

Guided Acquisition and Adaptive Feedback Microscopy



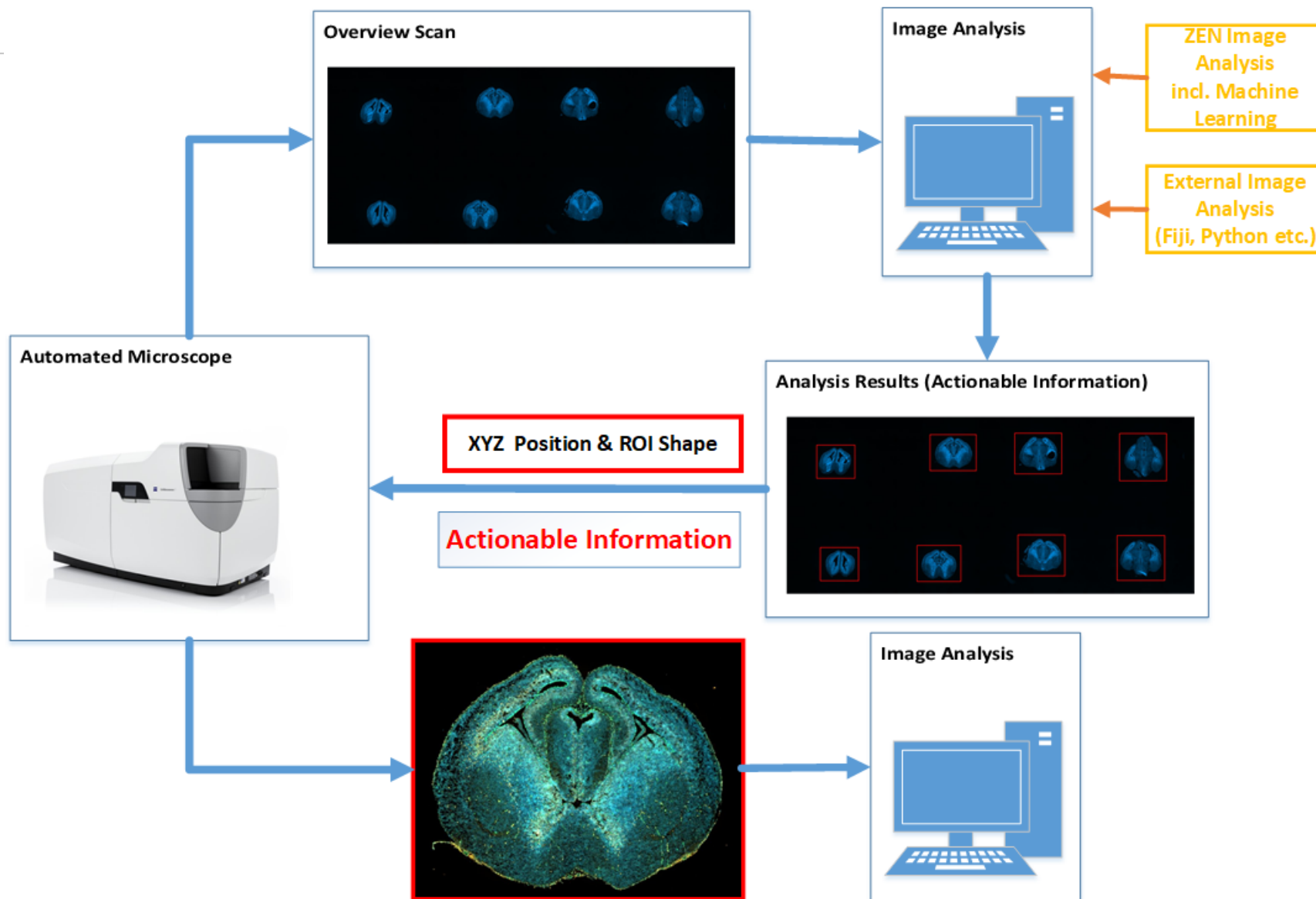
Guided Acquisition is one way to do Adaptive Feedback Microscopy



- It is all about creating **Actionable Information** and **Advanced Automated Microscopy**.
- **Actionable Information** = Use Image & Data Analysis to extract information guiding the next steps inside a workflow.
- Depending on the specific application online or offline **image analysis** is be required.

Guided Acquisition Workflow

Actionable Information



- Finding the “right” sample manually
 - is typically time consuming
 - can be really error prone
 - will bleach your sample unnecessarily
 - is sometimes close to impossible because the pure number of samples
 - can be tricky, because it is really hard to “find the needle inside the haystack”
- Reliable statistics benefit reproducible acquisition workflows
- “Hey, this cell looks nice, lets acquire more details here” – this approach can be problematic...
- **General Microscopy wisdom: “Everything that can be automated should be automated.”**
- Why should one always acquire data that are not needed (time, space and money)?

Guided Acquisition

What is it and what steps are required?



Guided Acquisition is a tool inside ZEN Blue realizing the concepts of Adaptive Feedback Microscopy

- Run an experiment (Overview Scan) to acquire the sample using any detector (LSM, Camera, ...)
- Use Image Analysis to detect “objects of interest” automatically using
 - built-in classical image analysis
 - built-in machine learning tools (ZEN Intellesis)
 - APEER modules (use language independent image analysis tools)
 - external software tools like Fiji etc.
- Retrieve the Image Analysis Results (Actionable Information) automatically and use it to guide the system
- Acquire data for every objects automatically (Detail Scan) with high resolution etc.
- Store all the data and manage them inside ZEN Connect

What tools and skills are needed to run a Guided Acquisition workflow?

The ZEN Toolbox has it all !

- **Open Application Development (OAD)** uses powerful Python Scripts to simplify, customize and automate your workflows
- **ZEN Image Analysis** with built-in classical and machine-learning algorithms using **ZEN Intellesis**
- **APEER** - digital microscopy platform enabling customized your workflows by leveraging Docker™ technology.
- The **CZI-API** for .NET (ZeissImgLib) / C++ (libCZI) and BioFormats (CZIReader) allow easy access to CZI files.
- **ZEN Connect** and **BioFormats Import** – Read any microscopy image and store them in a sample-centric manner.
- Smart” experiments with **Experiment Feedback** and modify the acquisition On-the-fly based on Online Image Analysis and External Inputs.

Guided Acquisition & Adaptive Feedback Microscopy

General Considerations



- What is the actual **nature of the desired feedback** and upon what **event** it should be triggered?
- What exactly is the **Actionable Information** to be extracted?
- On what **timescale** this feedback is required?
- Is **Online Image Analysis** available and is it sufficient to detect the feedback event?
- Which **interfaces** can be used to communicate with **external image analysis** tools or **external devices**?
- What is right **choice of hardware** and is it ready to be automated?
- What could go potentially **wrong** inside such an **automated workflow** and what be the **consequences**?

In the case where external image analysis is needed the “painless” exchange of data becomes crucial!

- CZI can be read easily by many open source and commercial software packages
 - Fiji, ImageJ, Python, KNIME, Icy
 - MATLAB, Imaris, Arivis, ORS
- Constant exchange with BioFormats team to keep **their** CZIReader up-to-date
- **ZEN has option for BioFormats import to read 3rd party images (paid module)**
- Zeiss offers two open available APIs to read CZI on any platform
 - libCZI (C++) for cross-platform applications (Windows, Linux, MacOS)
 - ZeissImgLib (c#) for Windows only
 - Python wrapper for libCZI is in progress

Adaptive Feedback Microscopy

What is the right system?



