Guided Acquisition and Adaptive Feedback Micoscopy

Adaptive Feedback Microscopy
What problems and the challenges of typical acquisition workflows?

- 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”

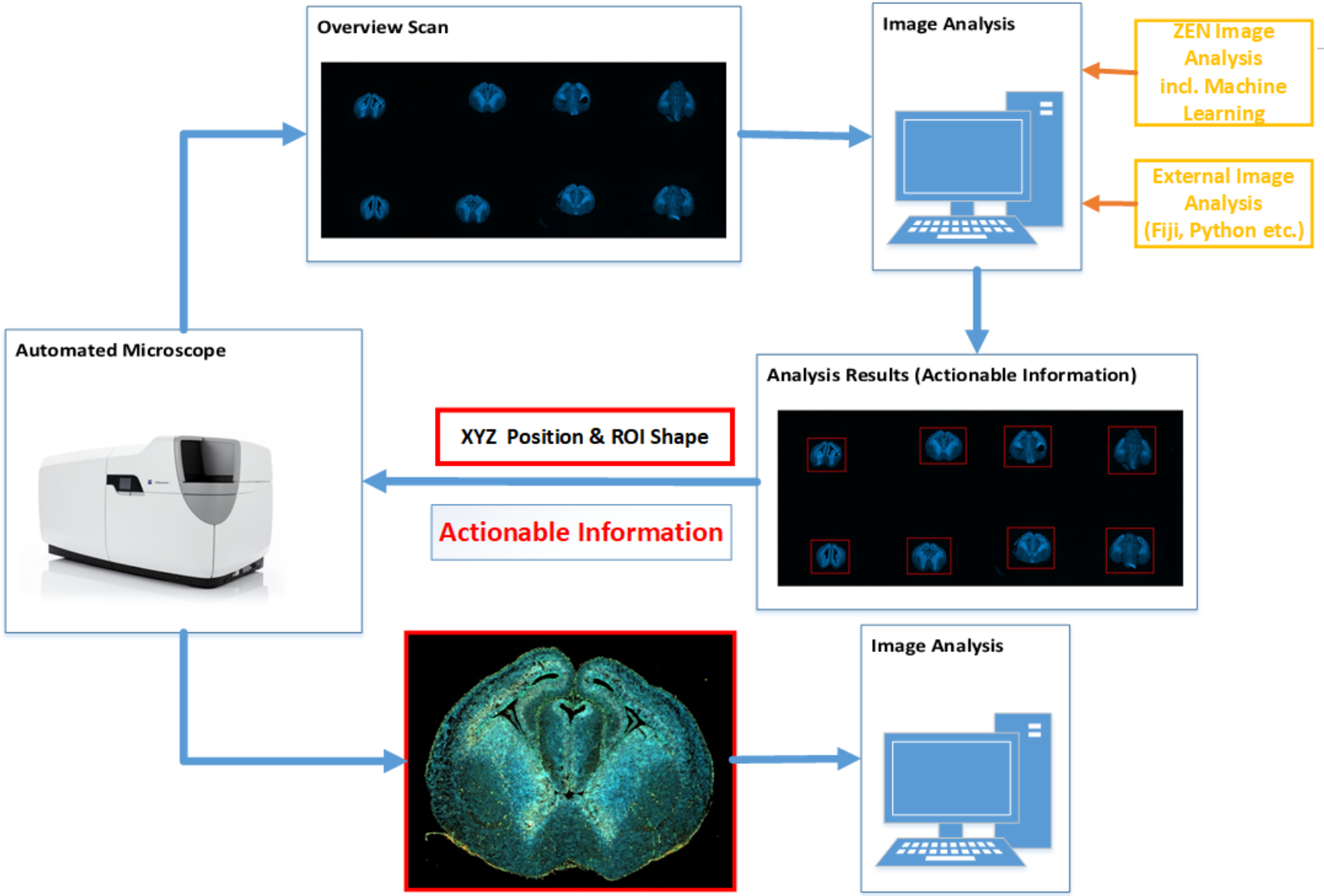
- Reliable statistics benefit reproducible acquisition workflows, so Automation is key.

- “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 be 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 !

Adaptive Feedback Microscopy
ZEN blue Toolbox

- **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.

Adaptive Feedback Microscopy
ZEN Blue Toolbox

- **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

Adaptive Feedback Microscopy
General Questions

- 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**?

