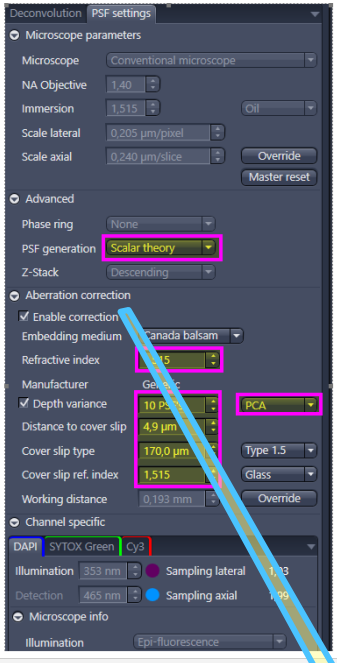
## Airyscan Processing



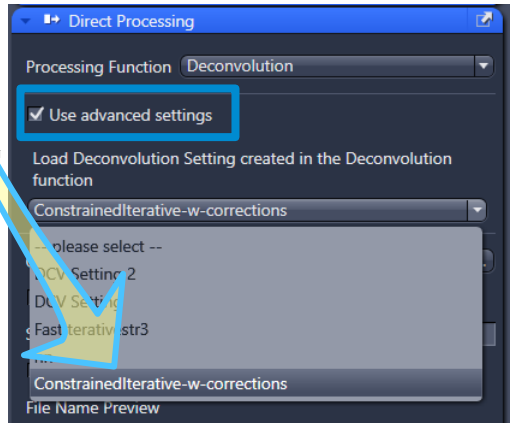




Channel specific processing

## Deconvolution




Use custom deconvolution settings in direct processing!



# ZEN Module 3Dxl

**What's new:**

3D View can now be used already during acquisition.
Bridge between ZEN and arivis Vision4D has been improved, select a 3D analysis pipeline in ZEN so that you can work immediately on the analysis in Vision4D.
A new 3D View has been created for ZEN Connect.

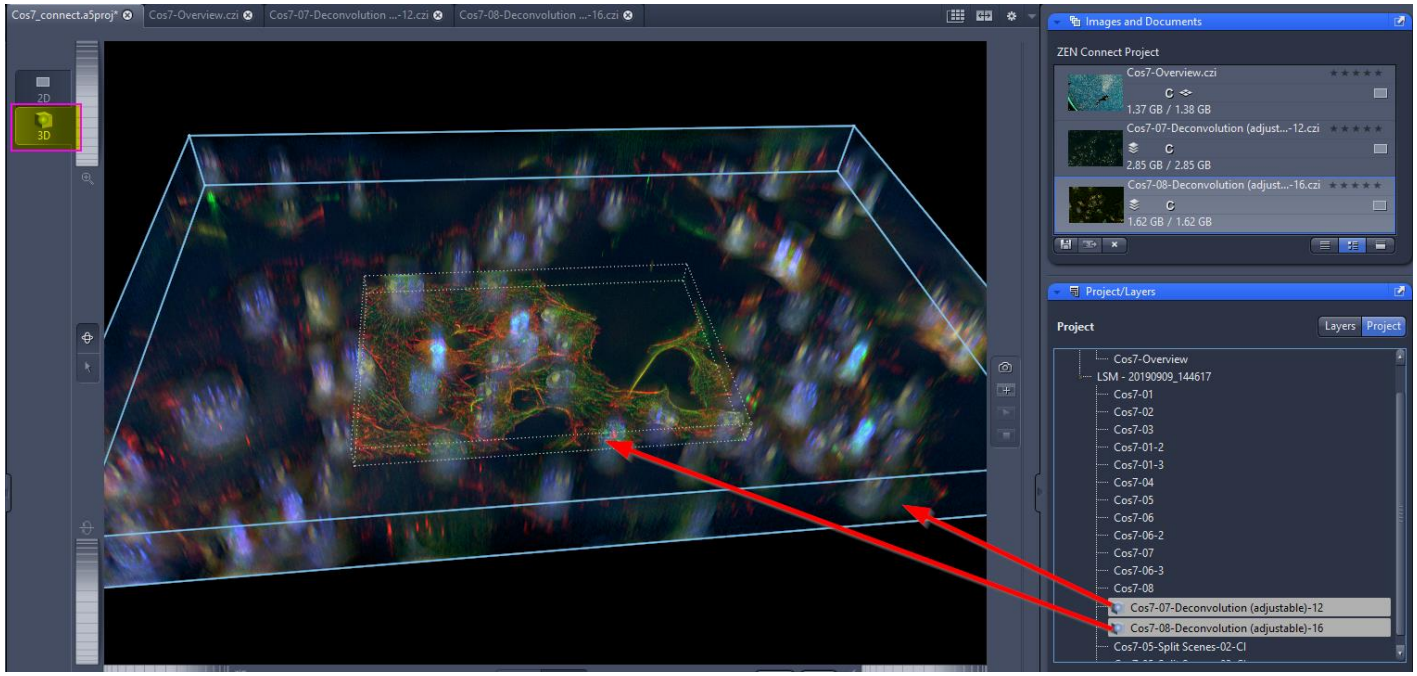
**Nice & Easy:**

Acquire a Z-stack / time series and switch to 3D View during acquisition.
Select an interesting 3D image such as one with vesicles, select a 3D analysis pipeline and use the bridge function to Vision4D.
You can now display two volumes of different resolutions directly in ZEN connect.

