

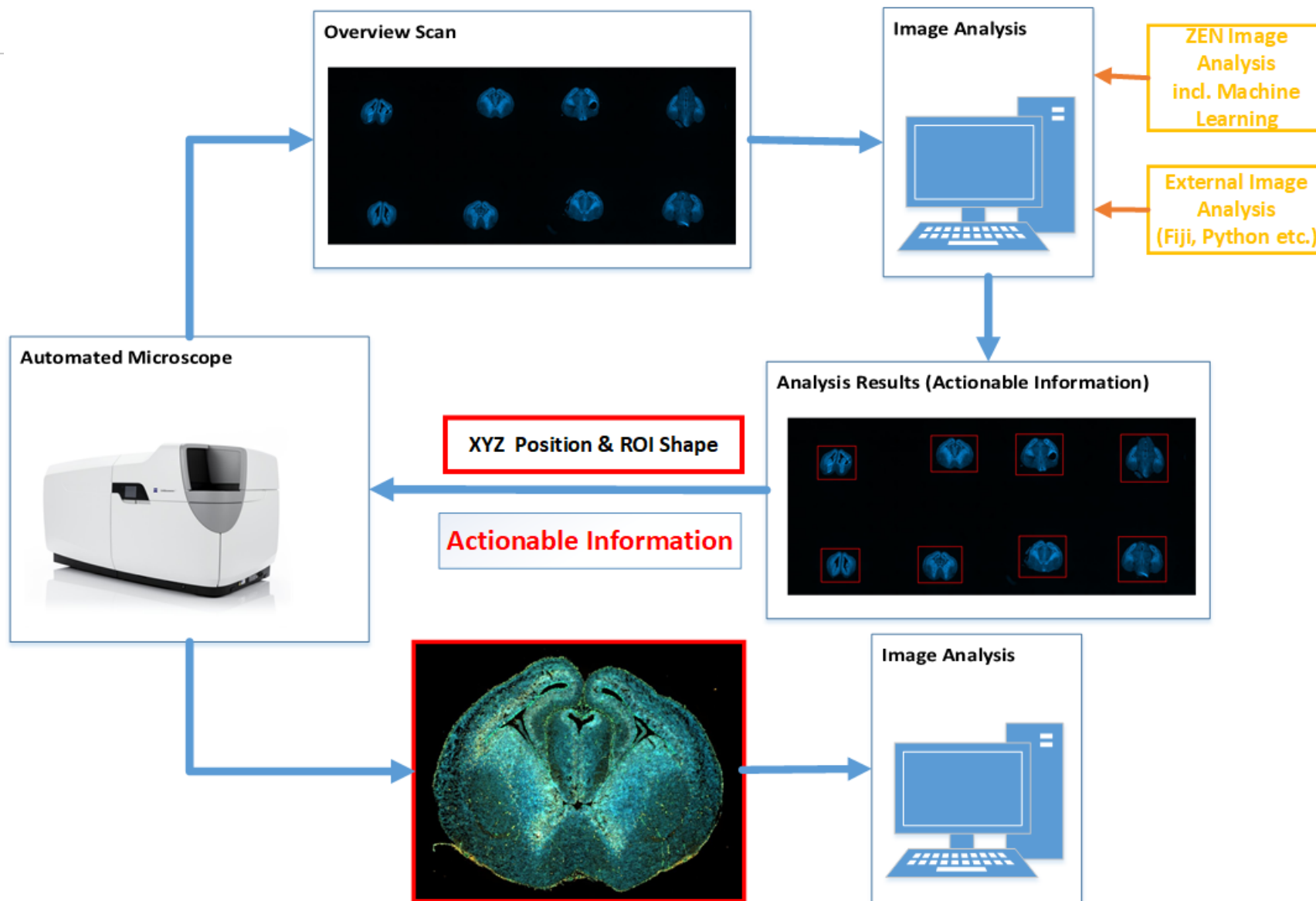
Guided Acquisition and Adaptive Feedback Microscopy



- 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, so Automation is key.
- “Hey, this cell looks nice, lets acquire more details here” – this approach can be (very) problematic...
- **General Microscopy wisdom: “Everything that can be automated should be automated.”**
- Why should one always acquire data that are not needed (time, data storage space and money)?

Adaptive Feedback Microscopy

Actionable Information



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 required.
- This can be done by internal or external image analysis tools.

Adaptive Feedback Microscopy

What are the typical steps inside such a workflow?



- 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 blue 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.

- **ZEN Image Analysis to extract Actionable Information**
 - classical segmentation and Machine Learning methods can be used
- **OAD Python Environment**
 - powerful and flexible python scripts to automate workflows
 - Call external software and exchange data
 - Create dialogs and User Interfaces for Ease-of-Use
- **Experiment Feedback**
 - Online Image Analysis during running experiments
 - Modification of Experiments on runtime
 - Sending or receiving signals to the “outside”
 - Powerful combinations with Experiment designer for heterogeneous acquisition workflows
- **TCP/IP Interface** to control ZEN from the “outside” or vice versa