Adaptive Feedback Microscopy
Data Format Considerations

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

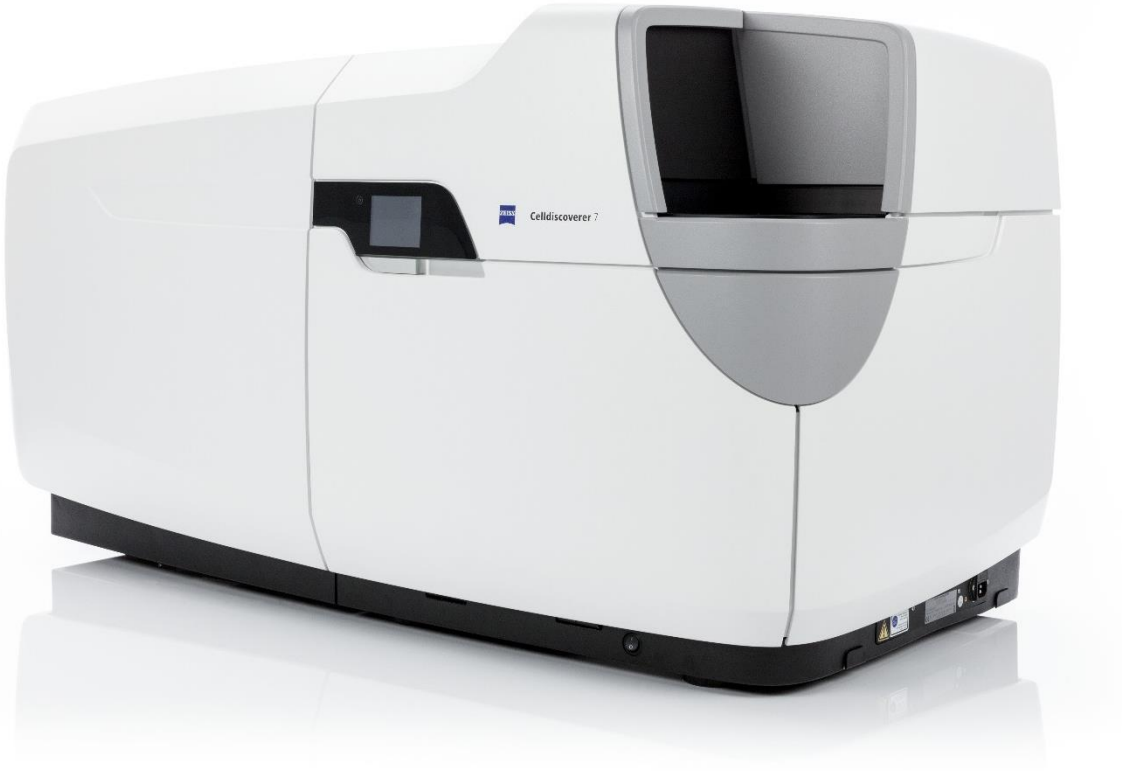
- Constant exchange with BioFormats team to keep **their** CZIReader up-to-date

- **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)

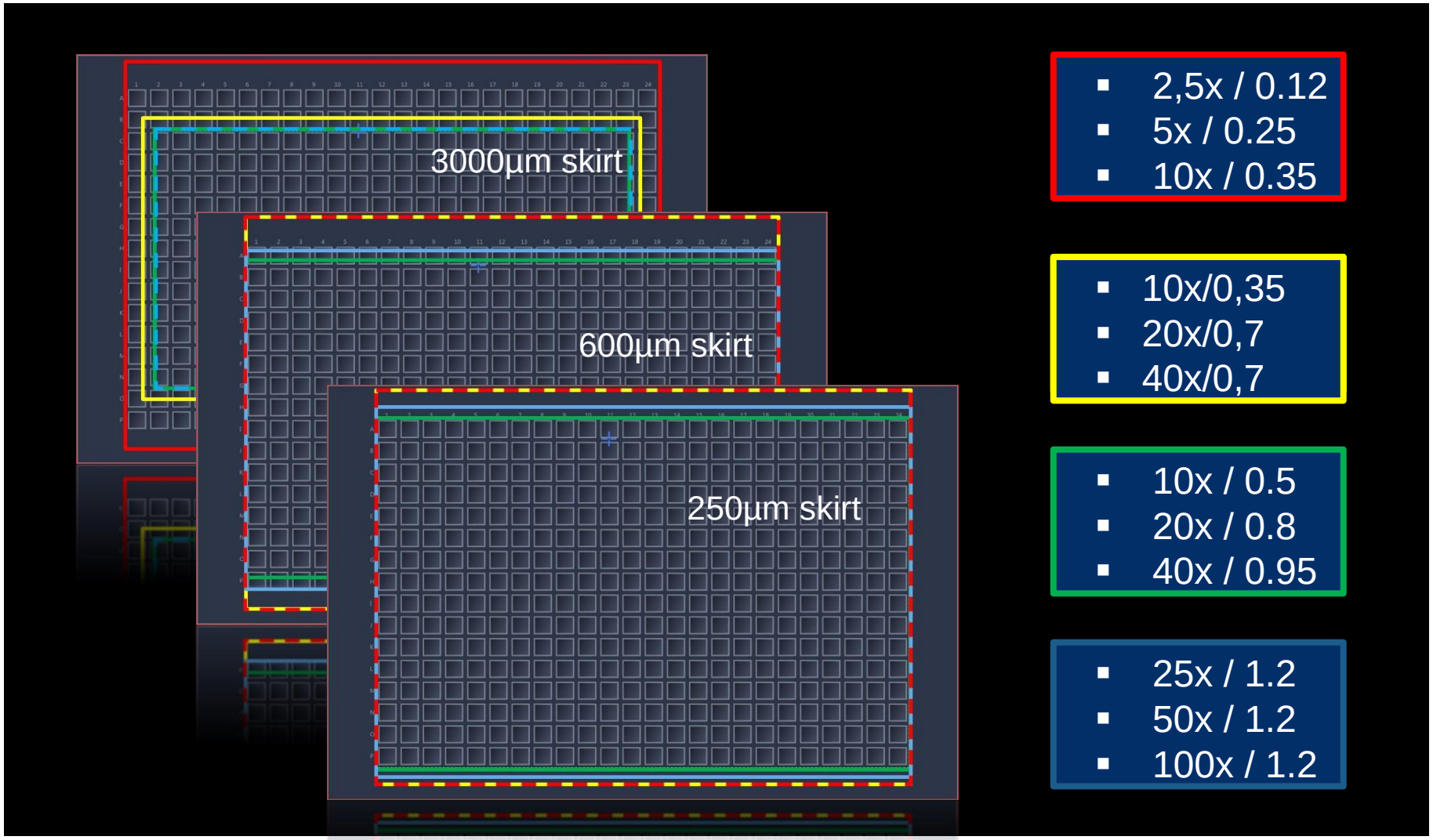
Adaptive Feedback Microscopy
System and Software Requirements