Well, it depends from your application ...



... but all motorized ZEN Blue systems can be used (not only CellDiscoverer 7)

Adaptive Feedback Microscopy

System and Software Requirements



- **motorized** and **calibrated** imaging system
- Fast and robust sample- and **focus-finding** mechanisms
- **secure** XYZ stage movements
- **open** data format
- built-in online **image analysis** with classical tools and **machine-learning**
- flexible **scripting language**
- **adaptable** experiments and hardware settings
- interfaces to “the outside world”
- interface for “real programmers” with access to “deeper” system layers

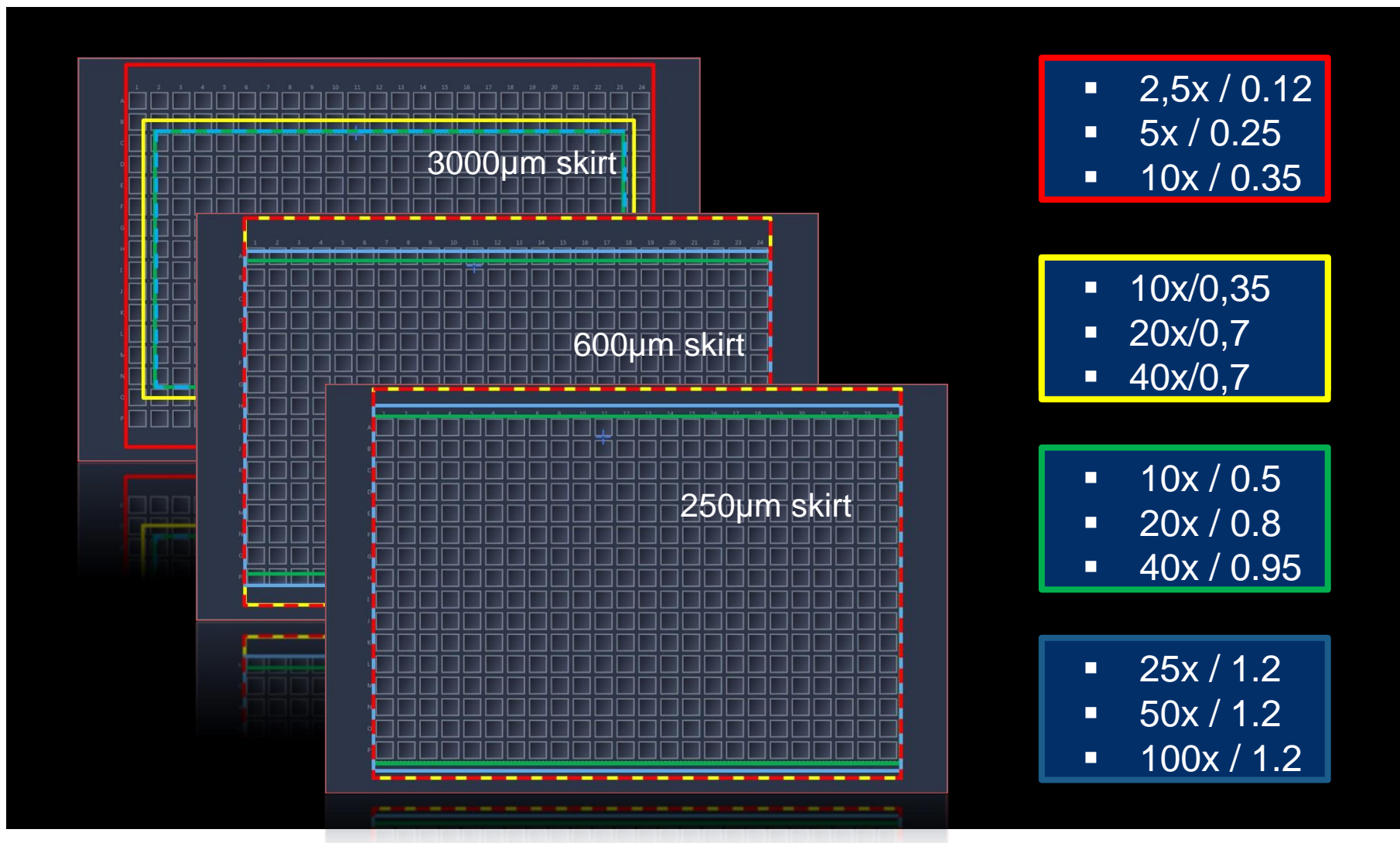
Adaptive Feedback Microscopy

Other requirements - when things go wrong ...



Adaptive Feedback Microscopy

When things go wrong ... Adaptive Lens Guard



So what is solution or answer to all those question and challenges when it come to ZEN Controlled systems?

???

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Guided Acquisition

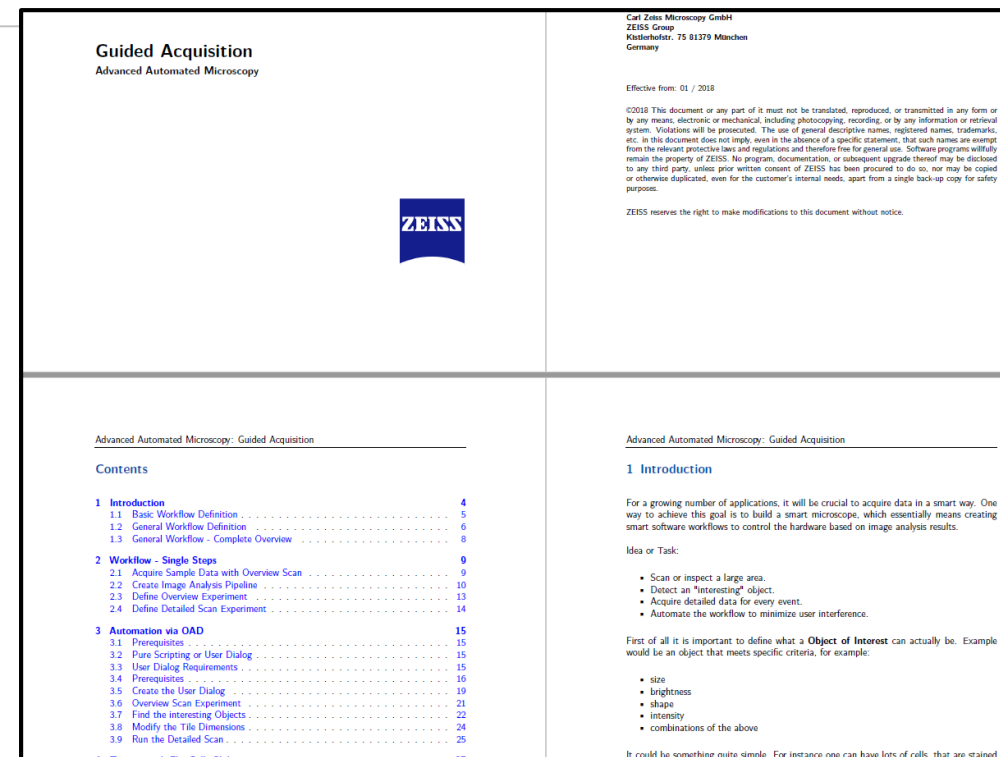
Guided Acquisition

Do I have to create this solution by myself?



```
#####
# File      : Guided_Acquisition_shortUI.py
# Version   : 7.1
# Author    : czsrb, czmla
# Date      : 12.04.2019
# Institution : Carl Zeiss Microscopy GmbH
#
# !!! Requires with ZEN >=2.6 HF3 - Use at your own Risk !!!
#
# Optimized for the use with Celldiscoverer 7 and DF2, but
# applicable for all motorized stands running in ZEN Blue.
# Please adapt focussing commands, especially FindSurface
# when using with other stands.
#
# 1) - Select Overview Scan Experiment
# 2) - Select appropriate Image Analysis Pipeline
# 3) - Select Detailed Scan Experiment
# 4) - Specify the output folder for the image and data tables
#
# Copyright(c) 2019 Carl Zeiss AG, Germany. ALL Rights Reserved.
#
# Permission is granted to use, modify and distribute this code,
# as long as this copyright notice remains part of the code.
#####
import time
from datetime import datetime
import errno
from System import Array
from System import ApplicationException
from System import TimeoutException
from System.IO import File, Directory, Path
import sys

# version number for dialog window
version = 7.1
# file name for overview scan
ovscan_name = 'OverviewScan.czi'
```