- 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

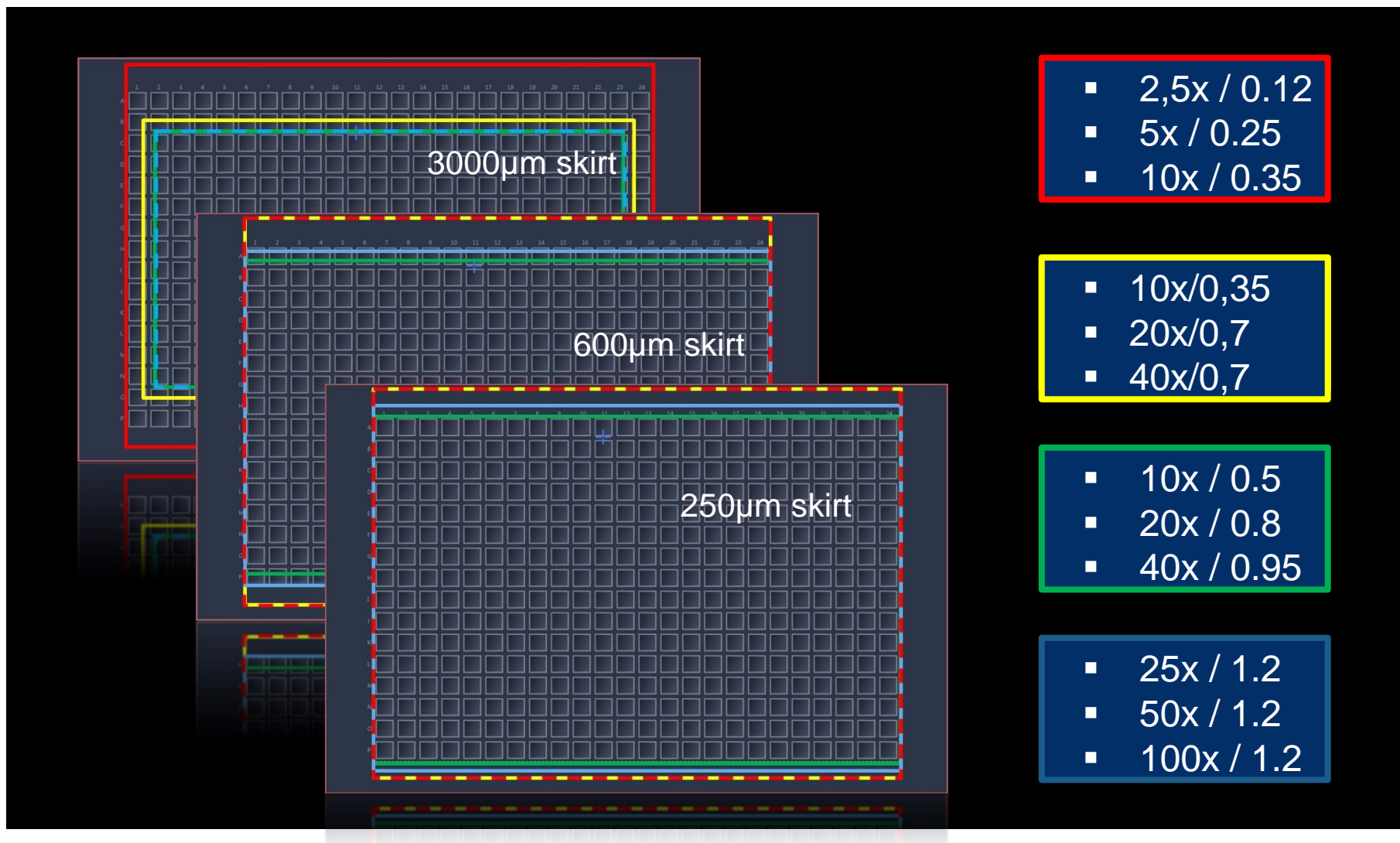
Adaptive Feedback Microscopy

Other requirements - when things go wrong ...



Adaptive Feedback Microscopy

When things go wrong ... Adaptive Lens Guard



So what is solution ZEN blue can offer?

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 sys import argv
from sys import ApplicationException
from sys import TimeoutException
from sys import File, Directory, Path
import sys

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

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|-------------------------------|---|---------------------------------|---|--|---|---------------------------|---|--|---|------------------------------------|----|--------------------------------|----|-------------------------------------|----|----------------------|----|-------------------|----|-----------------------------------|----|------------------------------|----|-------------------|----|----------------------------|----|------------------------------|----|----------------------------------|----|--------------------------------|----|---------------------------|----|--|
| <h3>Guided Acquisition</h3> <p>Advanced Automated Microscopy</p> | <p>Carl Zeiss Microscopy GmbH ZEISS Group Kittlerstr. 75 81379 München Germany</p> <p>Effective from: 01 / 2018</p> <p>©2018 This document or any part of it must not be translated, reproduced, or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or by any information or retrieval system. Violations will be prosecuted. The use of general descriptive names, registered names, trademarks, etc. in this document does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use. Software programs willfully remain the property of ZEISS. No program, documentation, or subsequent upgrade thereof may be disclosed to any third party, unless prior written consent of ZEISS has been procured to do so, nor may be copied or otherwise duplicated, even for the customer's internal needs, apart from a single back-up copy for safety purposes.</p> <p>ZEISS reserves the right to make modifications to this document without notice.</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <h4>Advanced Automated Microscopy: Guided Acquisition</h4> <h5>Contents</h5> <table><tr><td>1 Introduction</td><td>4</td></tr><tr><td>1.1 Basic Workflow Definition</td><td>5</td></tr><tr><td>1.2 General Workflow Definition</td><td>6</td></tr><tr><td>1.3 General Workflow - Complete Overview</td><td>8</td></tr><tr><td>2 Workflow - Single Steps</td><td>9</td></tr><tr><td>2.1 Acquire Sample Data with Overview Scan</td><td>9</td></tr><tr><td>2.2 Create Image Analysis Pipeline</td><td>10</td></tr><tr><td>2.3 Define Overview Experiment</td><td>13</td></tr><tr><td>2.4 Define Detailed Scan Experiment</td><td>14</td></tr><tr><td>3 Automation via OAD</td><td>15</td></tr><tr><td>3.1 Prerequisites</td><td>15</td></tr><tr><td>3.2 Pure Scripting or User Dialog</td><td>15</td></tr><tr><td>3.3 User Dialog Requirements</td><td>15</td></tr><tr><td>3.4 Prerequisites</td><td>16</td></tr><tr><td>3.5 Create the User Dialog</td><td>19</td></tr><tr><td>3.6 Overview Scan Experiment</td><td>21</td></tr><tr><td>3.7 Find the Interesting Objects</td><td>22</td></tr><tr><td>3.8 Modify the Tile Dimensions</td><td>24</td></tr><tr><td>3.9 Run the Detailed Scan</td><td>25</td></tr></table> | 1 Introduction | 4 | 1.1 Basic Workflow Definition | 5 | 1.2 General Workflow Definition | 6 | 1.3 General Workflow - Complete Overview | 8 | 2 Workflow - Single Steps | 9 | 2.1 Acquire Sample Data with Overview Scan | 9 | 2.2 Create Image Analysis Pipeline | 10 | 2.3 Define Overview Experiment | 13 | 2.4 Define Detailed Scan Experiment | 14 | 3 Automation via OAD | 15 | 3.1 Prerequisites | 15 | 3.2 Pure Scripting or User Dialog | 15 | 3.3 User Dialog Requirements | 15 | 3.4 Prerequisites | 16 | 3.5 Create the User Dialog | 19 | 3.6 Overview Scan Experiment | 21 | 3.7 Find the Interesting Objects | 22 | 3.8 Modify the Tile Dimensions | 24 | 3.9 Run the Detailed Scan | 25 | <h4>Advanced Automated Microscopy: Guided Acquisition</h4> <h5>1 Introduction</h5> <p>For a growing number of applications, it will be crucial to acquire data in a smart way. One way to achieve this goal is to build a smart microscope, which essentially means creating smart software workflows to control the hardware based on image analysis results.</p> <p>Idea or Task:</p> <ul style="list-style-type: none">• Scan or inspect a large area.• Detect an "interesting" object.• Acquire detailed data for every event.• Automate the workflow to minimize user interference. <p>First of all it is important to define what a Object of Interest can actually be. Example would be an object that meets specific criteria, for example:</p> <ul style="list-style-type: none">• size• brightness• shape• intensity• combinations of the above <p>It could be something quite simple. For instance one can have lots of cells, that are stained</p> |
| 1 Introduction | 4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1.1 Basic Workflow Definition | 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1.2 General Workflow Definition | 6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1.3 General Workflow - Complete Overview | 8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2 Workflow - Single Steps | 9 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2.1 Acquire Sample Data with Overview Scan | 9 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2.2 Create Image Analysis Pipeline | 10 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2.3 Define Overview Experiment | 13 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2.4 Define Detailed Scan Experiment | 14 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3 Automation via OAD | 15 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3.1 Prerequisites | 15 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3.2 Pure Scripting or User Dialog | 15 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3.3 User Dialog Requirements | 15 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3.4 Prerequisites | 16 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3.5 Create the User Dialog | 19 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3.6 Overview Scan Experiment | 21 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3.7 Find the Interesting Objects | 22 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3.8 Modify the Tile Dimensions | 24 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3.9 Run the Detailed Scan | 25 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

- 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 ? ✕

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 -----

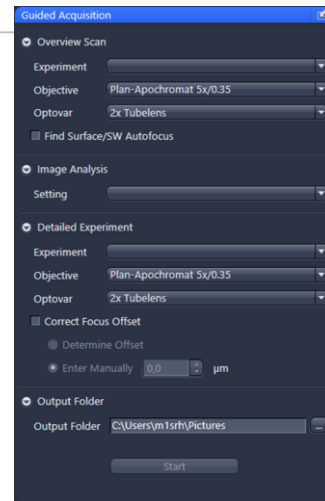
Basefolder for Images and Data Tables c:\Users\m1srh\Documents\Software\Bioformats\6.1.1\ 📁