- **motorized** and **calibrate**d 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 …

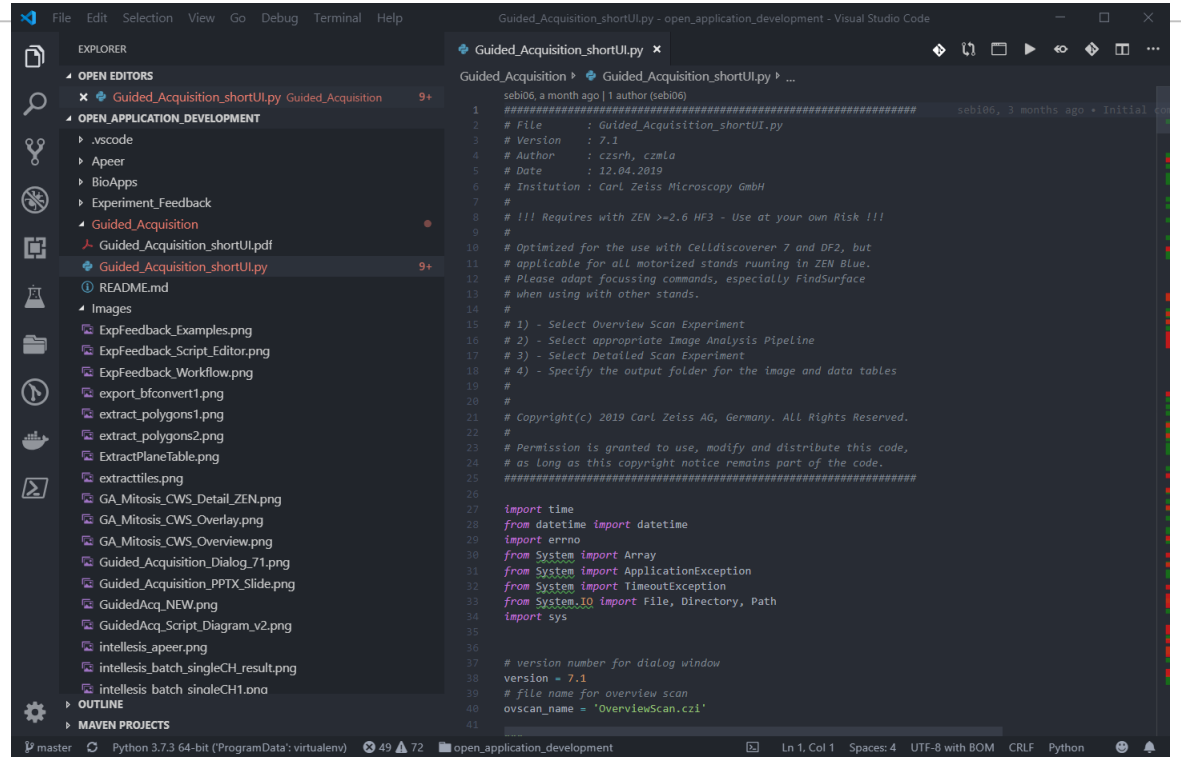
Adaptive Feedback Microscopy
When things go wrong … Adaptive Lens Guard

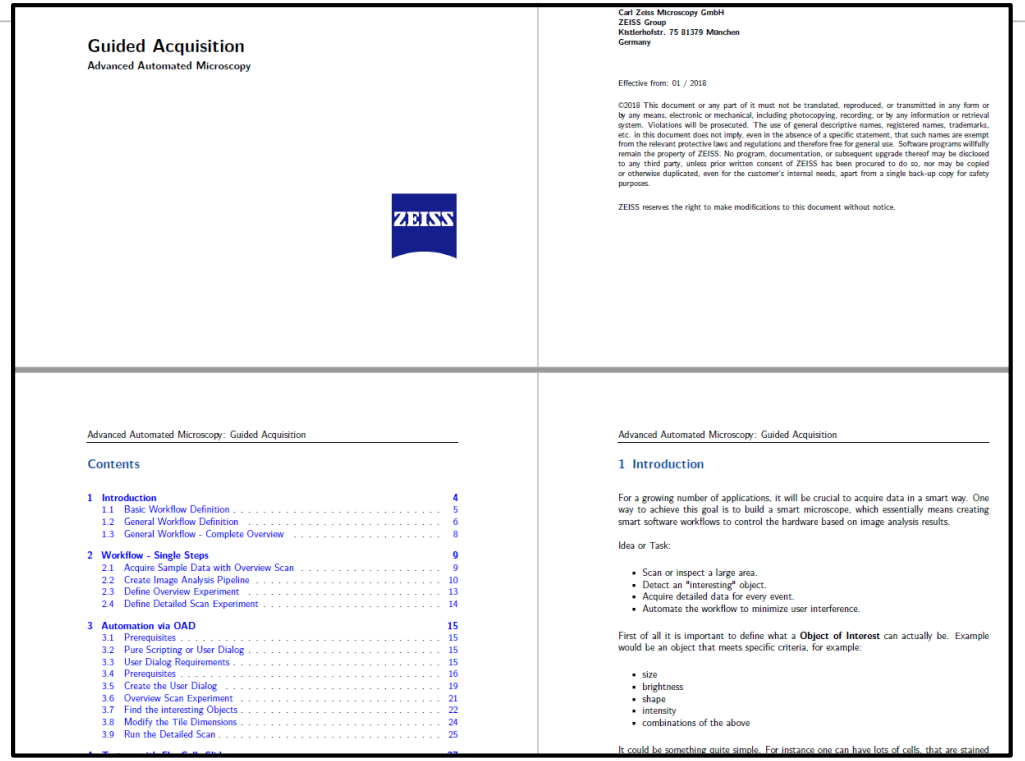


So what is solution ZEN blue can offer?