**What you might notice:**

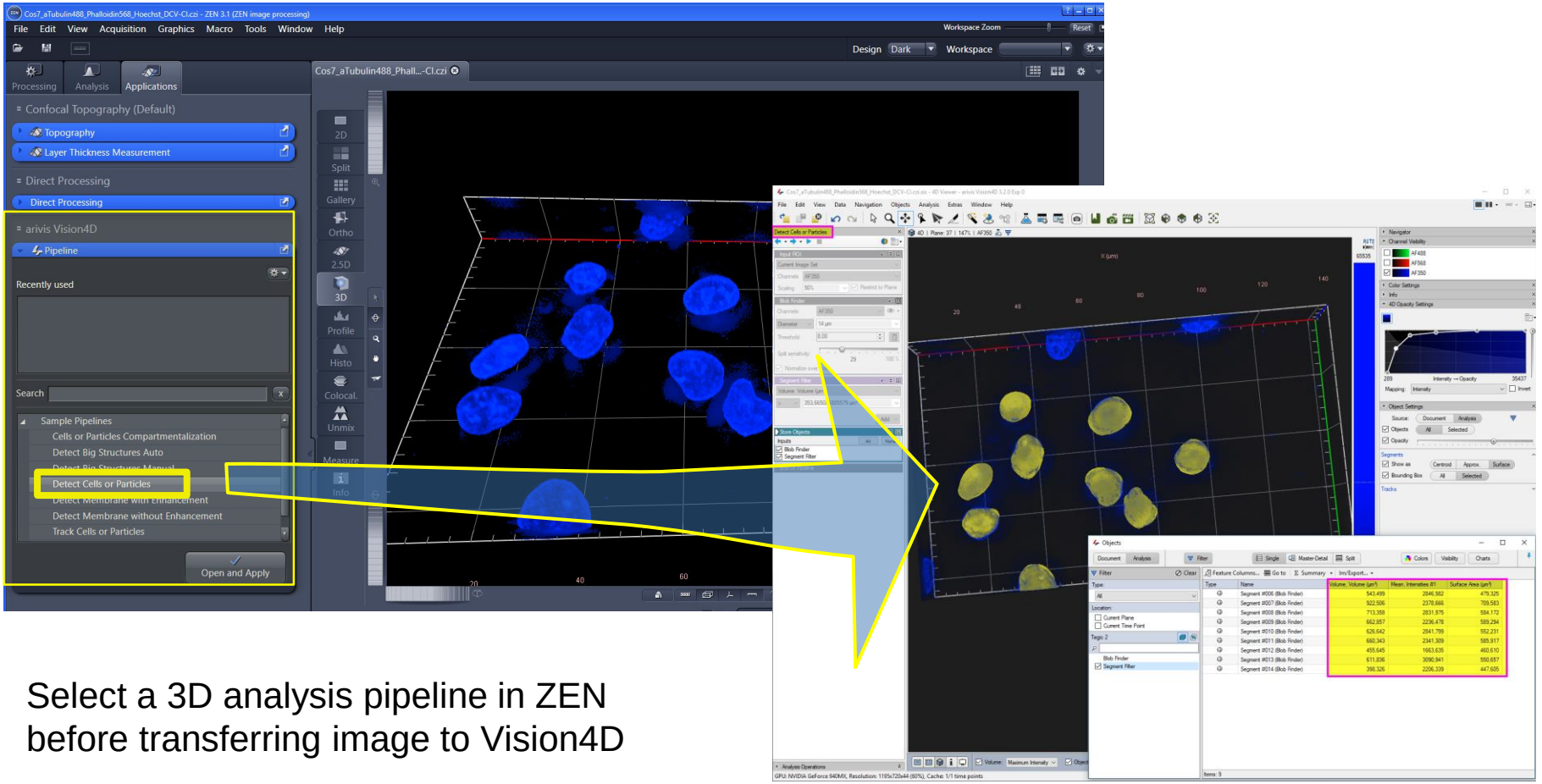
For the bridge to work you'll need a separate arivis Vision4D dongle. Be prepared before you try to show that or get arivis personnel involved to help with the demo.
3D View in ZEN Connect is a very first step only. We are working hard on creating a better usability experience possible with our upcoming new releases.

# 3D View in ZEN Connect
# → getting ready for correlative 3D work




**Multi-resolution 3D Viewing** of 2 volumes with different resolution

# Bridge ZEN → Vision4D




Select a 3D analysis pipeline in ZEN
before transferring image to Vision4D

# ZEN Connect:
# New module and product structure

**What's new:**

- The **ZEN Connect Entry** is now included also for ZEN lite and allows to open ZEN Connect projects also in the free version

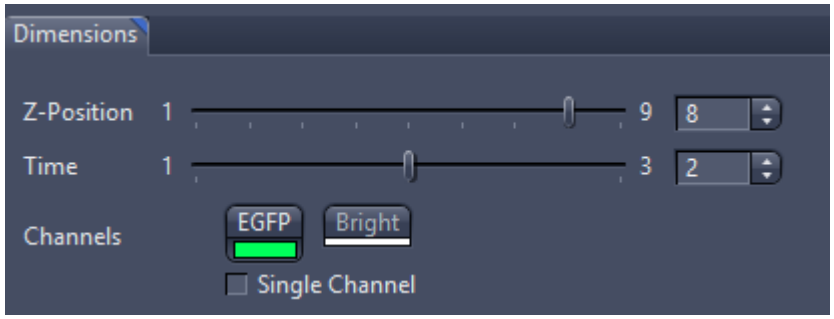
- The **ZEN Connect Advanced** module has been renamed to ZEN Connect

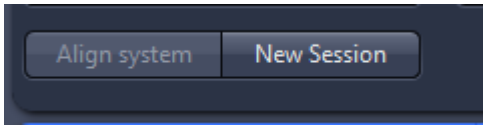
- The new module **ZEN Connect 2D** provides S&F functionality and will replace Shuttle&Find for ZEN blue

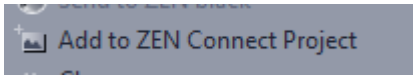
# ZEN Connect:
# Improvements to ZEN Connect

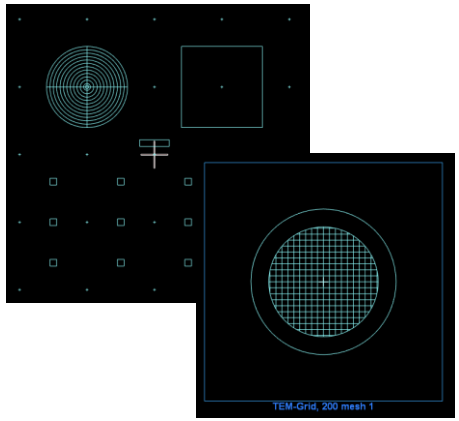
**What's new:**

- Direct control of display settings from ZEN Connect
- New shortcut to align the current system
- New function to add images opened in ZEN directly to a ZEN Connect project
- **New ZEN Connect holder for TEM grids and Argolight slides**
- Export to SerialEM format



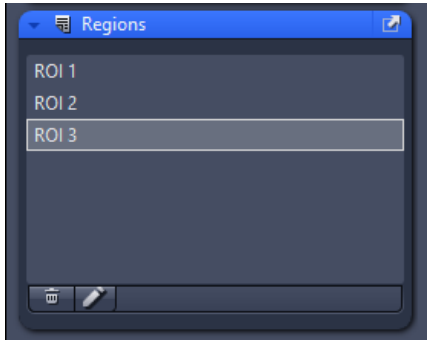



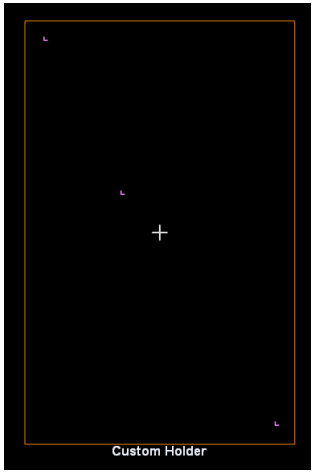



# ZEN Connect:
# ZEN Connect 2D Add-on

**What's new:**

- It is possible to define Regions of interest in ZEN Connect
- Custom correlative holders can be defined and used in ZEN Connect
- The standard holder calibration is available with ZEN Connect 2D Add-on
- The ZEN Connect 2D Add-on replaces Shuttle&Find for ZEN blue
- (No replacement for existing customers who will keep their S&F module)