- ReadMe: https://github.com/zeiss-microscopy/OAD/blob/master/Guided_Acquisition/README.md
- Source Code: https://github.com/zeiss-microscopy/OAD/blob/master/Guided_Acquisition/Guided_Acquisition_shortUI.py
- Tutorial: https://github.com/zeiss-microscopy/OAD/blob/master/Guided_Acquisition/Guided_Acquisition_shortUI.pdf

Guided Acquisition User Interface

Script- based for maximal flexibility and Ease-of-Use



Guided Acquisition - Version : 7.1

✓ Show All ? x

1) Select Overview Experiment -----

Overview Scan

Overview Scan Experiment 000_Overview.czexp

OPTION - FindSurface (DF only) before Overview ☐

OPTION - SWAF before Overview ☐

Initial SWAF Range before Overview [micron] 200

2) Select Image Analysis to detect objects -----

Image Analysis

Image Analysis Pipeline 000_GuidedAcq_OverView.czias

3) Select DetailScan Experiment -----

Detail Scan

Detailed Scan Experiment 001_Detail.czexp

OPTION - FindSurface (DF only) before Detail ☐

OPTION - SWAF before Detail ☐

Initial SWAF Range before Detail [micron] 100

OPTION - Use RecallFocus (DF only) before Detail ☐

4) Specify basefolder to save the images -----

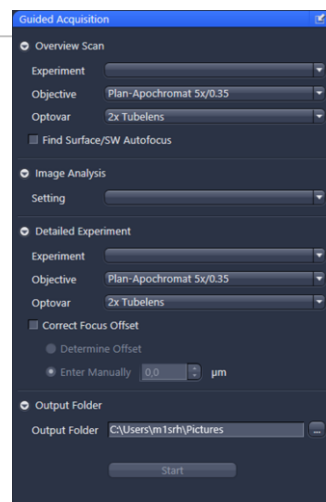
Basefolder for Images and Data Tables c:\Users\m1srh\Documents\Software\Bioformats\6.1.1\

OK Cancel

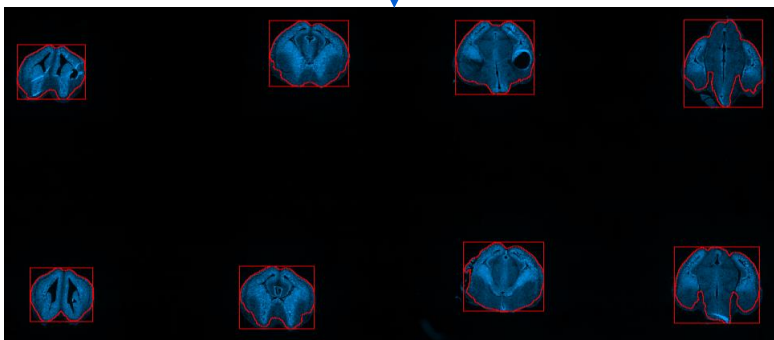
- Guided Acquisition UI is simple and user-friendly
- There is no need for the user to modify script ...
- ... but it can be customized for advanced applications
- Special focusing options depending on the used hardware
- Adaptable due to scripting for advanced applications

Guided Acquisition

Complete automated workflow



OAD - Automated Acquisition
and Image Analysis



	ID	Bound Center X Stage[μm]	Bound Center Y Stage[μm]	Bound Width[μm]	Bound Height[μm]
	A	B	C	D	E
1	2	40.786.876	10.236.734	3.014.505	2.511.325
2	3	47.835.969	10.371.678	2.996.208	2.799.510
3	4	30.956.571	10.934.324	2.584.515	2.085.909
4	5	56.540.981	10.604.970	2.996.208	3.320.987
5	6	31.372.838	19.403.757	2.392.392	2.035.591
6	7	48.176.759	18.745.048	3.046.525	2.639.407
7	8	56.309.976	19.044.669	3.238.649	2.909.295
8	9	39.556.373	19.508.967	2.831.530	2.383.243