Guided Acquisition

Guided Acquisition
Do I have to create this solution by myself?




Guided Acquisition
Advanced Automated Microscopy

Carl Zeiss Microscopy GmbH
ZEISS Group
Klettherhofstr. 75 81379 München
Germany

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1 Introduction

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.

Idea or Task:

- Scan or inspect a large area.
- Detect an "interesting" object.
- Acquire detailed data for every event.
- Automate the workflow to minimize user interference.

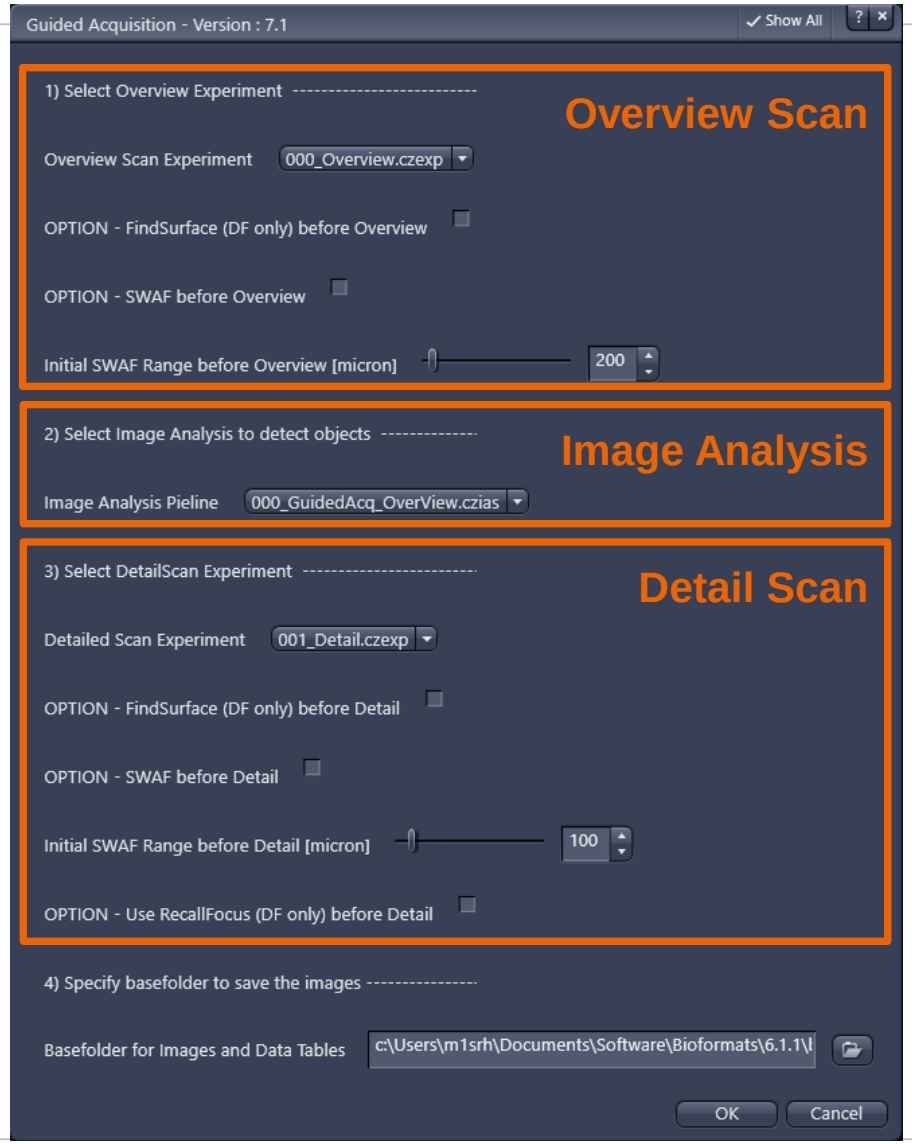
First of all it is important to define what a **Object of Interest** can actually be. Example would be an object that meets specific criteria, for example:

- size
- brightness
- shape
- intensity
- combinations of the above

It could be something quite simple. For instance one can have lots of cells, that are stained

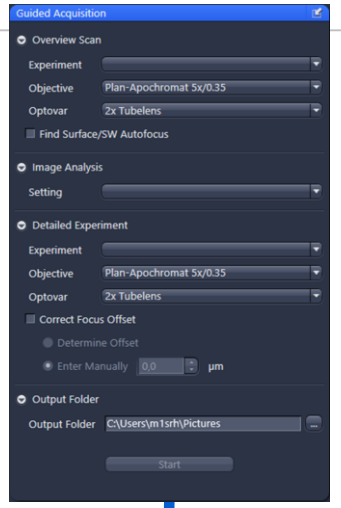
- ReadMe: https://github.com/zeiss-microscopy/OAD/blob/master/Guided_Acquisition/README.md
- Source Code: https://github.com/zeiss-microscopy/OAD/blob/master/Guided_Acquisition/Guided_Acquisition_shortUI.py
- Tutorial: https://github.com/zeiss-microscopy/OAD/blob/master/Guided_Acquisition/Guided_Acquisition_shortUI.pdf