# ZEN Module “Intellesis Trainable Segmentation”

**What's new:**

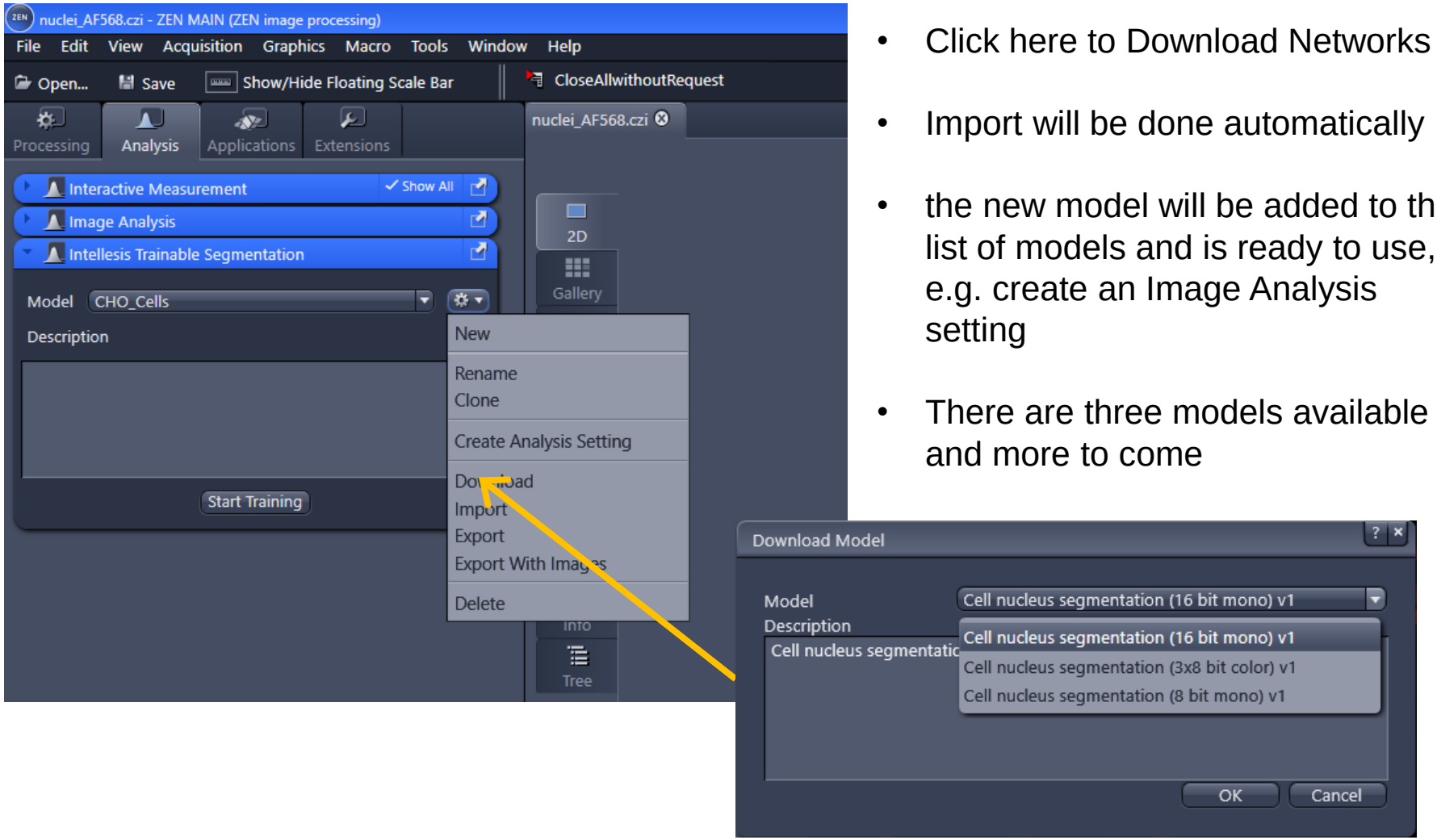
Option to use deep neural networks for segmentation. As a first application example there will be 3 different pre-trained networks to detect cell nuclei.

Other improvements are the **visualization of the brush cursor**, more **binary post-processing options** for Image Analysis settings crested directly from a trained model and an **extended python scripting interface** to automate various workflows regarding Intellesis incl. an easy way to compare feature extractors.

**Nice & Easy:**

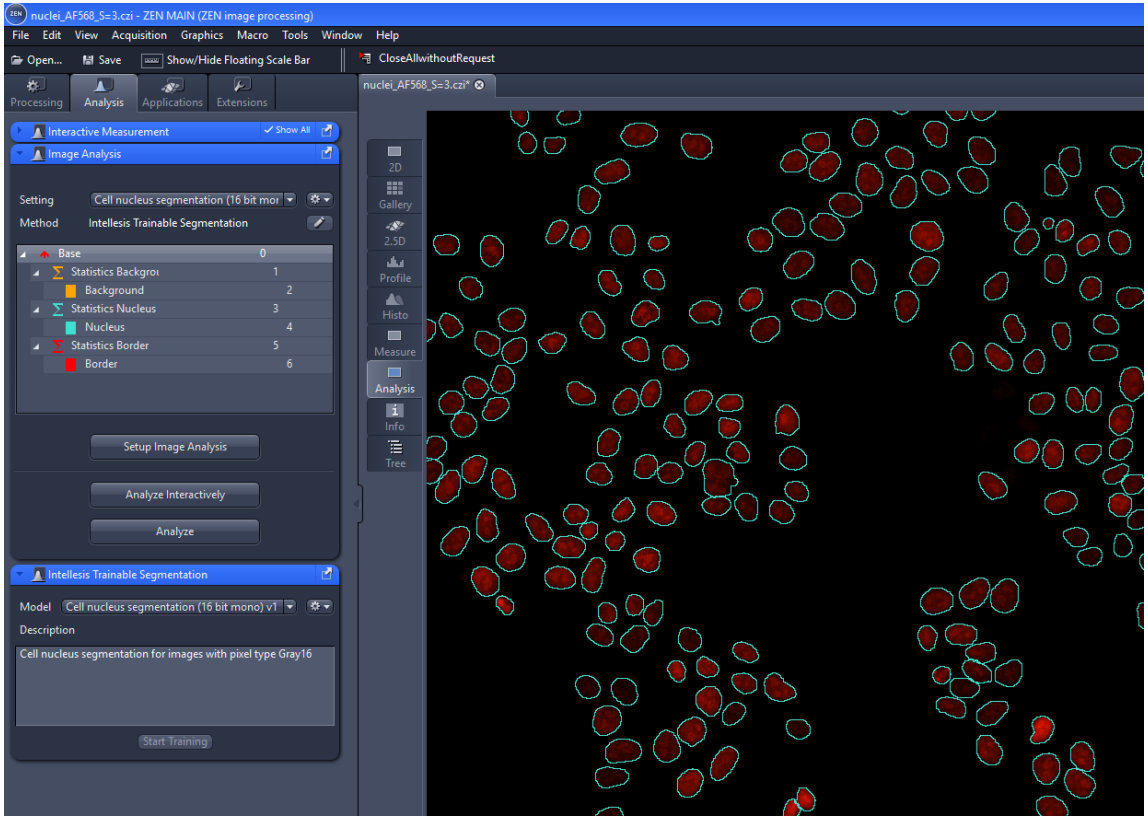
- Connect the system to the internet and download n network to detect nuclei. Open an single channel image with cell nuclei stained with DAPI, Hoechst, … and use the pre-trained nucleus detector to segment the cell nuclei without any training.
- Create an Image Analysis Setting using a multi-channel image, where one channel contains cell nuclei and use the pre-trained network to segment use via Class Segmentation.
- Start script for Feature Comparison and demonstrate the results for 2 different feature extractors using the MultiView.