OK Cancel

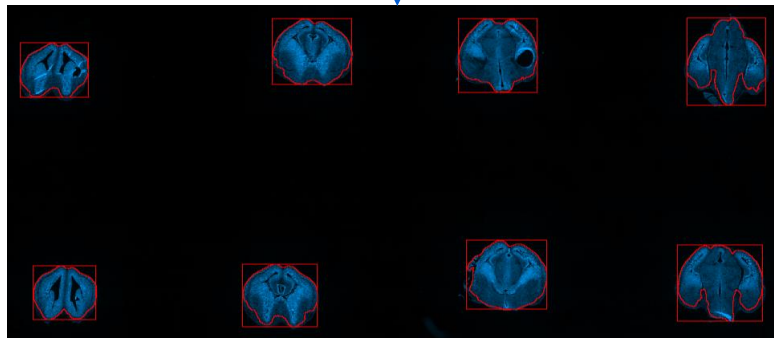
- Guided Acquisition UI is simple and user-friendly
- There is no need for the user to modify script ...
- ... but it can be customized for advanced applications
- Special focusing options depending on the used hardware
- Adaptable due to scripting for advanced applications

Guided Acquisition

Complete automated workflow

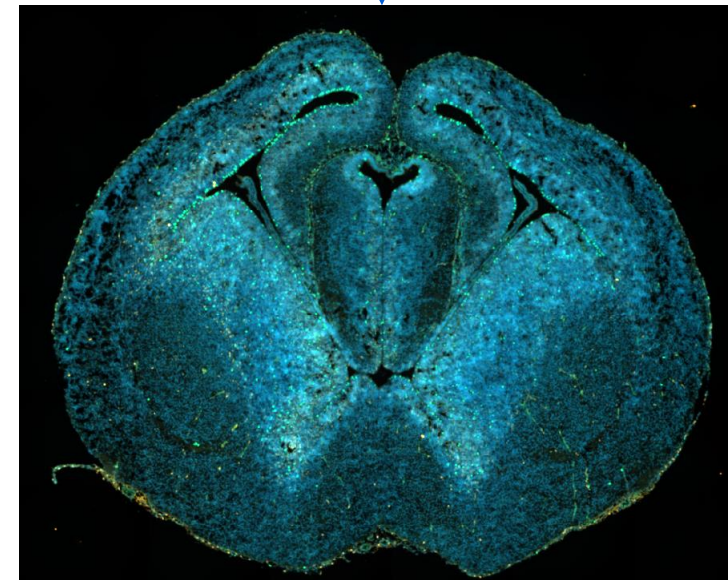


OAD - Automated Acquisition
and Image Analysis



| | 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

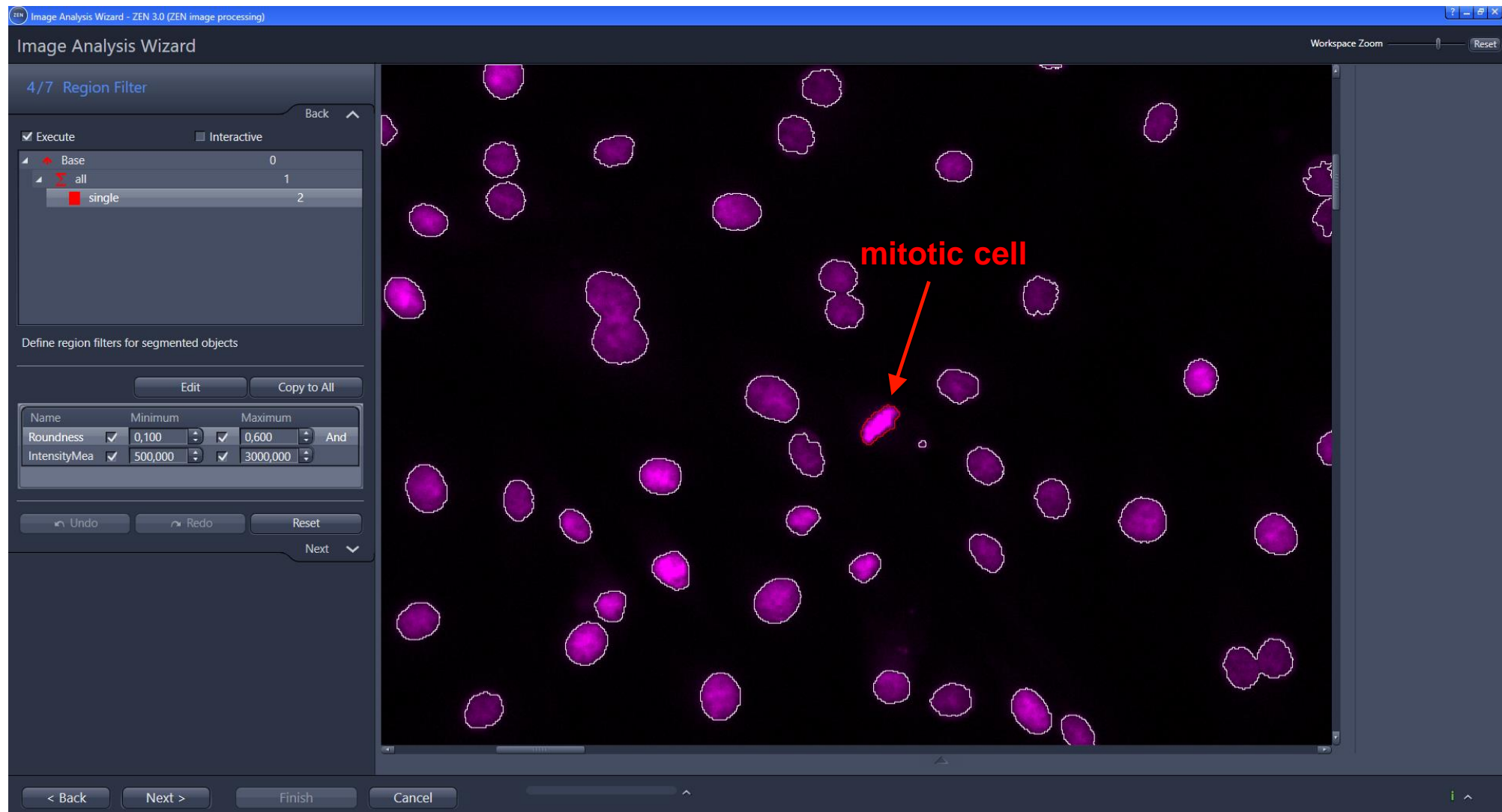
Detect mitotic cells



- 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 ... 😊

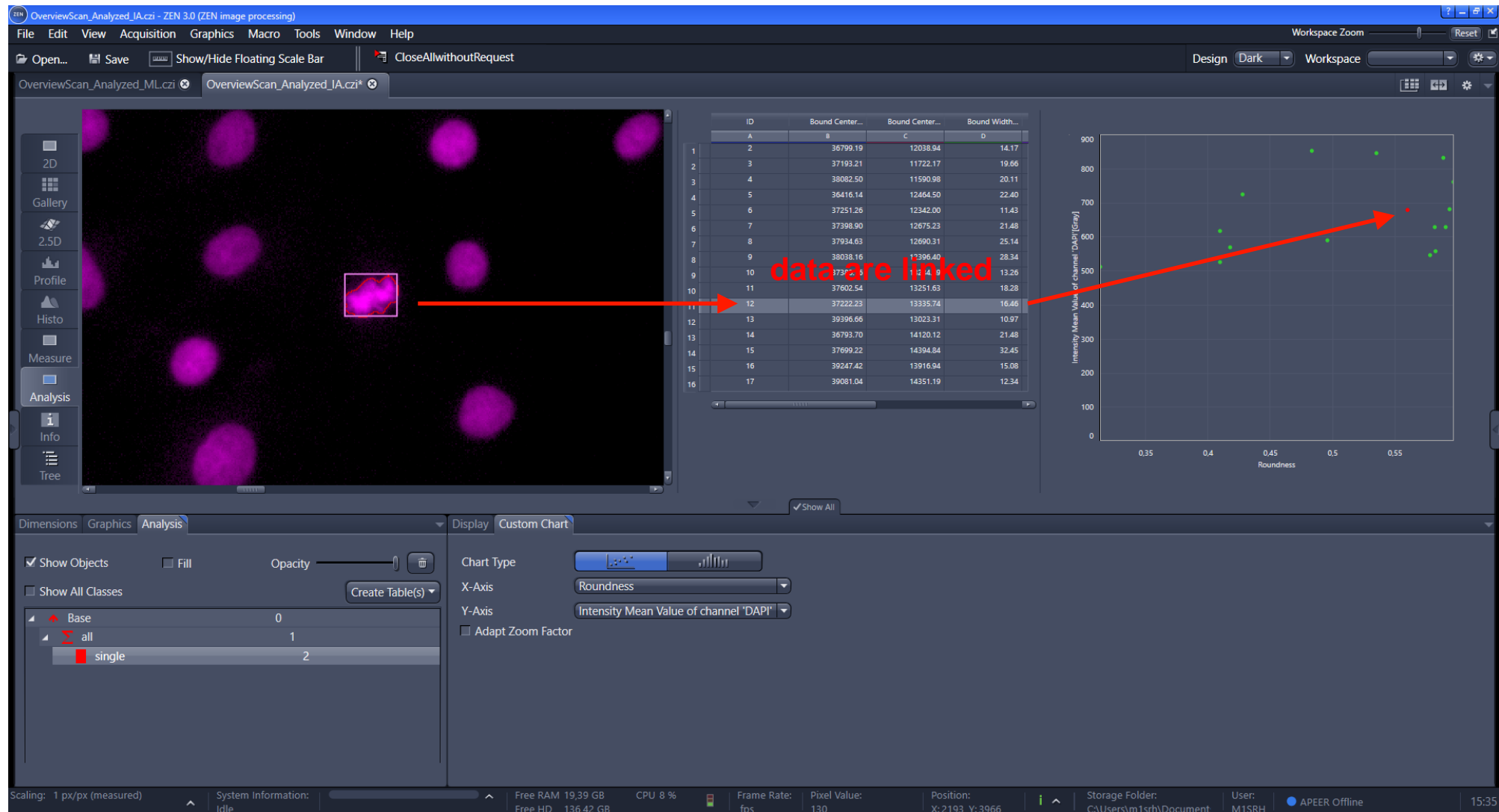
Guided Acquisition Example

Detect mitotic cells (detected using ZEN Image Analysis)