Guided Acquisition User Interface
Script- based for maximal flexibility and Ease-of-Use

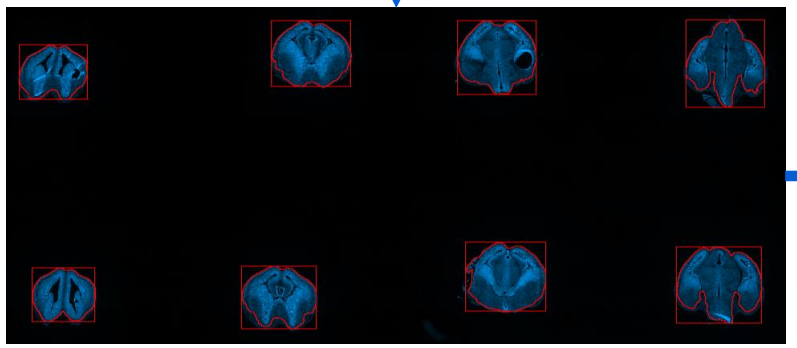


- Guided Acquisition UI is simple and user-friendly
- There is no need for the user to modify script …
- … but it can be customized for advanced applications
- Special focusing options depending on the used hardware
- Adaptable due to scripting for advanced applications

Guided Acquisition
Complete automated workflow



OAD - Automated Acquisition and Image Analysis



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	A	B	C	D	E
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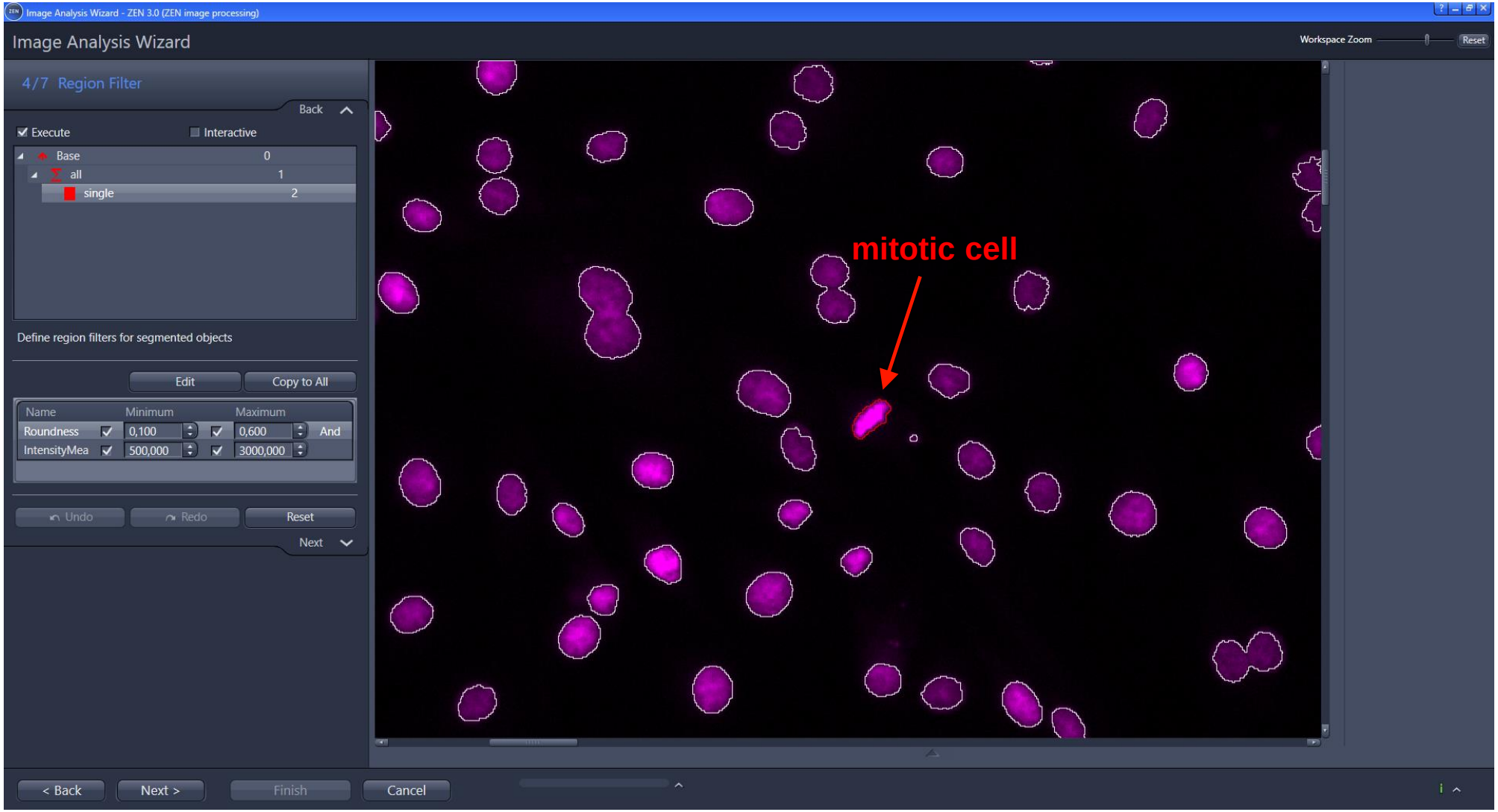
2	3	47.835,969	10.371,678	2.996,208	2.799,510
3	4	30.956,571	10.934,324	2.584,515	2.085,909
4	5	56.540,981	10.604,970	2.996,208	3.320,987
5	6	31.372,838	19.403,757	2.392,392	2.035,591
6	7	48.176,759	18.745,048	3.046,525	2.639,407
7	8	56.309,976	19.044,669	3.238,649	2.909,295
8	9	39.556,373	19.508,967	2.831,530	2.383,243