# ZEN Intellesis – Download networks



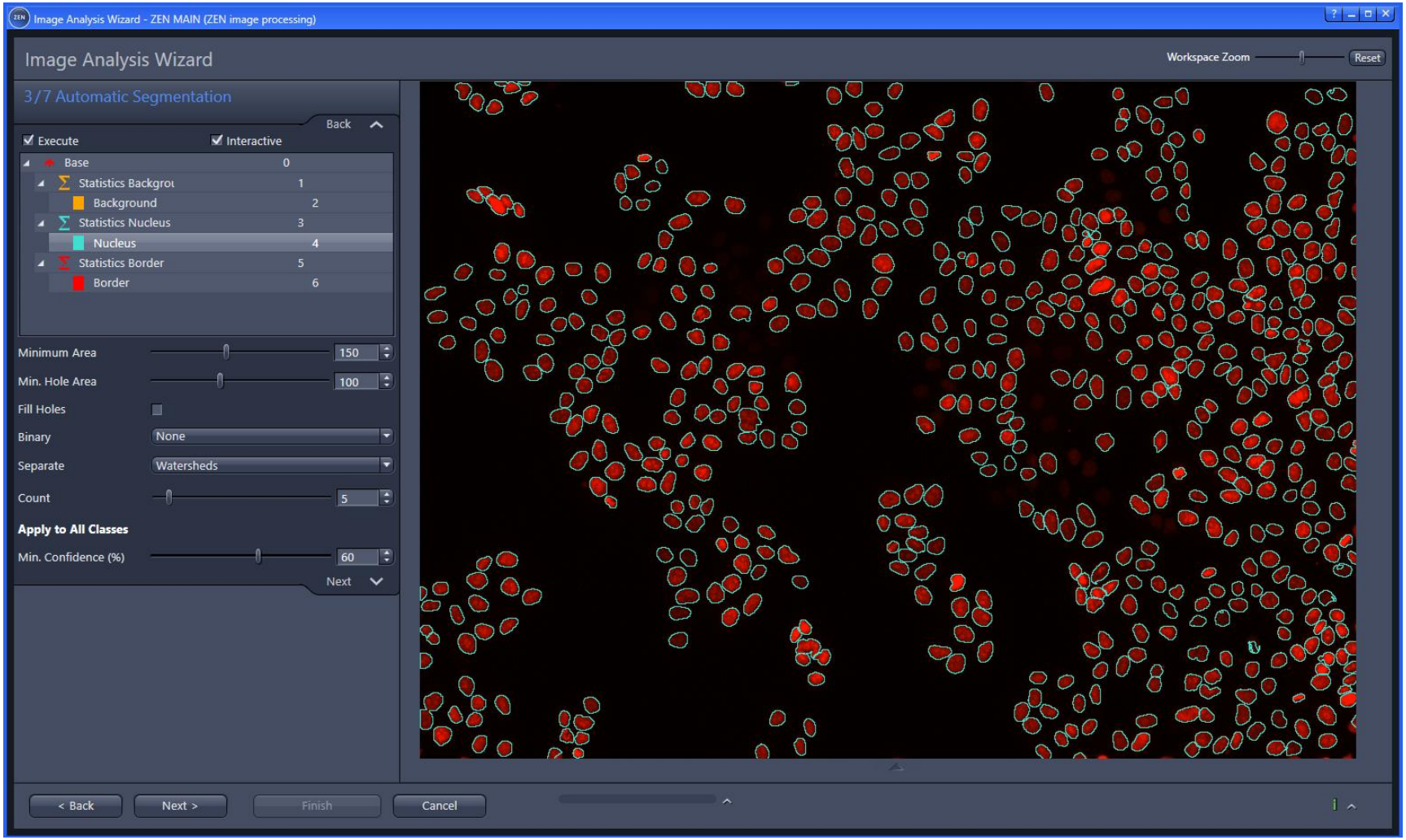

- Click here to Download Networks
- Import will be done automatically
- the new model will be added to the list of models and is ready to use, e.g. create an Image Analysis setting
- There are three models available and more to come

# ZEN Intellesis – Use trained network for Image Analysis

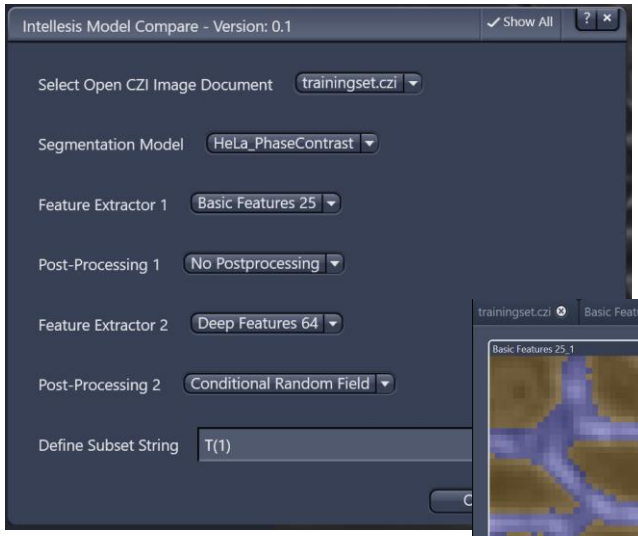



- Network can be “converted” to a fully functional Image Analysis Pipeline by one single click
- Such settings will now also have the advanced binary postprocessing options
- Confidence Threshold is also available
- There are three models available and more to come