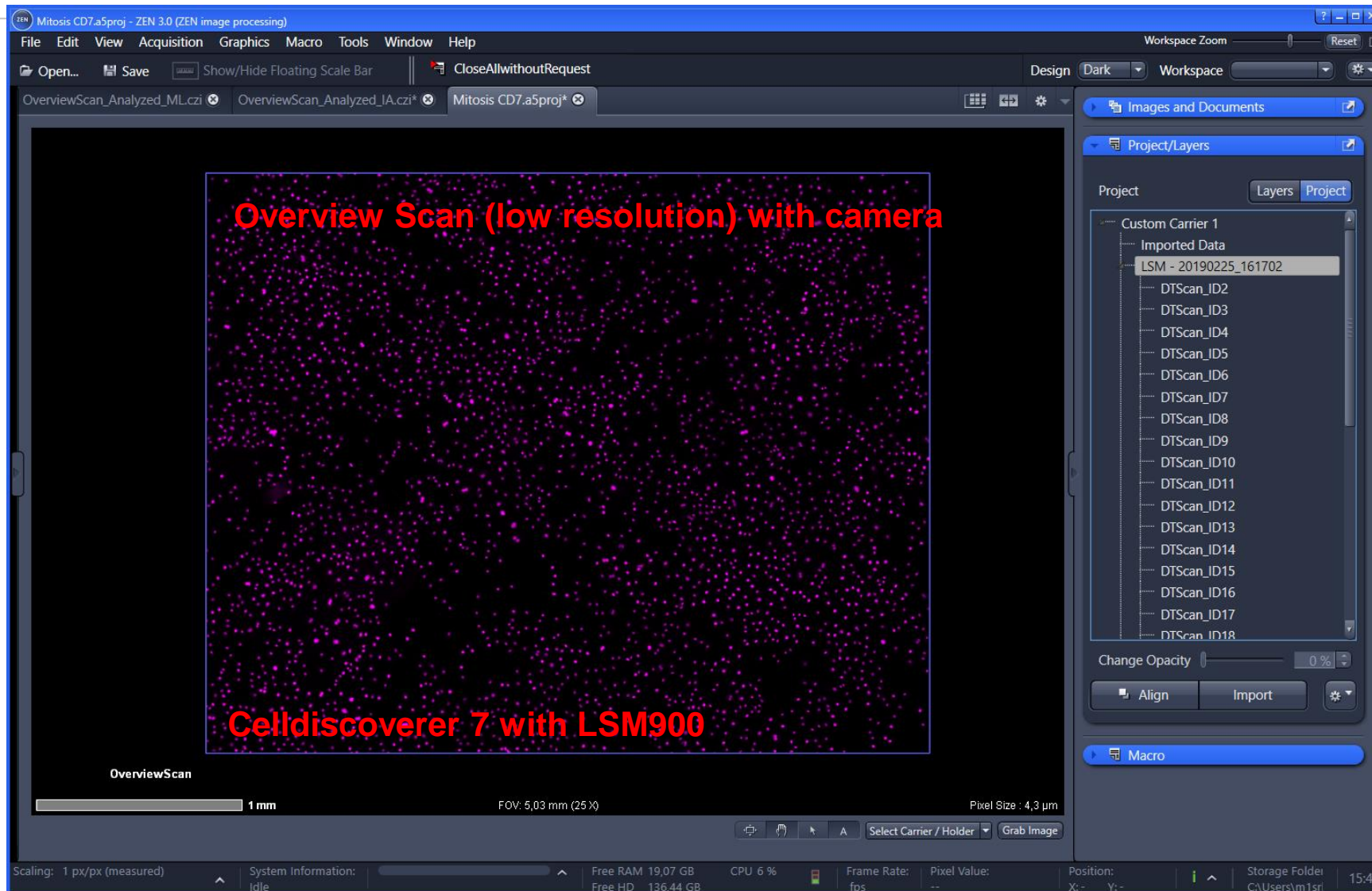
Guided Acquisition Example

Detect mitotic cells – Use interactive tools to create object filters



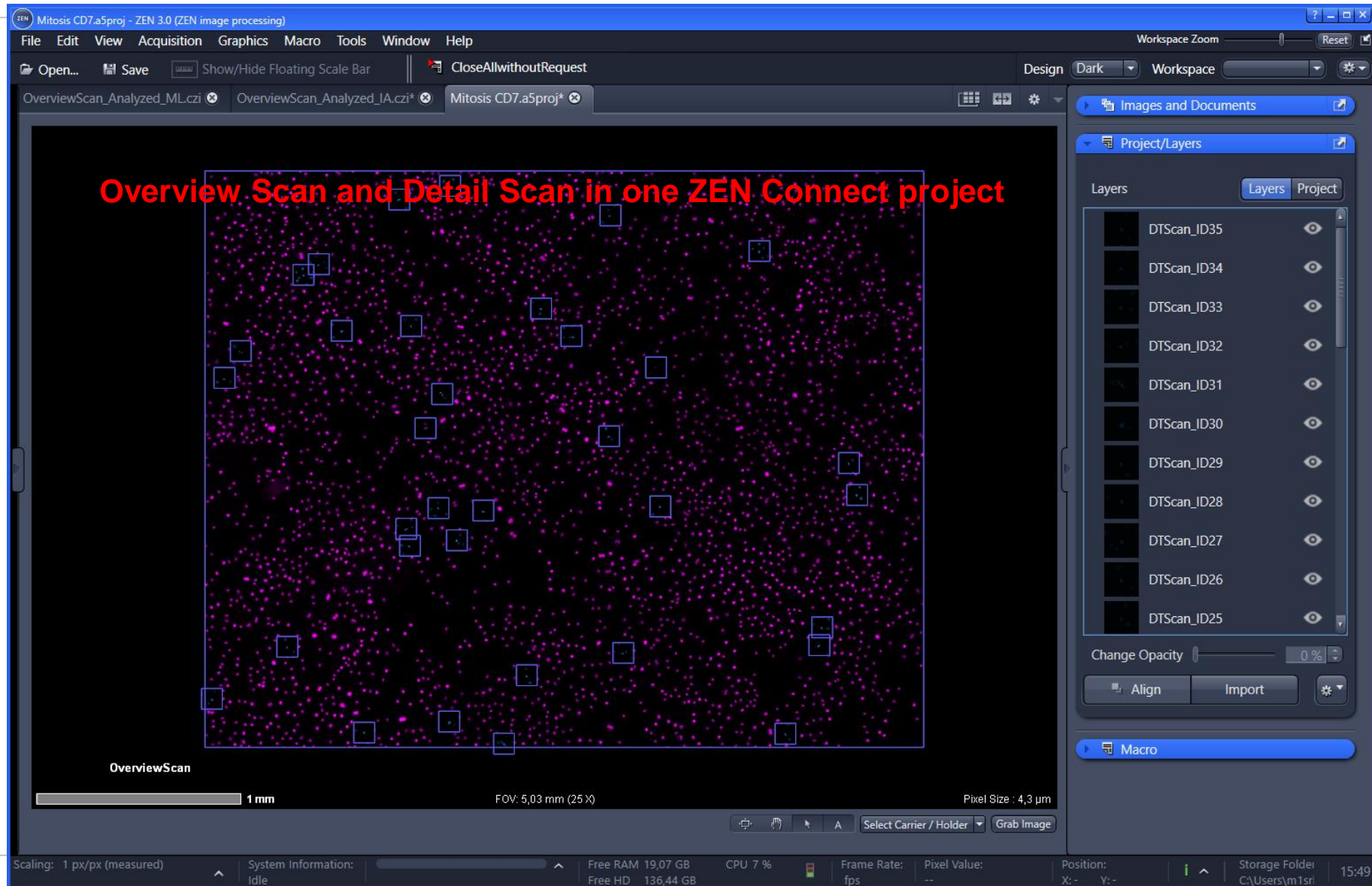
Guided Acquisition Example

Use ZEN Connect for sample-centric data storage



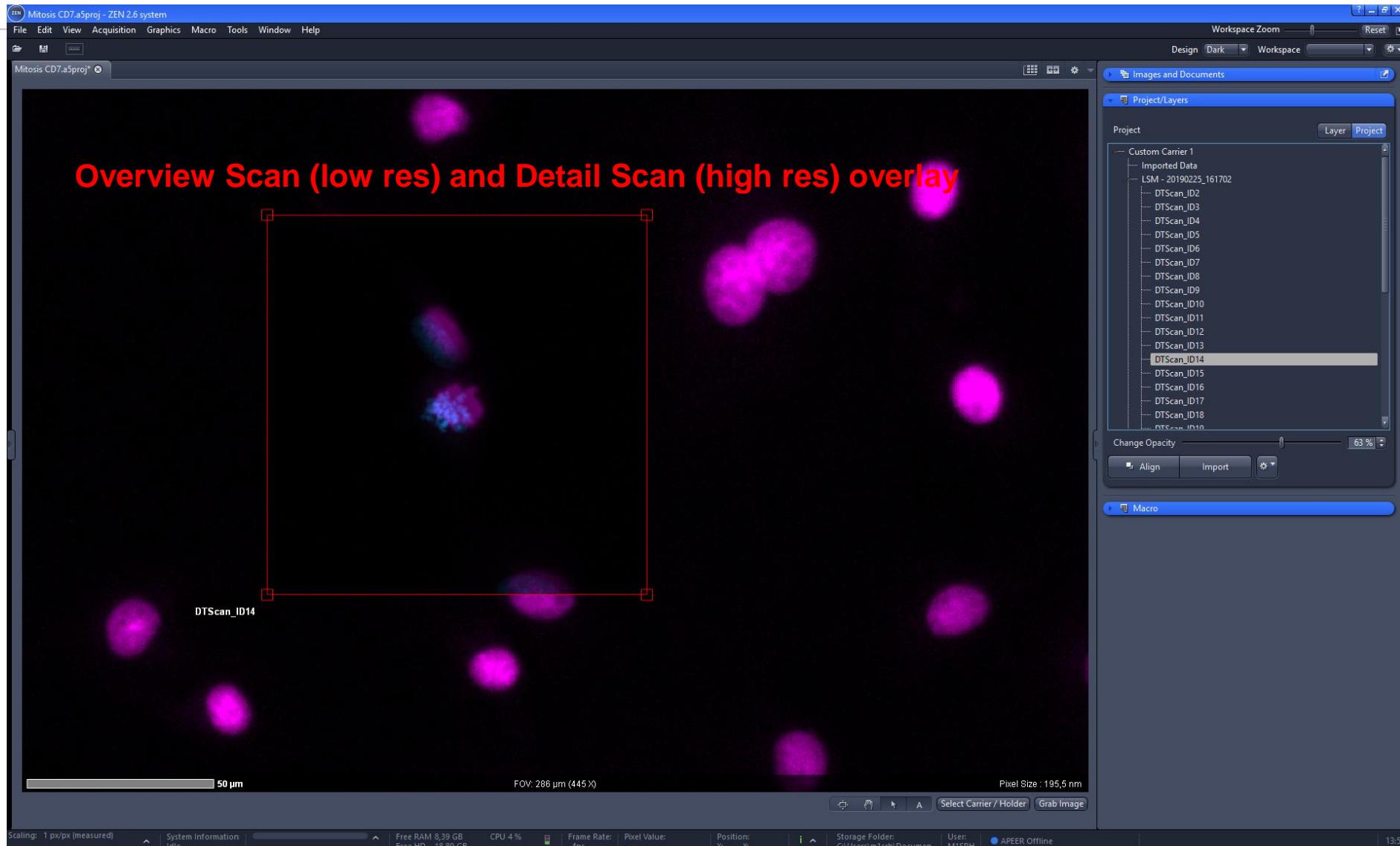
Guided Acquisition Example

Use ZEN Connect for sample-centric data storage