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

Guided Acquisition Example
Detect mitotic cells

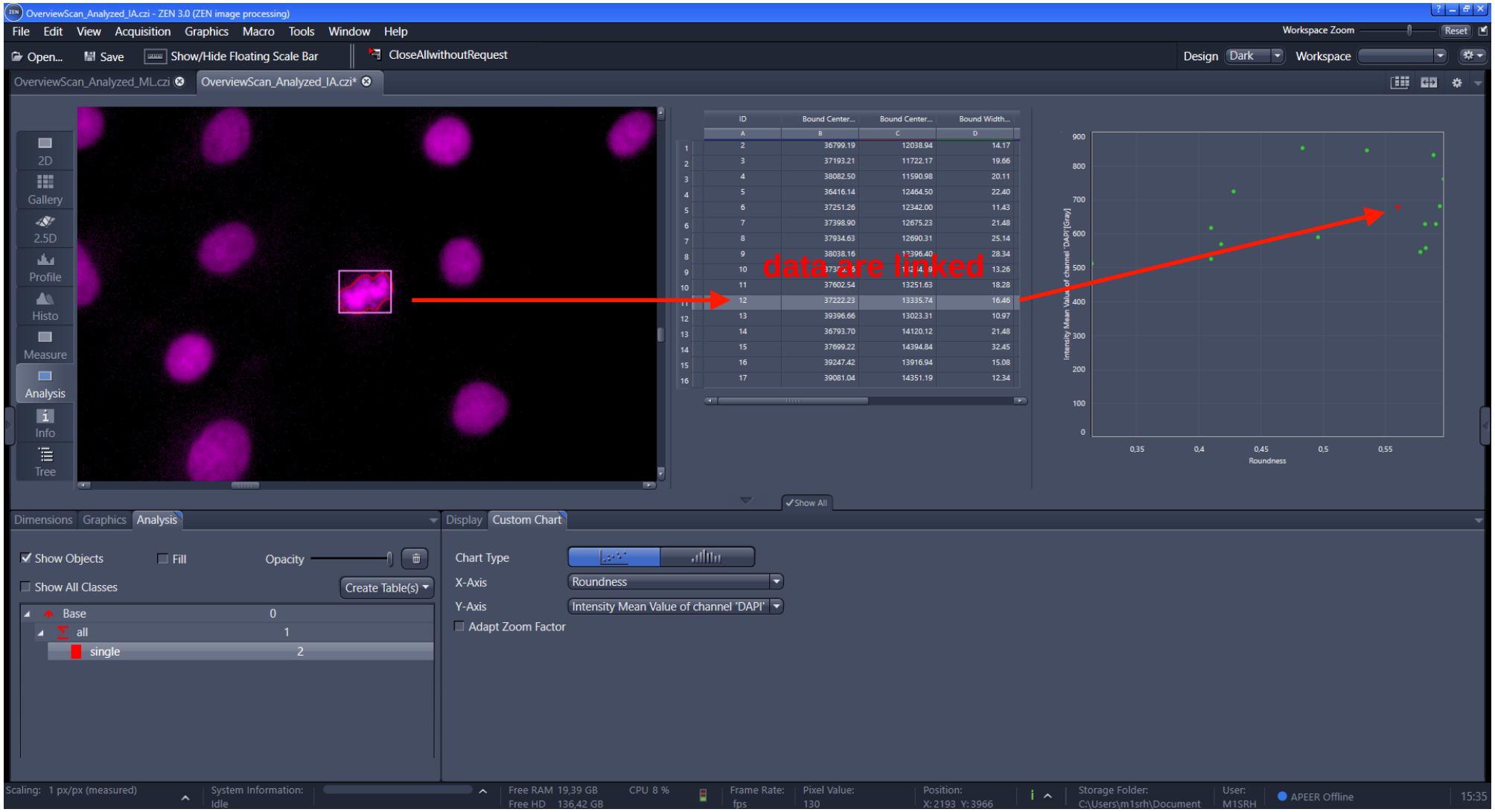
- Acquire a test image with many cells
- Setup an Image Analysis Setting to segment the desired objects of interests or
- Train a Intellesis model that can be used to segment the objects
- “Finetune” the Image Analysis by using Region Filters
- Specify the required features for the Guided Acquisition
- 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)