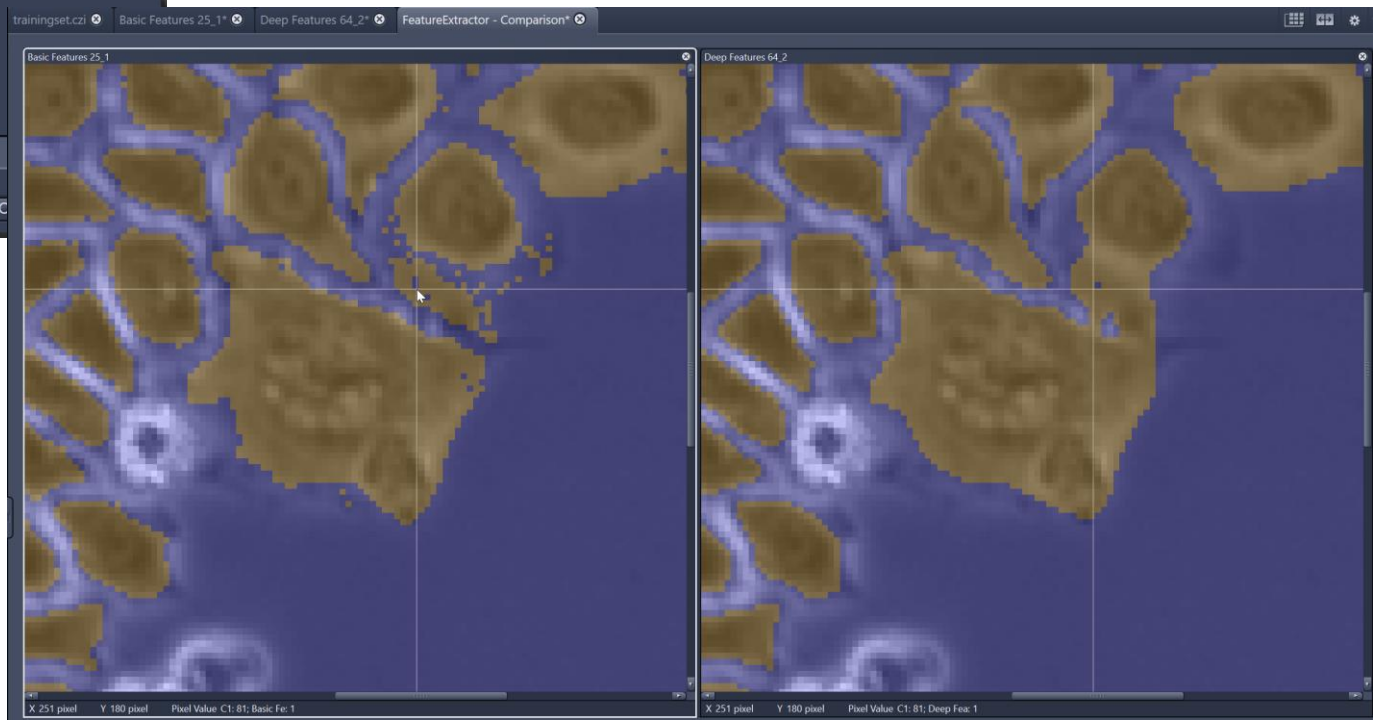
# ZEN Intellesis – Download networks



# ZEN Intellesis – Extended Scripting Interface




- Retrain model using different feature extractors from script
- Toggle postprocessing options
- Automatically display result side-by-side



# Take home message: Intellesis
# Selected new features ZEN 3.1 blue edition

**ZEN Intellesis:** Now supports using trained Deep Neural Networks (DNNs) for Segmentation.

- Such networks can be downloaded  are will be not shipped with the DVD → can be updated easily, when a new application example becomes available
- The 1st example will be a Nucleus Detector, that can be used directly without any additional training (works best on fluorescence images).
- The nucleus Detector is based on the “famous” U-Net architecture
- More applications example will come (Confluency Measurements, Simple Cell Counting, Segmenting Cell Compartments inside BF image) depending on the need and the availability of taring data and labels

**Small bite size features**:

- More advanced binary post-processing option for IA settings created directly from a trained model.
- Brush cursor inside Intellesis Training UI is now visualized.
- Greatly extended python scripting inferface for Intellesis related functions, allowing to automate more aspects of working with models and using them → Example: Compare Feature Extractors

# New Features & Functions

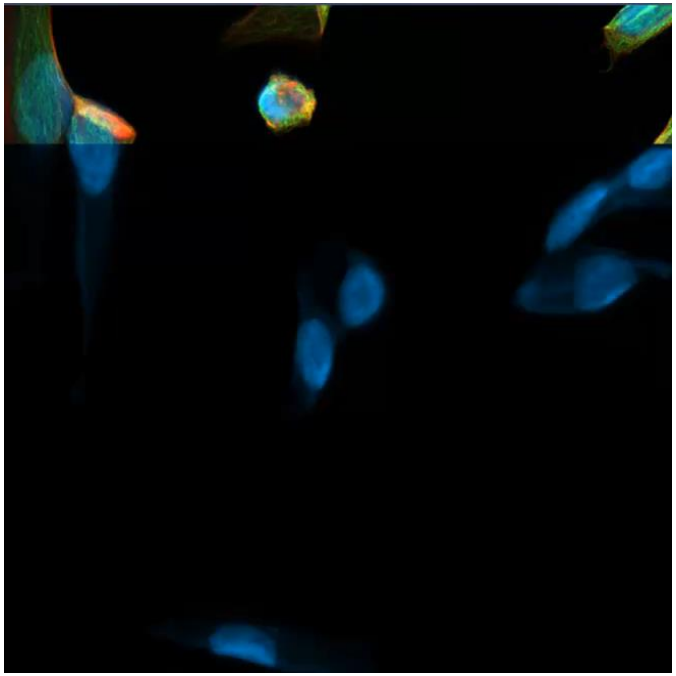
**Celldiscoverer 7 with LSM 900  in ZEN 3.1**

# Celldiscoverer 7 with LSM 900: MIXED Mode Acquisition for Seamless Combination of Camera and LSM

The Celldiscoverer 7 allows for combined acquisition of camera and LSM tracks. The unique **Mixed Mode Acquisition** ensures the precise overlay of the widefield and confocal/Airyscan images.

**Features**

- Overview image (Prescan image), **plus**:
- Combined multichannel acquisition of camera tracks and LSM tracks (Widefield, LSM confocal, Airyscan HS and Airyscan MPLX)
- One frame including all **aligned** channels for seamless analysis workflows



**Application benefits**

- Sample navigation, **plus**:
- Gentle and fast multichannel acquisition
  - Widefield channel for localization purpose
  - Confocal / Airyscan channels for high resolution / superresolution imaging
- Precise fusion and combination of widefield, confocal & Airyscan workflows

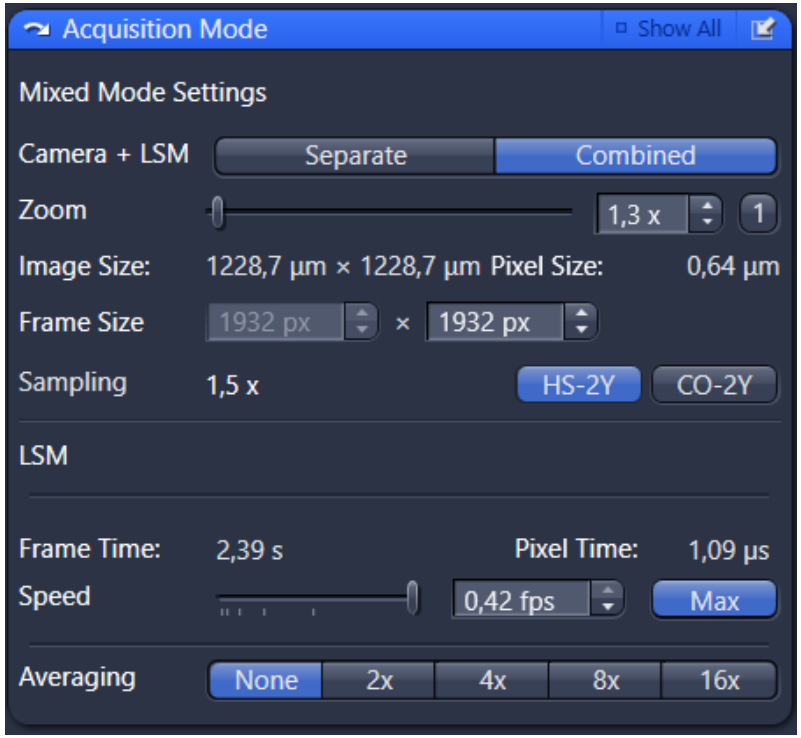
# Celldiscoverer 7 with LSM 900:
# Mixed mode acquisition for widefield and MPLX tracks

**What's new:**

The Mixed mode acquisition was **improved** to enable the **combination of Widefield and Airyscan MPLX tracks for fast image acquisition**

The following tracks can be combined:

- Widefield + LSM confocal
- Widefield + LSM confocal + Airyscan HS
- Widefield + Airyscan HS
- **Widefield + Airyscan MPLX HS**