OAD – Cycle through list, modify
experiment and acquire Detailed Scan



Guided Acquisition Example

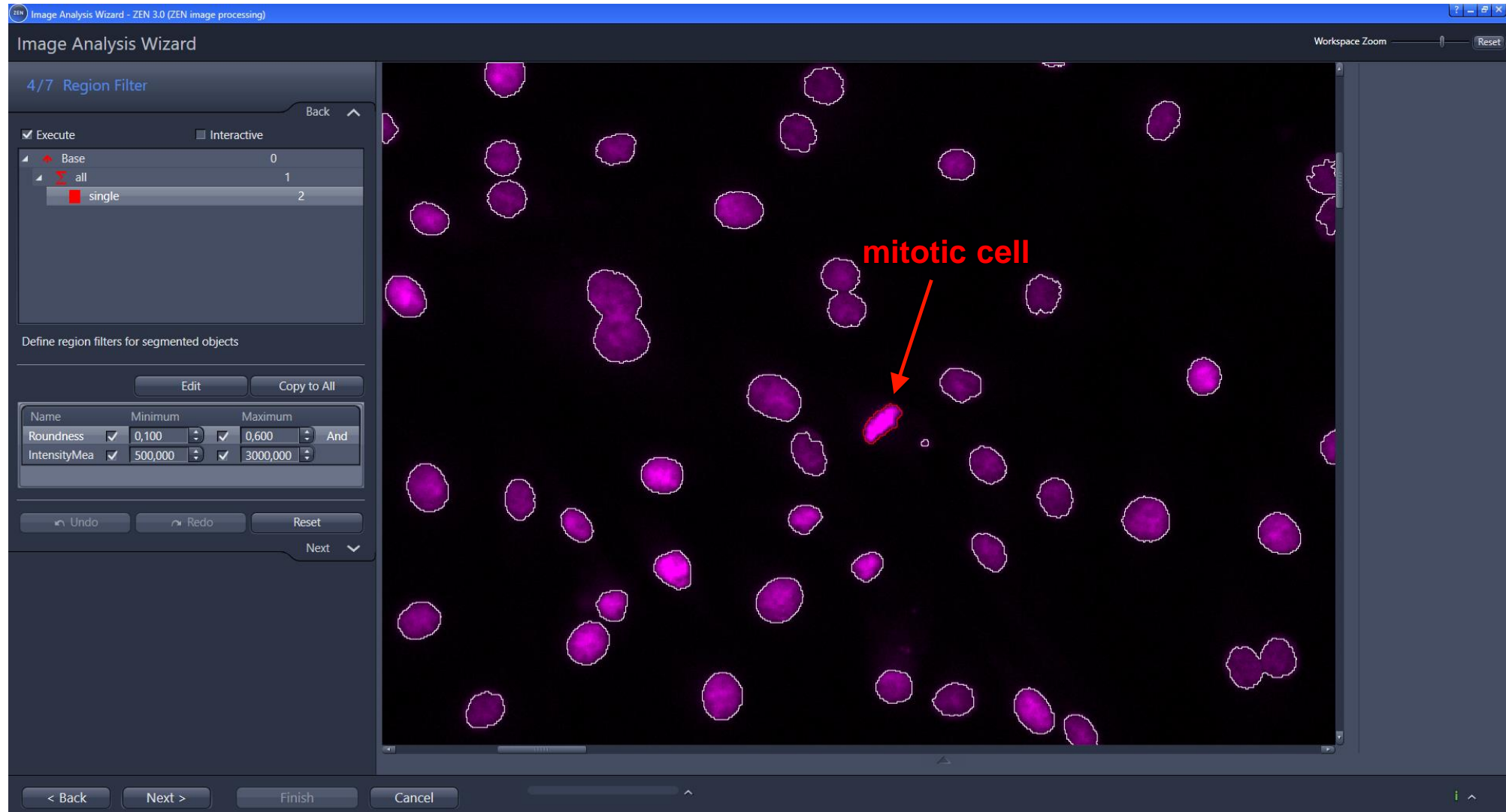
Detect mitotic cells



- Acquire a test image with many cells
- Setup an Image Analysis Setting to segment the desired objects of interests or
- Train a Intellesis model that can be used to segment the objects
- “Finetune” the Image Analysis by using Region Filters
- Specify the required features for the Guided Acquisition
- Test the Image Analysis with more test images
- Rather acquire a few false positives than missing objects – it is all automated ... 😊