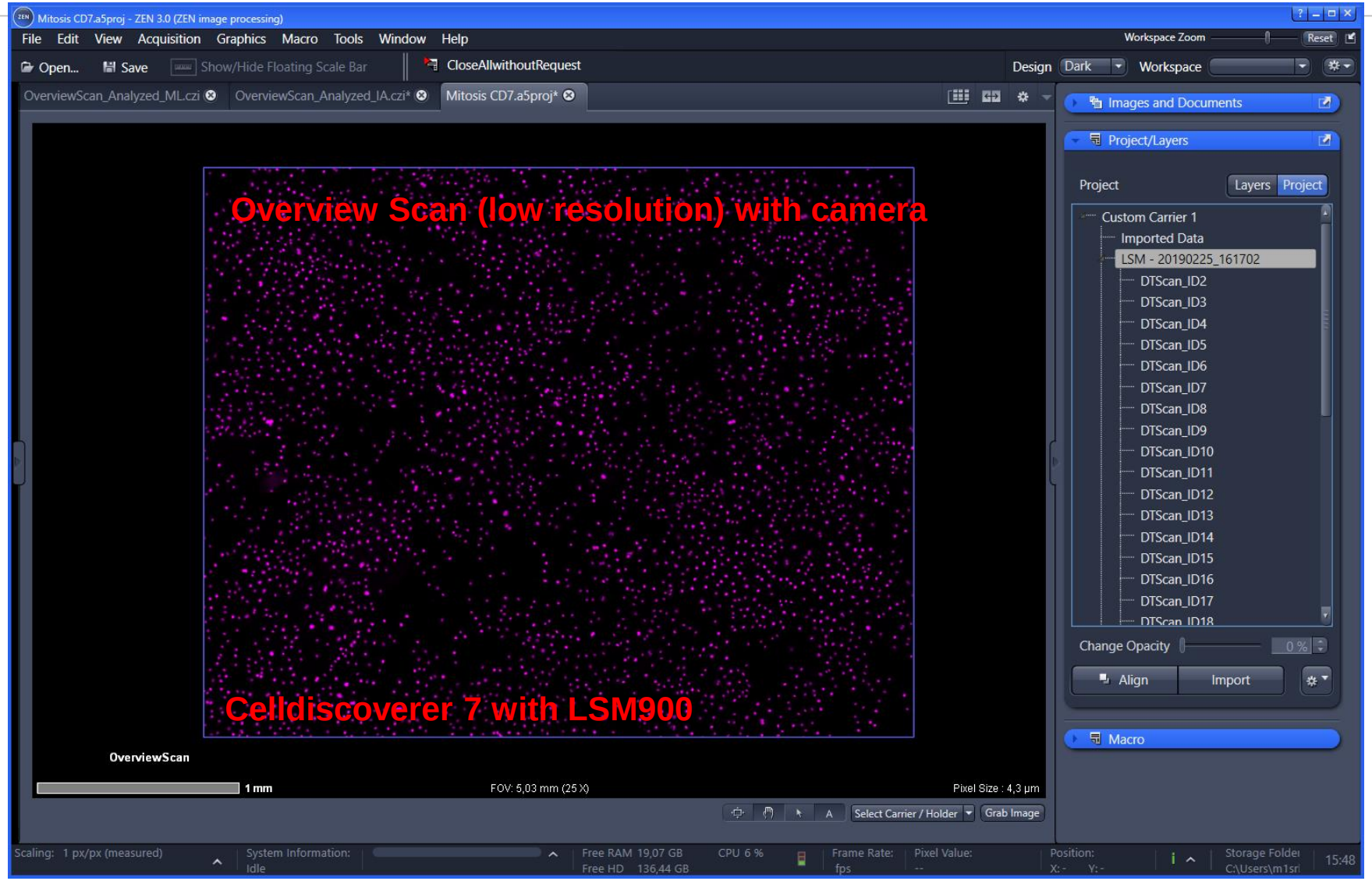
Guided Acquisition Example

Detect mitotic cells – Use interactive tools to create object filters



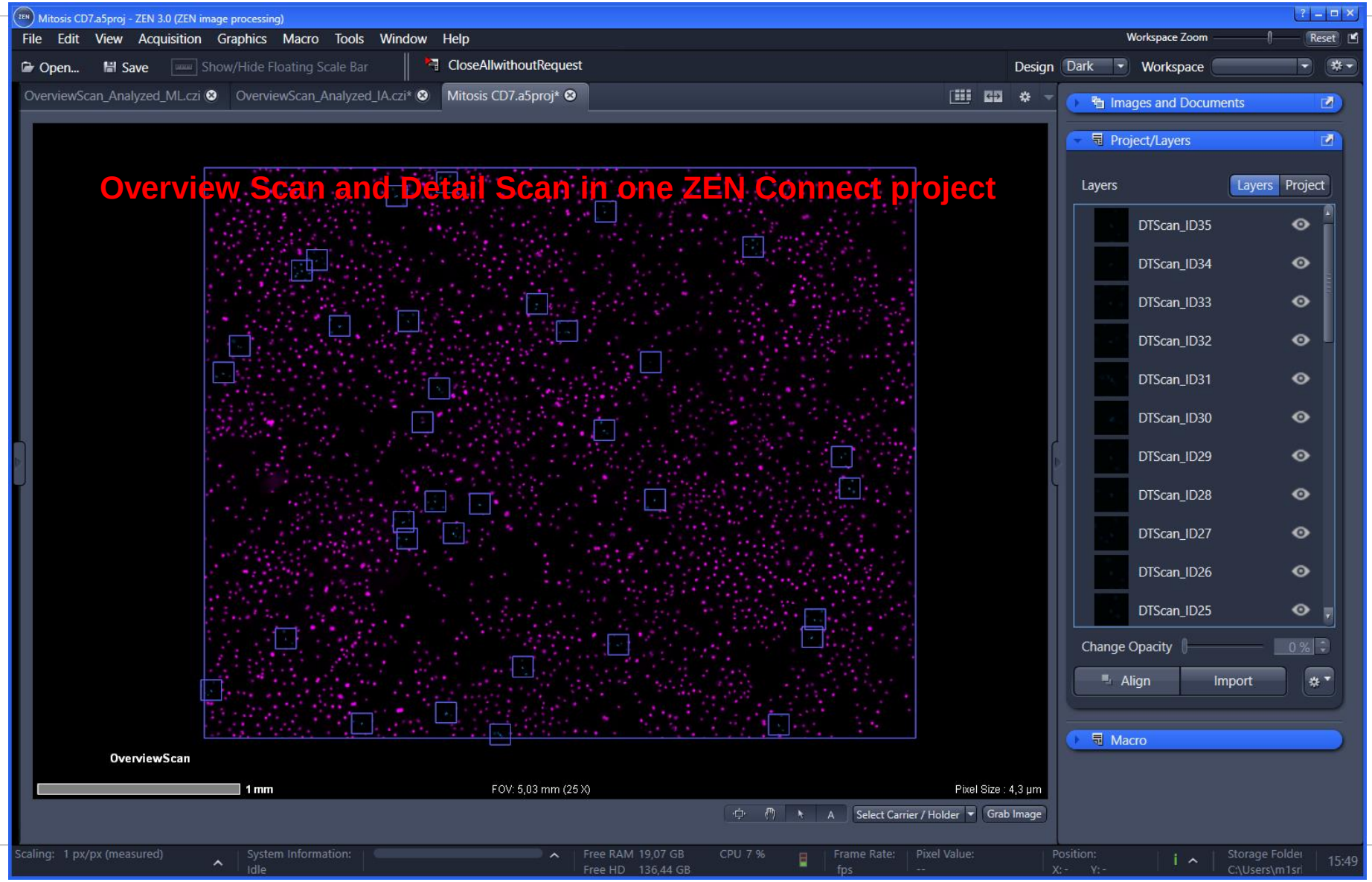