# Celldiscoverer 7 with LSM 900:
# ZEN Module Automated Photomanipulation

**What's new:**

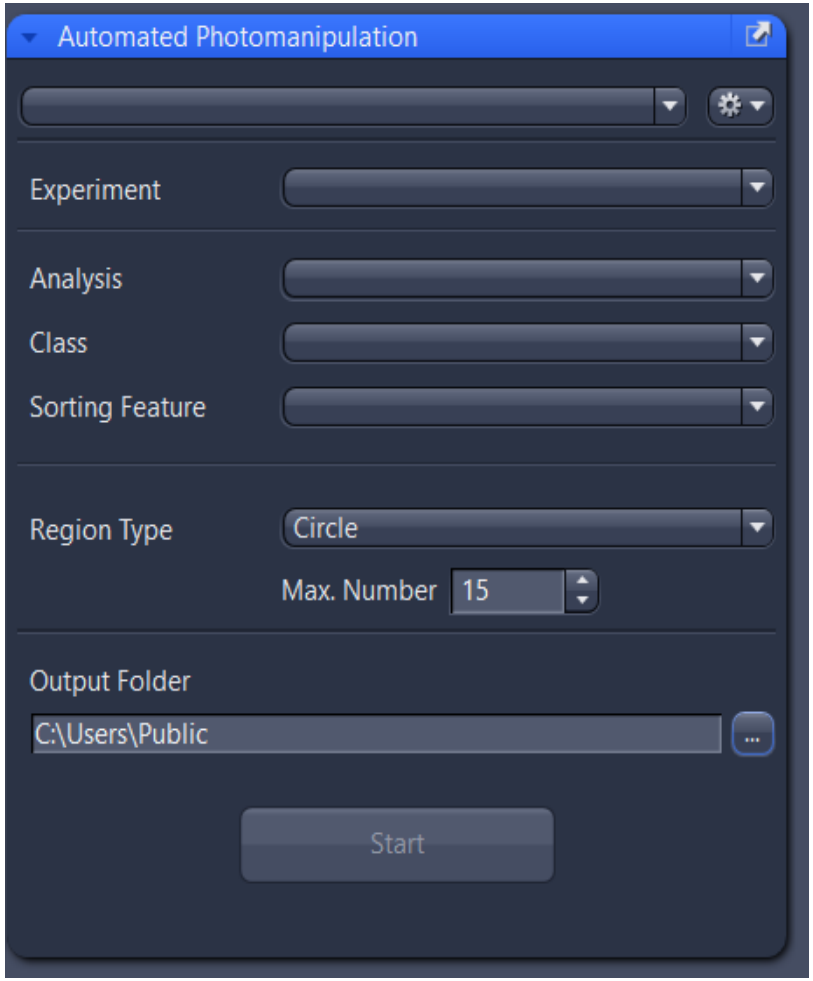
This **NEW** ZEN module is exclusively available for the Celldiscoverer 7 and allows for **automated photoactivation and bleaching at multiple positions**.

The photomanipulation can be executed at:

- All Tile Regions per Time Point (e.g. for photoactivation)
- Full Time Series per Tile Region (e.g. for photobleaching).

Using this module, the system is executing the following experiment steps automatically without user interaction:

1. Acquisition of a multiposition image
2. Identification of the photomanipulation ROIs based on a customized image analysis
3. Photomanipulation experiment



# Celldiscoverer 7 with LSM 900:
# ZEN Module Automated Photomanipulation

**Nice & Easy:**

For a successful Automated Photomanipulation experiment, you need to prepare a photomanipulation experiment at multiple positions and an image analysis setting:

- Activate the Automated Photomanipulation module under Tools > Modules Manager > Automated Photomanipulation.
- Create and save a suitable experiment for photomanipulation at multiple positions (including Tiles, Bleaching and Time Series).
  - Define the experiment positions in Navigation&Tiles
  - Define the photomanipulation settings in the Timed Bleaching Tool Window
- Create a suitable image analysis setting using the Image Analysis Wizard or an OAD macro that detects the regions of interest where the photomanipulation should be executed
  - **Important**: the classes in the analysis and their corresponding channel names must exactly fit to the channel names in the experiment

**What you might notice:**

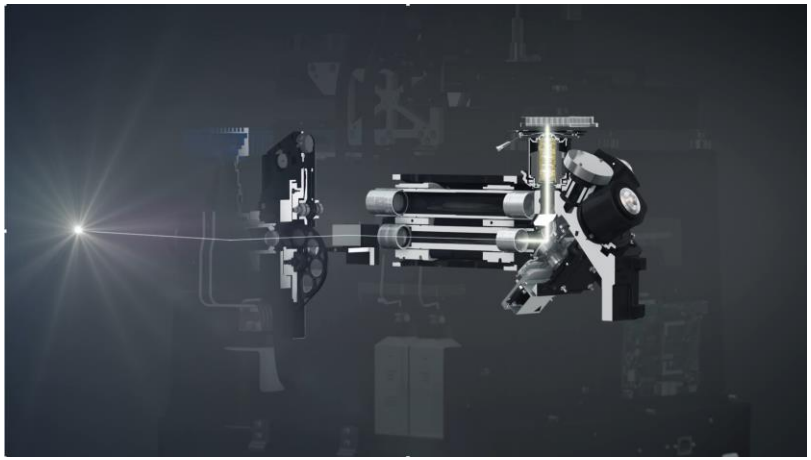
Automated Photomanipulation in Tile regions is not supported

Activate the module in Tools -> Modules Manager -> Optional Software

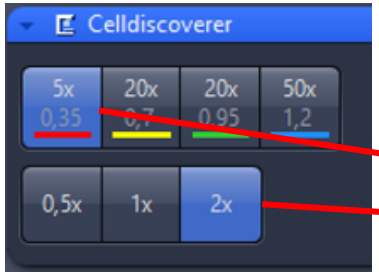
# Celldiscoverer 7 with LSM 900:
# Display of relative laser power using different magnifications

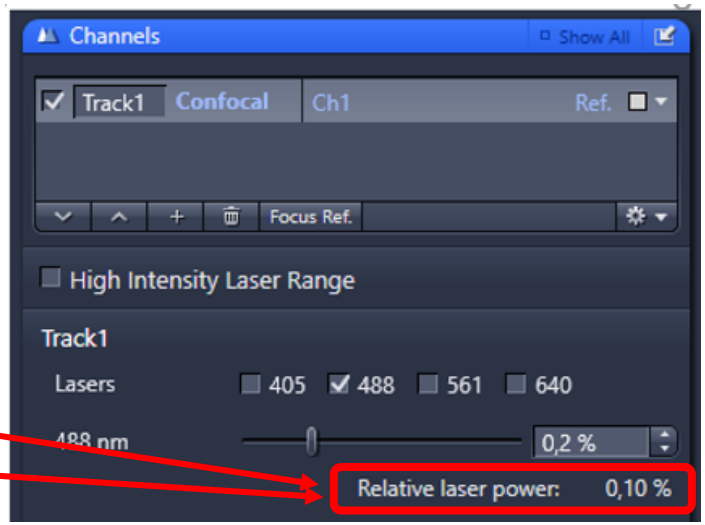
**What's new:**

- Using certain Objective/magnification changer combinations, only a portion of the laser light is applied to the sample due to the optical design of the system



- This information is now displayed in the Channels Tool Window as:
  - **relative laser power** (for LSM tracks)
  - **relative laser power in confocal mode** (for Airyscan MPLX tracks)



# Celldiscoverer 7 with LSM 900:
# Display of relative laser power using different magnifications

**Nice & Easy :** Use lower magnification and higher zoom

The laser power is calculated as:

- Relative laser power (%) = applicable laser power depending on objective/magnification changer * adjusted laser power (confocal tracks)
- Relative laser power in confocal mode (%) = applicable laser power depending on objective/magnification changer * equivalent laser power in confocal mode (Airyscan tracks)

**Applicable laser power (relative):**

| Objective / Magnification changer | 5x/0.35 | 20x/0.7 | 20x/0.95 | 50x/1.2 |
|---|---|---|---|---|
| 0.5x | 100% | 49% | 100% | 92% |
| 1x | 100% | 49% | 64% | 23% |
| 2x | 49% | 12% | 23% | 6% |

# Celldiscoverer 7 with LSM 900:
# Automatic image crop in experiments using more than one MBS

**What's new:**

- Beam splitter shifts in multi-MBS experiments are causing image shifts of the single channels
- The Celldiscoverer 7 is using the beam splitter calibration to correct for that shift and to calculate an exact image overlay
- The **images are** now **automatically cropped** **to improve the image display and to enable seamless analysis workflows**
- The resulting **cropped image** shows **no black boundaries**
- For experiments using image tiles, the tile overlap is adapted accordingly

# New Features & Functions

**Axio Scan.Z1 in ZEN 3.1**

# Introduction - Axio Scan.Z1 in ZEN 3.1

- ZEN slidescan is part of ZEN 3.1 release called ZEN 3.1 slidescan
- This is not a product care, thus only some minor changes / feature were done. This document will describe these.
- ZEN slidescan 3.1 will be released for Windows 10 and **not** for Windows 7
- Changes done in generic ZEN 3.1/3.0 affecting ZEN slidescan will also migrate to ZEN 3.1 slidescan as in most cases standard controls are used. These changes will not be described.
- The upgrade from every ZEN slidescan version to 3.1 is free of charge.
- You will get a separate information regarding the Z840 Windows upgrade with more specific information than mentioned in this document.

# New lamp released also for Axio Scan.Z1

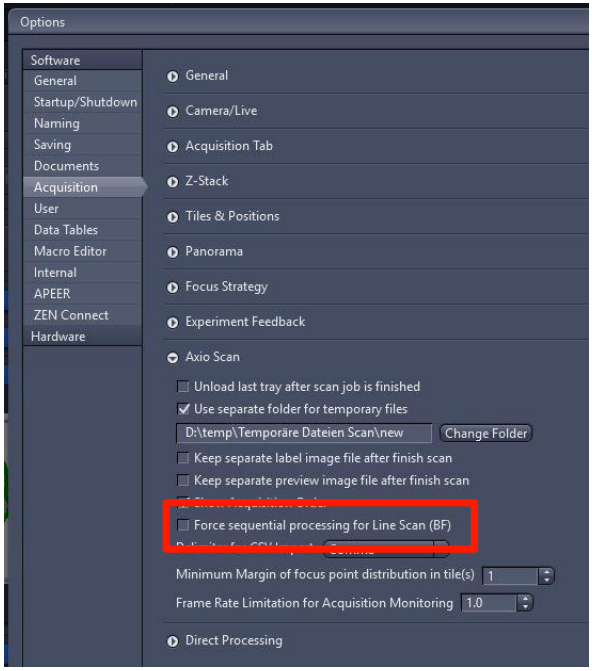
- The **X-Cite Xylis** lamp from Excelitas is now also released for Axio Scan.Z1 w/ the same functionality / limitations as seen with generic ZEN 3.0 / 3.1

- **X-Cite exacte** from Excelitas is now again released for Axio Scan.Z1 w/ the same functionality / limitations as seen with generic ZEN 3.0 / 3.1

- These lamps are **currently not in our price list** and needs to be ordered directly from Excelitas.

# New functions / improvements ZEN 3.1 Windows 10

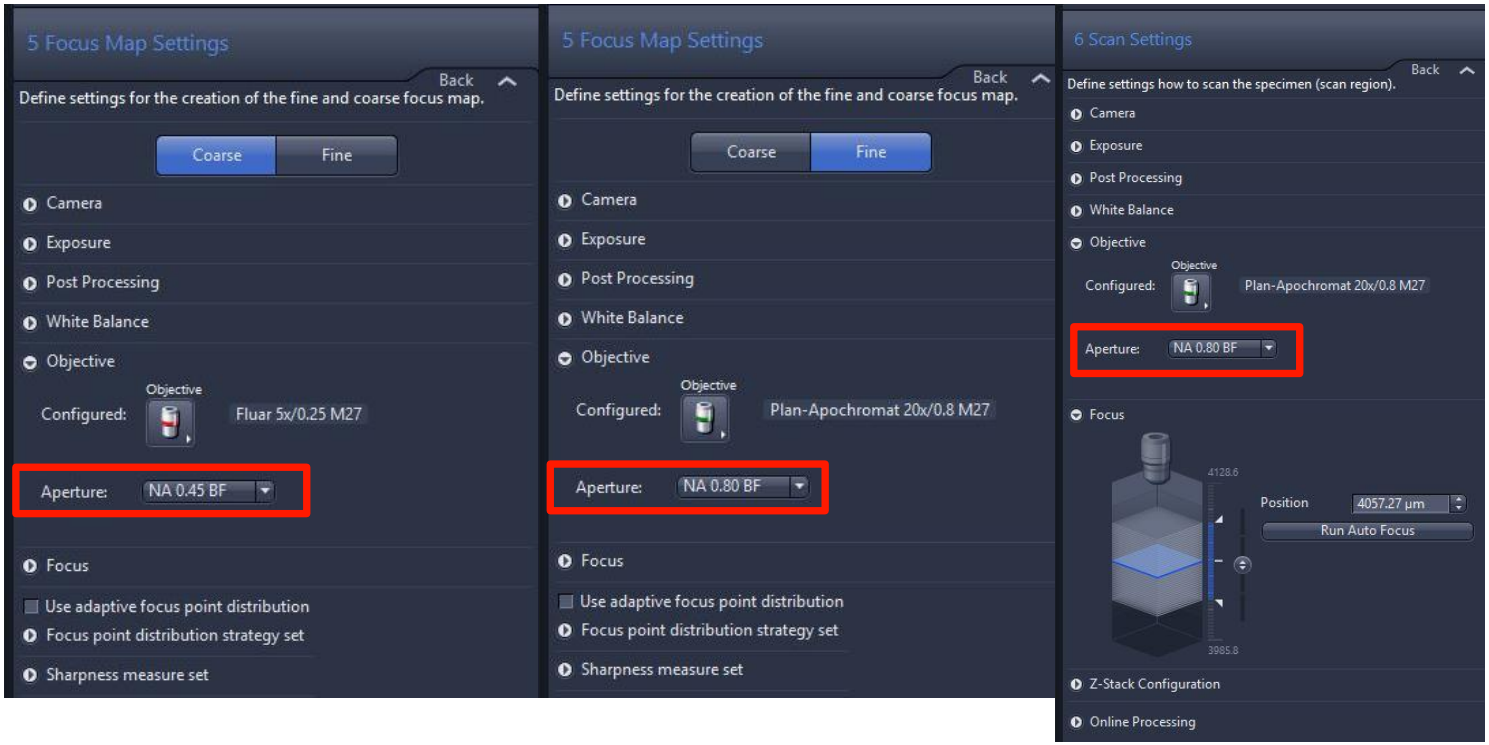
- With 2.6 HF 5 we introduced the Setting „Force sequential processing for Line Scan (BF)“ under Options -> Acquisition -> Axio Scan
- If this setting is not active, the software will activate this feature automatically only if the older windows 10 version is used and not for Windows 7 and Windows subversion 1809 and higher.
- Activate this setting only if the user experiences issues with scan interruptions and report the issue to techsupport.
- If this setting is activated the sequential acquisition is always activated. This would reduce the scan speed for bright field (so-called: line scan)!