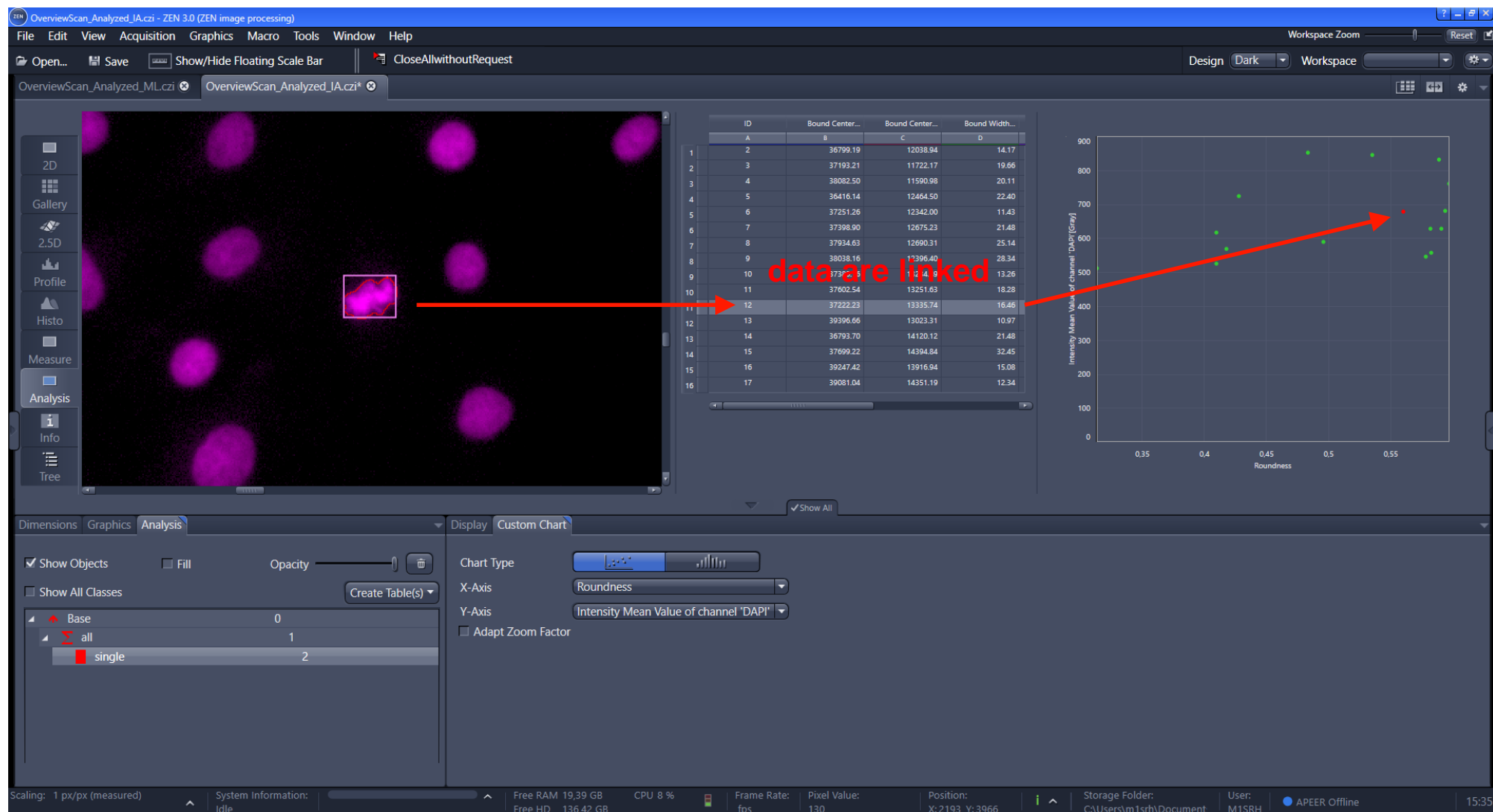
Guided Acquisition Example

Detect mitotic cells – Image Analysis



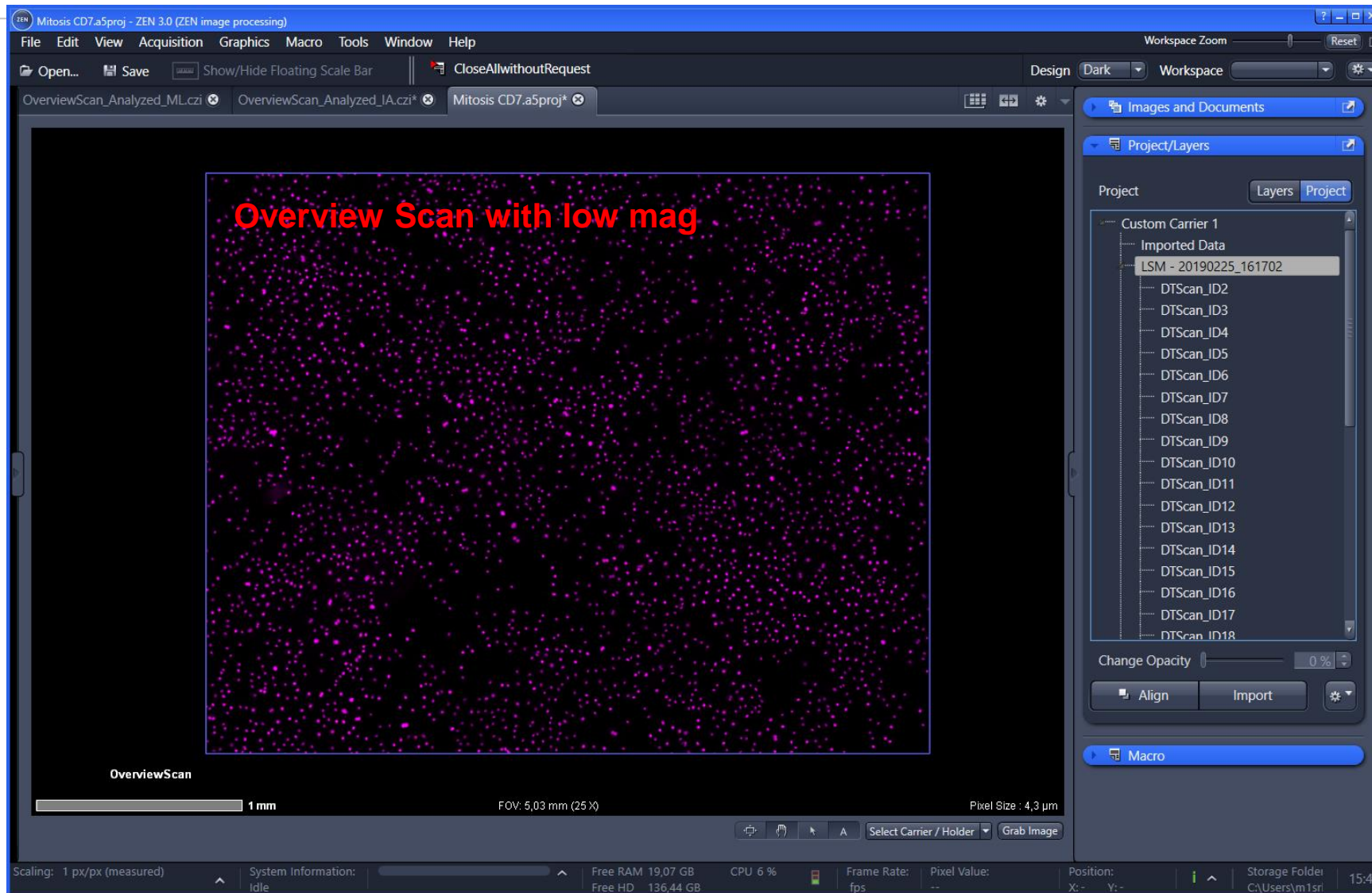
Guided Acquisition Example

Detect mitotic cells – Image Analysis



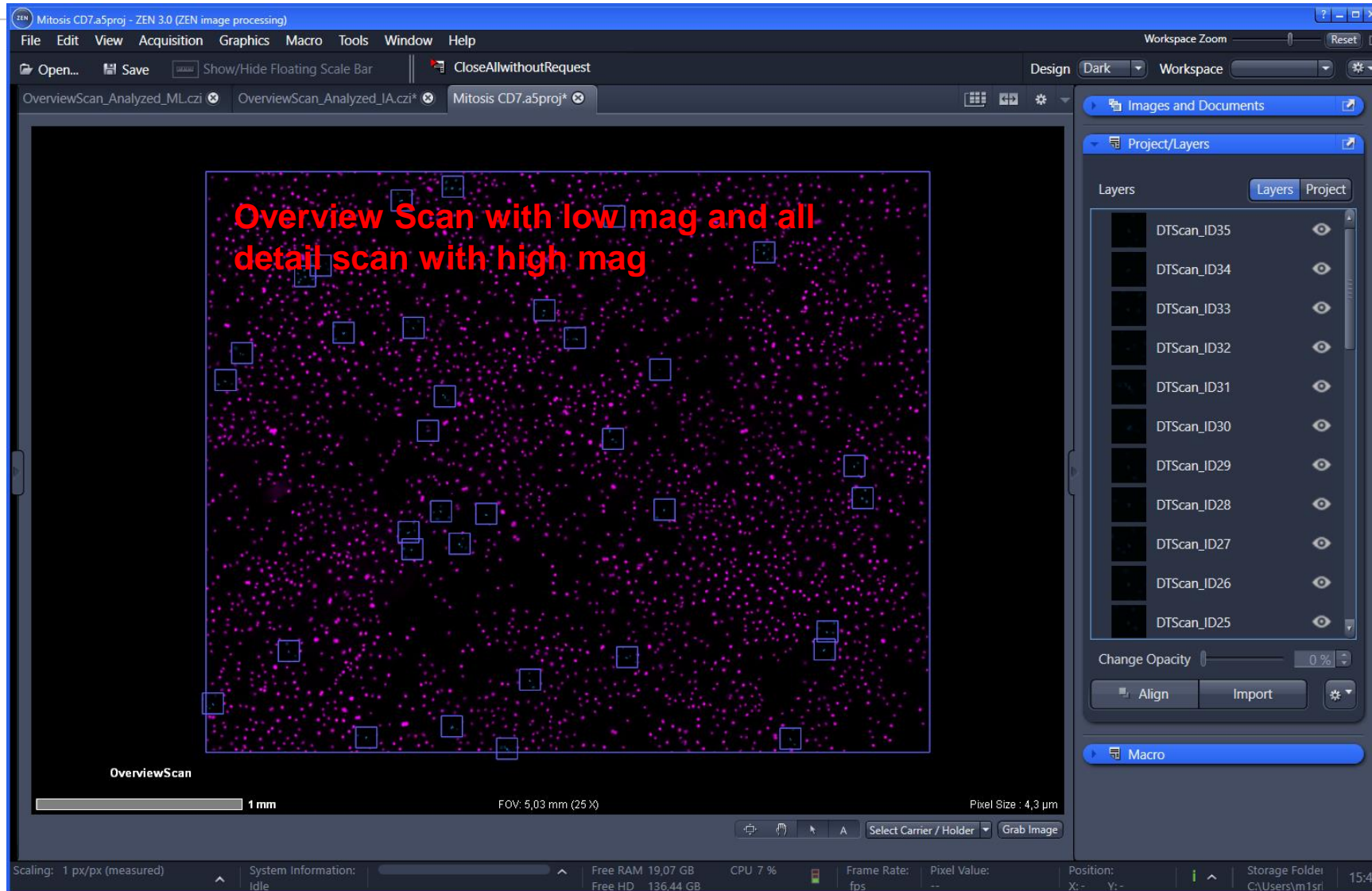
Guided Acquisition Example

ZEN Connect for sample-centric data storage



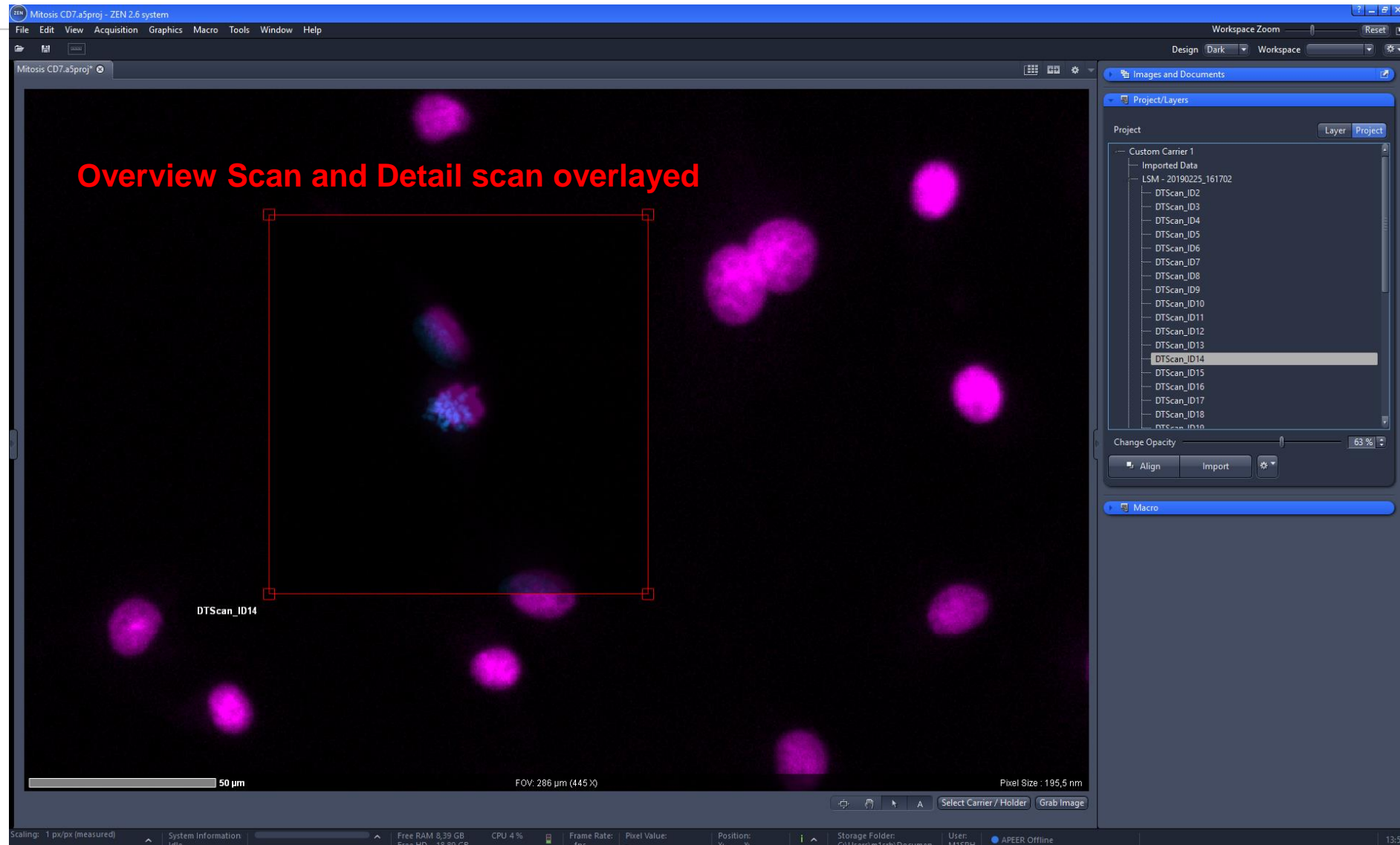