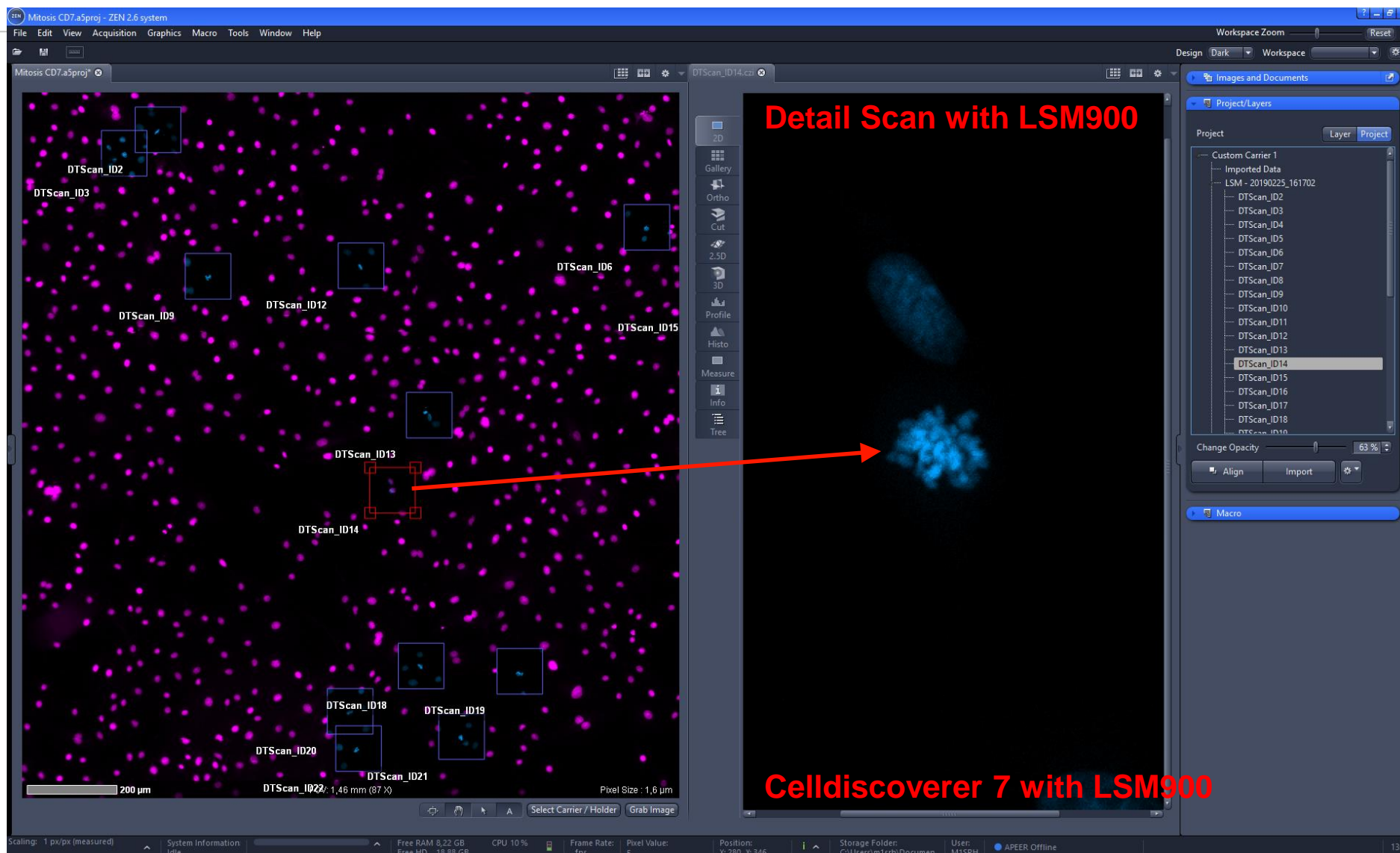
Guided Acquisition

ZEN Connect for sample-centric data storage



Guided Acquisition

ZEN Connect for sample-centric data storage

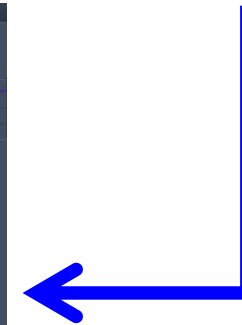
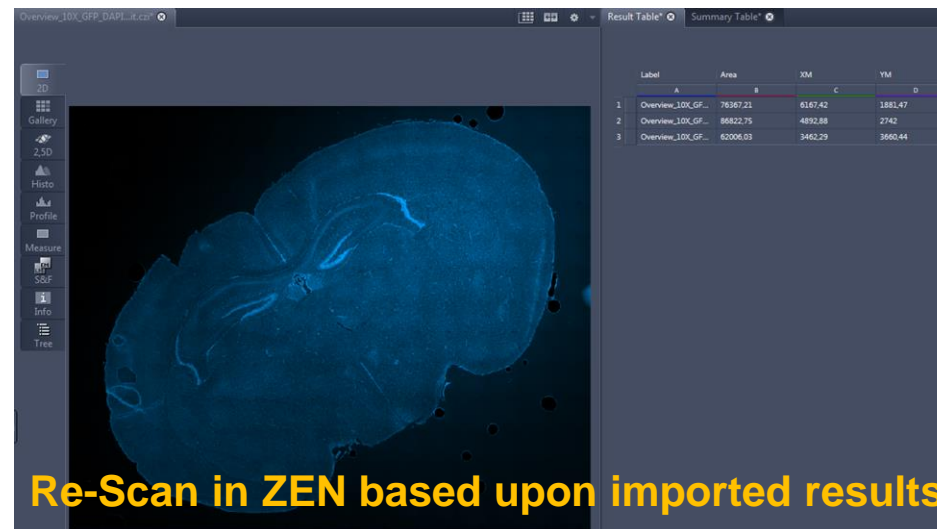


Guided Acquisition - Advanced

Incorporating external image analysis (Fiji)