# New functions / improvements ZEN 3.1
# BF workflow – Condenser position can now be set

- It is now possible to select the condenser for Coarse Focus / Fine Focus and Scan Settings (in the Expander “Objective”).

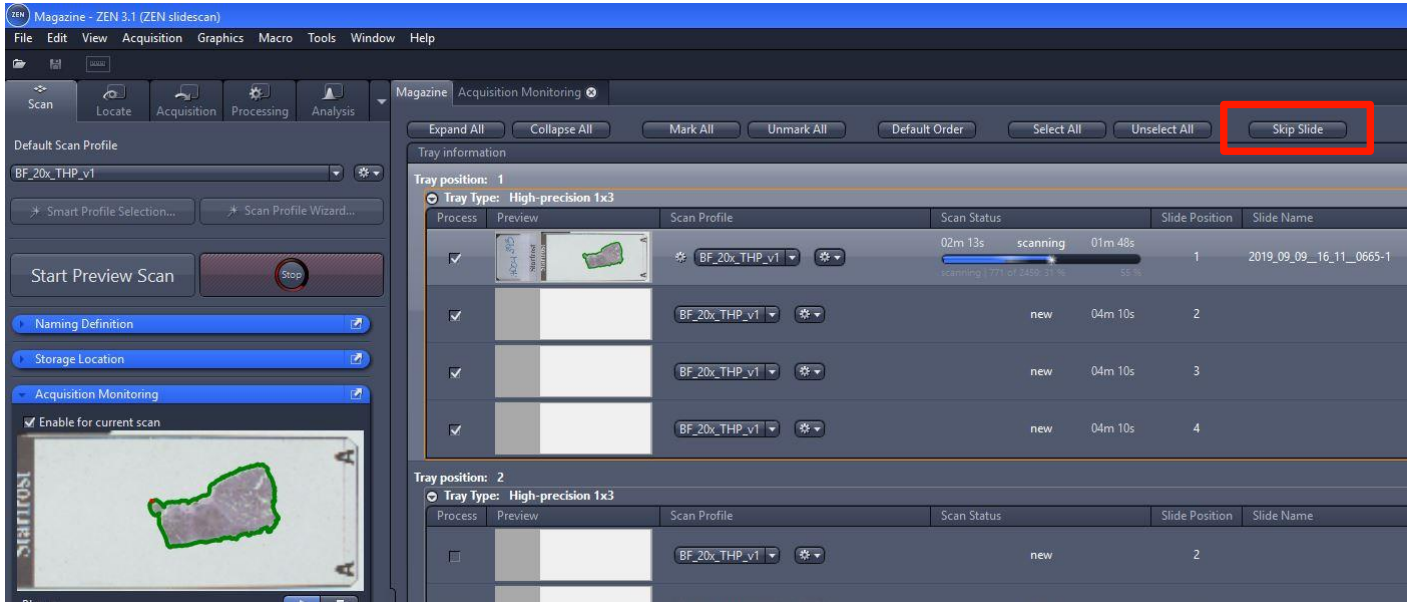
- This important e.g. to create images to the expectation of the user (e.g. if he wants to create an image which has a higher contrast and is willing to sacrifice the resolution)



# New functions / improvements ZEN 3.1
# Skip Slide

- With 2.6 we introduced the Acquisition Monitoring to check the image quality during the scan. Until now it was needed to stop the workflow and start it again if the user wanted to skip a certain slide.

- With 3.1 it is now possible to skip the current slides which is scanned with a mouse click. In the upper part of the magazine tab an additional button is available “Skip Slide”. If a user press this button the system will stop to process this slide and will continue with the next one.



# New High-end Axiocam models

**Introducing the Axiocam 705 & 712!**

# New Axiocam 705 and 712 Models with modern CMOS Sensors with a Massive Performance Improvement

**What's new:**

- We are introducing **four new high end Axiocam models,** offering a **massive performance improvement.**

- The new models based on the existing camera platform and **will replace existing models***:

  - **Axiocam 705 color** will replace the **Axiocam 503 color**
  - **Axiocam 705 mono** will replace the **Axiocam 503 mono**
  - **Axiocam 712 color** will replace the **Axiocam 512 color**
  - **Axiocam 712 mono** will replace the **Axiocam 512 mono**

* *Please note: the Axiocam 506 color/mono will remain available*

# New Axiocam 705 and 712 Models
# Established Basic Plattform Quality Parameters Remain Unchanged

- **Next Generation Camera Platform with following unchanged features:**

  - Same housing
  - Same interface USB3.0 + USB2.0 and standard cabeling
  - Same active thermo-electrical cooling down to 18°deg sensor temp.
  - Same HW trigger option
  - Same global shutter architecture
  - Same camera user interface
  - Same QE of 72%
  - Same wide range of exposure times form 100µs up to 60 s (very unusual for CMOS!!!)

  - **Compatibility to ZEN blue 3.1** and **ZEN core 2.7**

# New Axiocam 705 and 712 Models
# Camera System Value Drivers Clearly Improved

- **Camera specific improvements:**
  - High performance CMOS global shutter sensors
  - Extremly low noise floor from 2,2e down to 1,5e readout noise for best sensitivity
  - Speed improvements like 60 fps at 5 MP or 24 fps at 12 MP
  - 30 fps live image for all models
  - Doubled Dynamic at gain1x range of 1:5000 intensity values
  - HDR Mode with 1:25.000 intensitiy values
  - In case of 705: better resolution than predecessor 503
  - 5 MP version (instead of 3)

- **Noise Reduction Breakthrough** in CMOS blinking pixel noise suppression
  - No speed reduction!
  - No resolution loss!

  - **In spite of the dramatic performance improvement, the price point for the new 7xx models will remain unchanged.**

# Axiocam 712 and Axiocam 705
# Technical Data

| Key Features | Axiocam 705 color | Axiocam 705 mono | Axiocam 712 color | Axiocam 712 mono |
|---|---|---|---|---|
| Replaced camera | Axiocam 503 color | Axiocam 503 color | Axiocam 512 color | Axiocam 512 mono |
| Pixel Count | **5 MP** | **5 MP** | **12 MP** | **12 MP** |
| Pixel Size | **3,45 µm** | **3,45 µm** | **3,45 µm** | **3,45 µm** |
| Sensor Size diag. | **11mm = 2/3“** | **11mm = 2/3“** | **17,5mm =1,1“** | **17,5mm =1,1“** |
| Frames/s full | **62 fps** | **62 fps** | **23 fps** | **23 fps** |
| HDTV ROI Speed | **115 fps** | **115 fps** | **63 fps** | **63 fps** |
| Dynamic Ra | **1:5000** | **1:5000** | **1:5000** | **1:5000** |
| Read Noise down to | **1,15e** | **1,15e** | **1,15e** | **1,15e** |
| QE % | | **72%** | | **72%** |