Guided Acquisition Example

ZEN Connect for sample-centric data storage



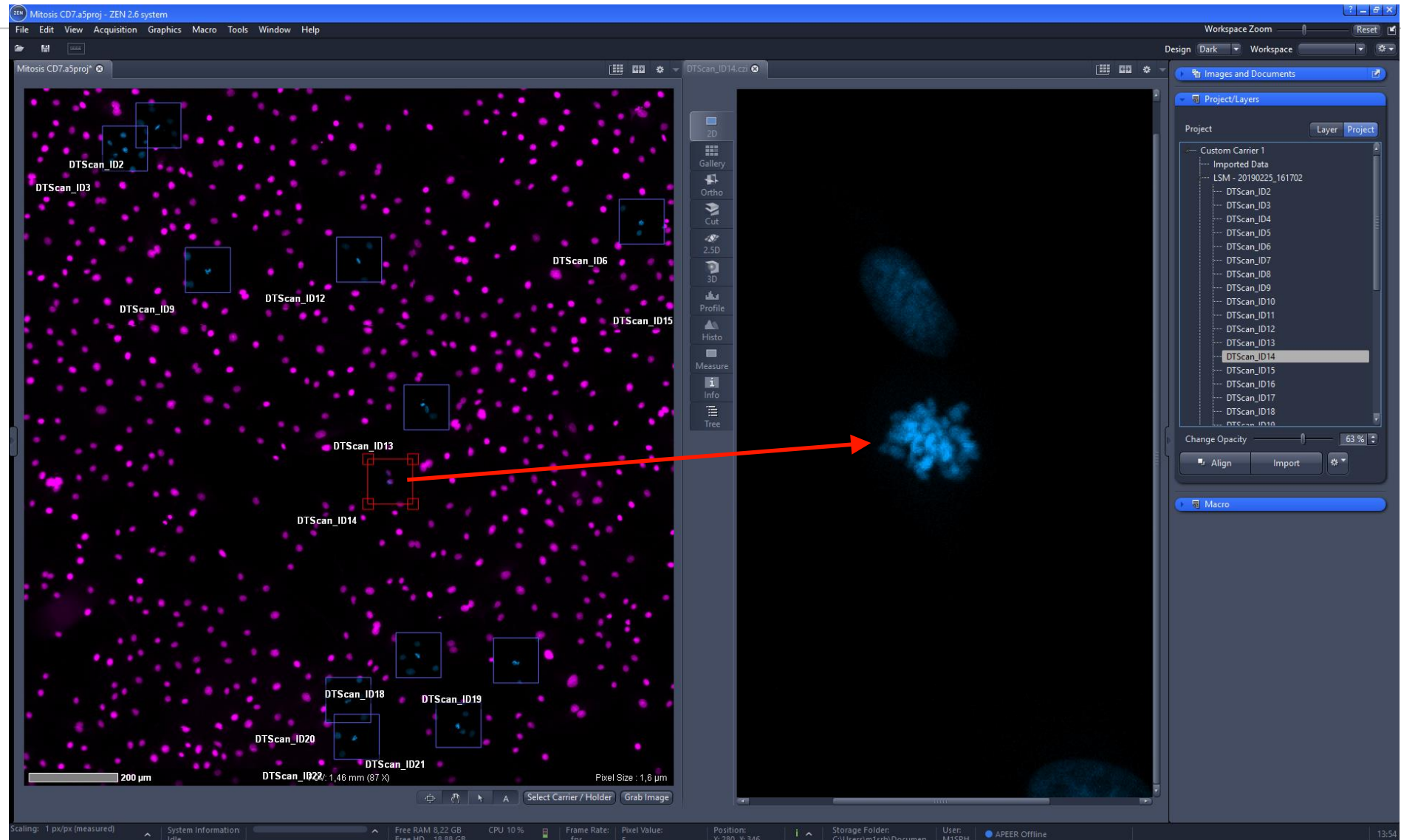
Guided Acquisition

ZEN Connect for sample-centric data storage



Guided Acquisition

ZEN Connect for sample-centric data storage

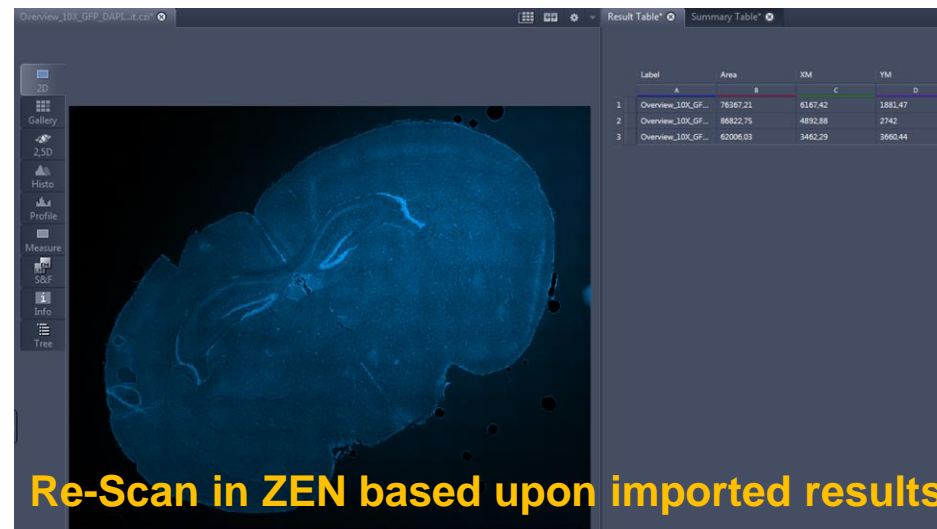


Guided Acquisition Advanced

Incorporating external image analysis (Fiji)