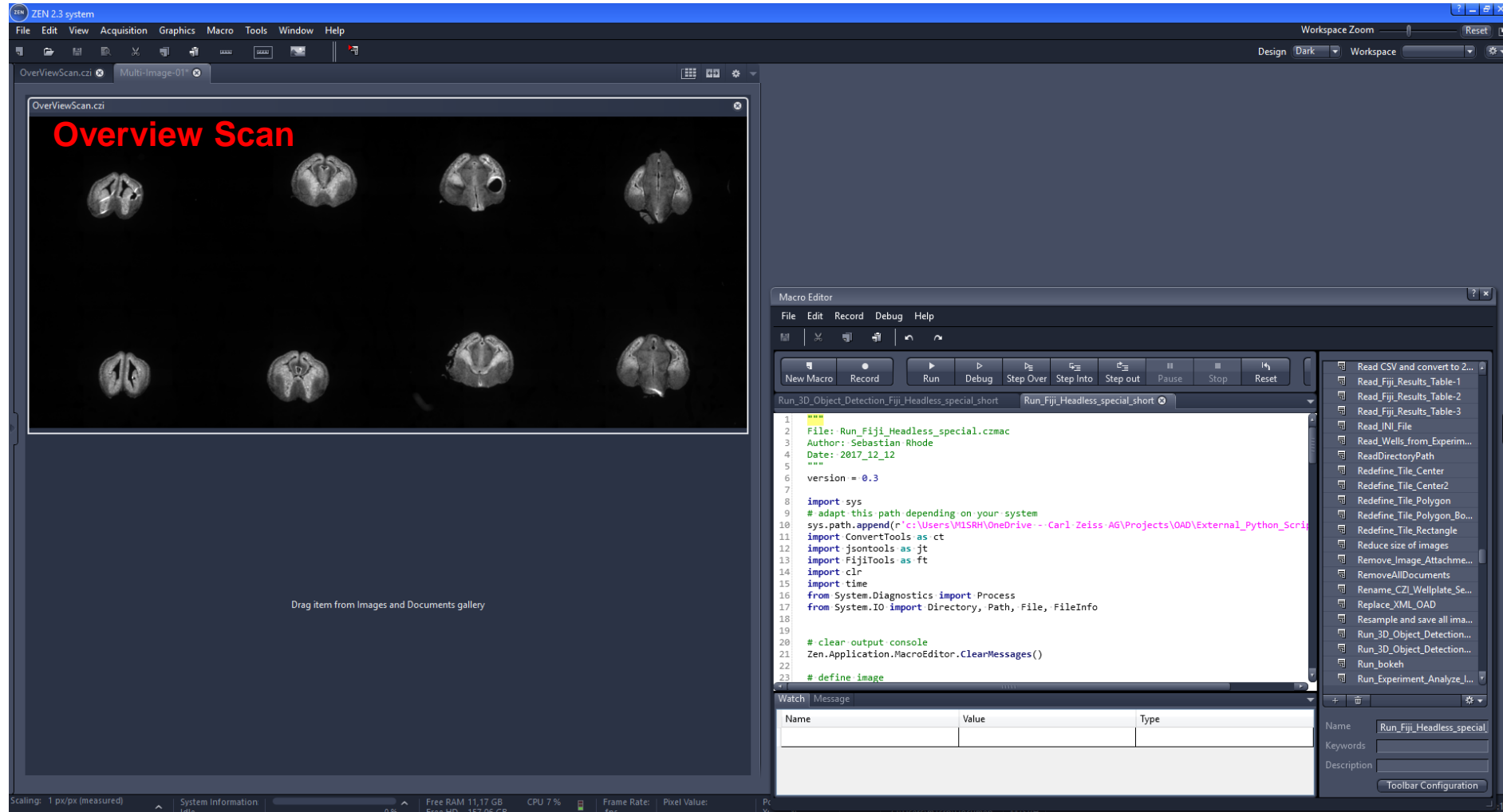
Open in Fiji



Import Results

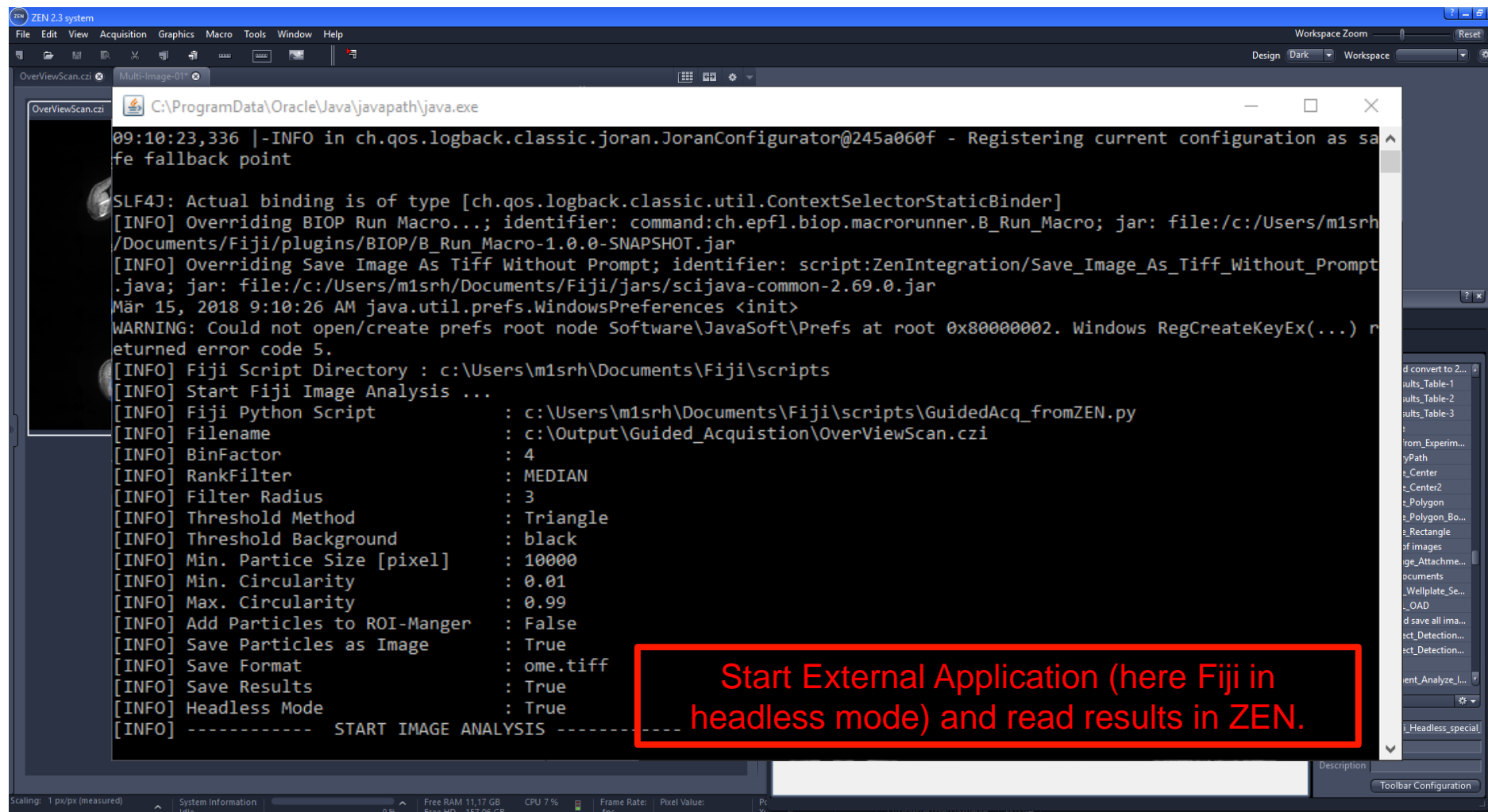
Guided Acquisition Advanced

Run overview scan



Guided Acquisition Advanced

Start external image analysis



The screenshot shows the ZEN 2.3 system interface with a Java command prompt window open. The window title is "C:\ProgramData\Oracle\Java\javapath\java.exe". The log output includes the following text:

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

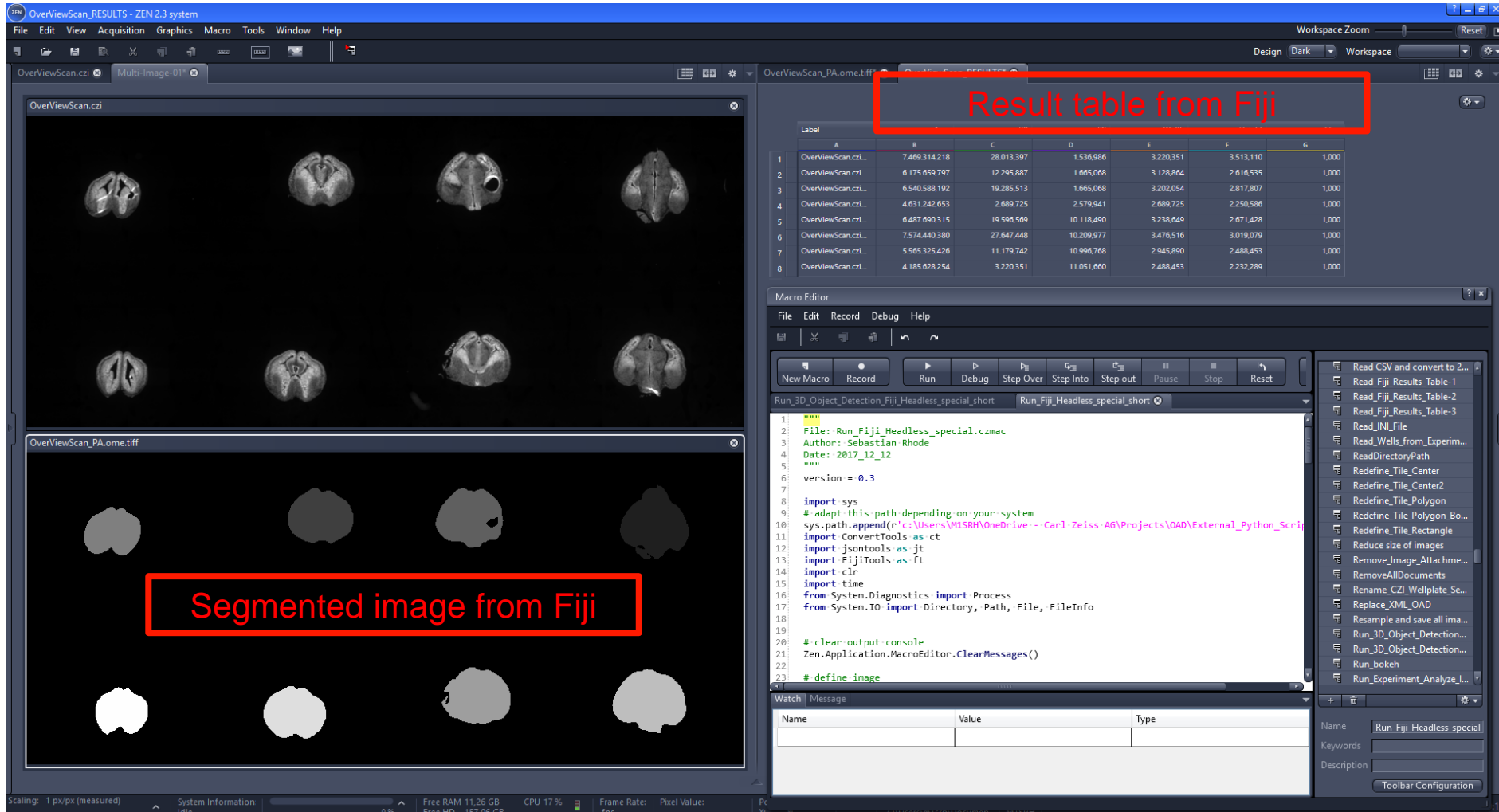
SLF4J: Actual binding is of type [ch.qos.logback.classic.util.ContextSelectorStaticBinder]
[INFO] Overriding BIOP Run Macro...; identifier: command:ch.epfl.biop.macrorunner.B_Run_Macro; jar: file:/c:/Users/m1srh/Documents/Fiji/plugins/BIOP/B_Run_Macro-1.0.0-SNAPSHOT.jar
[INFO] Overriding Save Image As Tiff Without Prompt; identifier: script:ZenIntegration/Save_Image_As_Tiff_Without_Prompt.java; jar: file:/c:/Users/m1srh/Documents/Fiji/jars/scijava-common-2.69.0.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                : c:\Output\Guided_Acquisition\OverViewScan.czi
[INFO] BinFactor               : 4
[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-Manger : False
[INFO] Save Particles as Image   : True
[INFO] Save Format               : ome.tiff
[INFO] Save Results              : True
[INFO] Headless Mode             : True
[INFO] ----- START IMAGE ANALYSIS -----
```

A red rectangular box highlights the following text in the log output:

Start External Application (here Fiji in headless mode) and read results in ZEN.

Guided Acquisition Advanced

Read the results and take action



The screenshot displays the Zen 2.3 system interface. The top-left panel shows a grid of eight brain scan images. The bottom-left panel shows a grid of eight segmented brain images, with a red box highlighting the text "Segmented image from Fiji". The top-right panel shows a table of results, with a red box highlighting the text "Result table from Fiji". The bottom-right panel shows the Macro Editor with a script for running a Fiji macro.

Result table from Fiji

| Label | A | B | C | D | E | F | G |
|-----------------------|---------------|------------|------------|-----------|-----------|-------|---|
| 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 | |

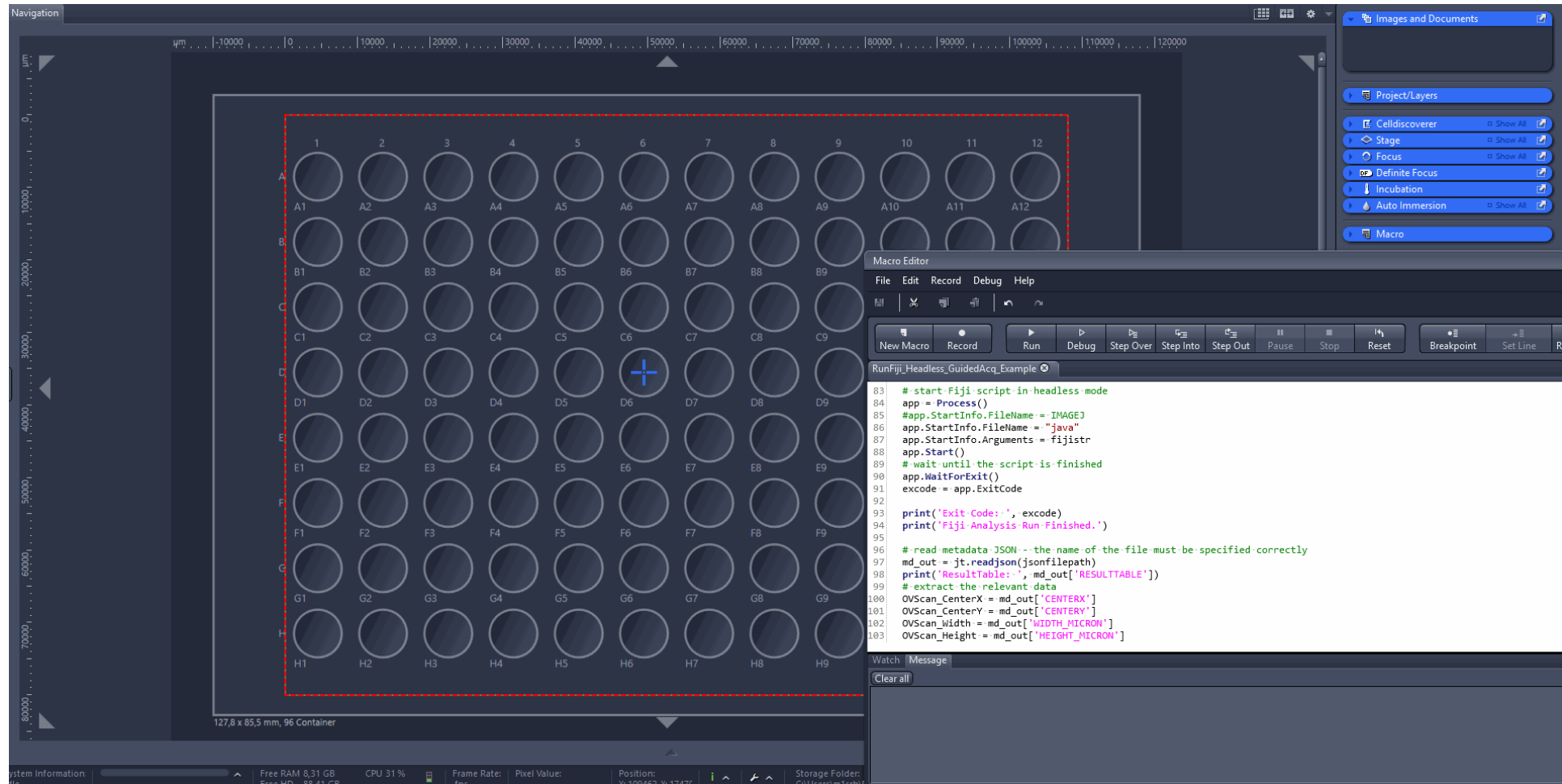
Segmented image from Fiji

Macro Editor

```
1 File: Run_Fiji_Headless_special.czm
2 Author: Sebastian Rhode
3 Date: 2017_12_12
4 version = 0.3
5
6 import sys
7 # adapt this path depending on your system
8 sys.path.append(r'c:\Users\W15SRH\OneDrive -- Carl-Zeiss AG\Projects\OAD\External_Python_Scripts')
9 import ConvertTools as ct
10 import jsonTools as jt
11 import FijiTools as ft
12 import clr
13 import time
14 from System.Diagnostics import Process
15 from System.IO import Directory, Path, File, FileInfo
16
17 # clear output console
18 Zen.Application.MacroEditor.ClearMessages()
19
20 # define image
```

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>