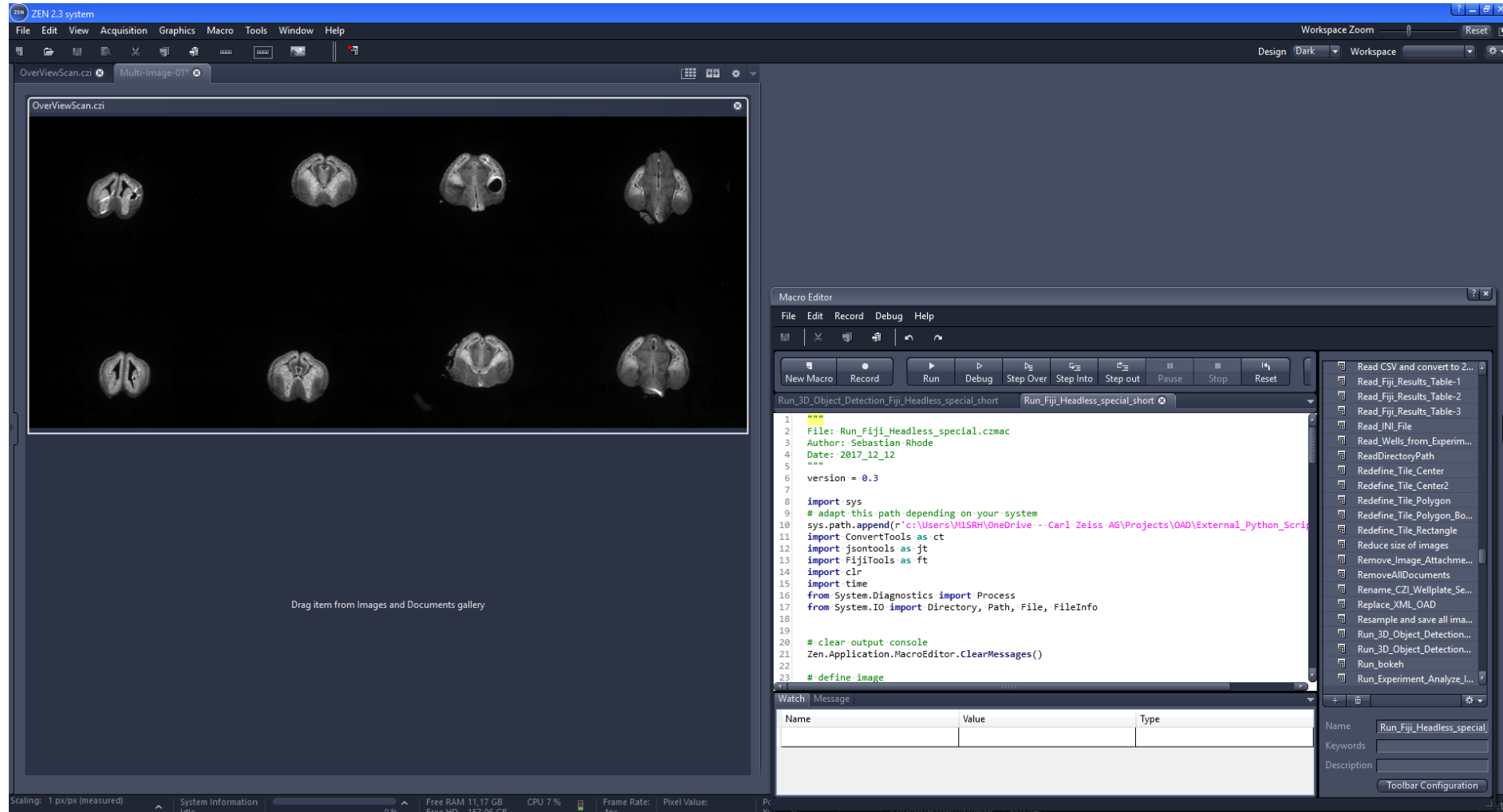
Open in Fiji



Import Results

Guided Acquisition Advanced

Run overview scan



Guided Acquisition Advanced

Start external image analysis



The screenshot shows the ZEN 2.3 system interface with a Java command window open. A yellow box highlights the following text in the command window:

```
09:10:23,336 |-INFO in ch.qos.logback.classic.joran.JoranConfigurator@245a060f - Registering current configuration as safe fallback point

SLF4J: Actual binding is of type [ch.qos.logback.classic.util.ContextSelectorStaticBinder]
[INFO] Overriding BIOP Run Macro...; identifier: command:ch.epfl.biop.macrorunner.B_Run_Macro; jar: file:///C:/Users/m1srh/Documents/Fiji/plugins/BIOP/B_Run_Macro-1.0.0-SNAPSHOT.jar
[INFO] Overriding Save Image as Tiff Without Prompt; identifier: script:ZenIntegration/Save Image As Tiff Without Prompt.java; jar: file:///C:/Users/m1srh/Documents/Fiji/jars/scijava-common-2.19.0.jar
Mar 15, 2018 9:10:26 AM java.util.prefs.WindowsPreferences <init>
WARNING: Could not open/create prefs root node Software\JavaSoft\Prefs at root 0x80000002. Windows RegCreateKeyEx(...) returned error code 5.
[INFO] Fiji Script Directory : c:\Users\m1srh\Documents\Fiji\scripts
[INFO] Start Fiji Image Analysis ...
[INFO] Fiji Python Script : c:\Users\m1srh\Documents\Fiji\scripts\GuidedAcq_fromZEN.py
[INFO] Filename : \\Output\Guided_Acquisition\OverViewScan.czi
[INFO] BinFactor : 1
[INFO] RankFilter : MEDIAN
[INFO] Filter Radius : 3
[INFO] Threshold Method : Triangle
[INFO] Threshold Background : black
[INFO] Min. Partice Size [pixel] : 10000
[INFO] Min. Circularity : 0.01
[INFO] Max. Circularity : 0.99
[INFO] Add Particles to ROI-Manager : False
[INFO] Save Particles as Image : True
[INFO] Save Format : ome.tiff
[INFO] Save Results : TRUE
[INFO] Headless Mode : True
[INFO] ----- START IMAGE ANALYSIS -----
```

Overlaid on the screenshot is the text: **Start External Application (here Fiji in headless mode) and read results in ZEN.**

Guided Acquisition Advanced

Read the results and take action



OverViewScan_RESULTS - ZEN 2.3 system

File Edit View Acquisition Graphics Macro Tools Window Help

OverViewScan.czi Multi-Image-01 OverViewScan_PA.ome.tif* OverViewScan_RESULTS*

Workspace Zoom Design Dark Workspace

OverViewScan.czi

OverViewScan_PA.ome.tif

	Label	A	B	C	BX	BY	Width	Height	Slice
1	OverViewScan.czi...	7,469,314,218	28,013,397	1,536,986	3,220,351	3,513,110	1,000		
2	OverViewScan.czi...	6,175,659,797	12,295,887	1,665,068	3,128,864	2,616,535	1,000		
3	OverViewScan.czi...	6,540,588,192	19,285,513	1,665,068	3,202,054	2,817,807	1,000		
4	OverViewScan.czi...	4,631,242,653	2,689,725	2,579,941	2,689,725	2,250,586	1,000		
5	OverViewScan.czi...	6,487,690,315	19,596,569	10,118,490	3,238,649	2,671,428	1,000		
6	OverViewScan.czi...	7,574,440,380	27,647,448	10,209,977	3,476,516	3,019,079	1,000		
7	OverViewScan.czi...	5,565,325,426	11,179,742	10,996,768	2,945,890	2,488,453	1,000		
8	OverViewScan.czi...	4,185,628,254	3,220,351	11,051,660	2,488,453	2,232,289	1,000		

Macro Editor

File Edit Record Debug Help

New Macro Record Run Debug Step Over Step Into Step out Pause Stop Reset

Run_3D_Object_Detection_Fiji_Headless_special_short Run_Fiji_Headless_special_short

```
1 """
2 File: Run_Fiji_Headless_special.czm
3 Author: Sebastian Rhode
4 Date: 2017_12_12
5 """
6 version = 0.3
7
8 import sys
9 # adapt this path depending on your system
10 sys.path.append(r'c:\Users\VIISRH\OneDrive -- Carl-Zeiss AG\Projects\OAD\External_Python_Scripts')
11 import ConvertTools as ct
12 import jsontools as jt
13 import FijiTools as ft
14 import clr
15 import time
16 from System.Diagnostics import Process
17 from System.IO import Directory, Path, File, FileInfo
18
19 # clear output console
20 Zen.Application.MacroEditor.ClearMessages()
21
22 # define image
```

Watch Message

Name	Value	Type

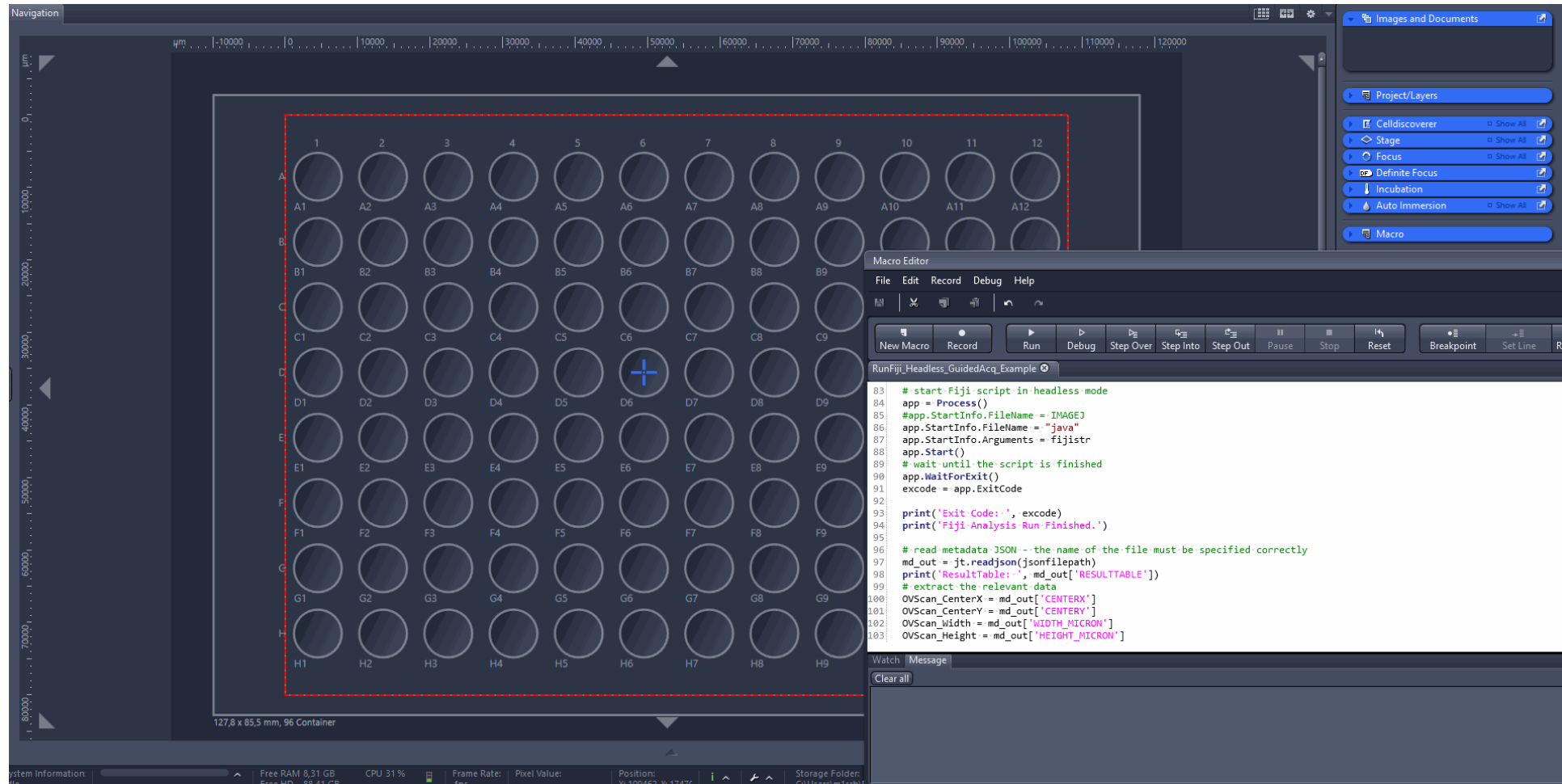
Read CSV and convert to 2...
Read_Fiji_Results_Table-1
Read_Fiji_Results_Table-2
Read_Fiji_Results_Table-3
Read_INI_File
Read_Wells_from_Experim...
ReadDirectoryPath
Redefine_Tile_Center
Redefine_Tile_Center2
Redefine_Tile_Polygon
Redefine_Tile_Polygon_Bo...
Redefine_Tile_Rectangle
Reduce size of images
Remove_Image_Attachme...
RemoveAllDocuments
Rename_CZI_Wellplate_Se...
Replace_XML_OAD
Resample and save all ima...
Run_3D_Object_Detection...
Run_3D_Object_Detection...
Run_bokeh
Run_Experiment_Analyze_I...

Name Run_Fiji_Headless_special
Keywords
Description
Toolbar Configuration

Scaling: 1 px/px (measured) System Information: Free RAM 11.26 GB CPU 17 % Frame Rate: Pixel Value: 0 % Free HD 157.06 GB

Guided Acquisition Advanced

Read the results and take action



- Open Application Development (OAD) : <https://github.com/zeiss-microscopy/OAD>
- Guided Acquisition : https://github.com/zeiss-microscopy/OAD/tree/master/Guided_Acquisition
- Experiment Feedback : https://github.com/zeiss-microscopy/OAD/tree/master/Experiment_Feedback
- APEER: <https://www.apeer.com>
- ZEN Intellesis : <https://www.zeiss.com/microscopy/int/products/microscope-software/zen-intellesis-image-segmentation-by-deep-learning.html>
- ZEN Connect : <https://www.zeiss.com/microscopy/int/products/microscope-software/zen-connect-image-overlay-and-correlative-microscopy.html>
- libCZI : <https://github.com/zeiss-microscopy/libCZI>