Guided Acquisition Example

Use ZEN Connect for sample-centric data storage

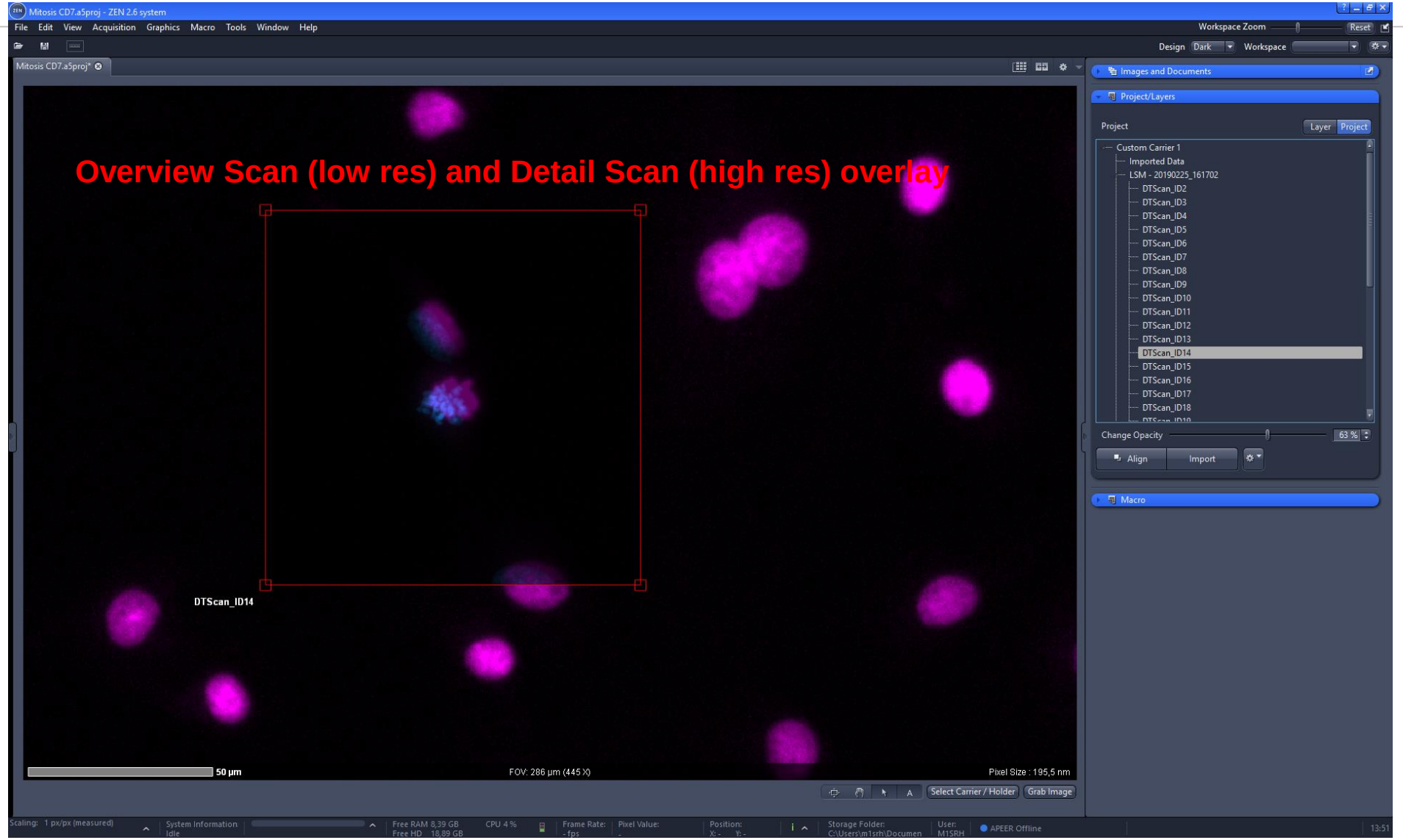


Guided Acquisition Example

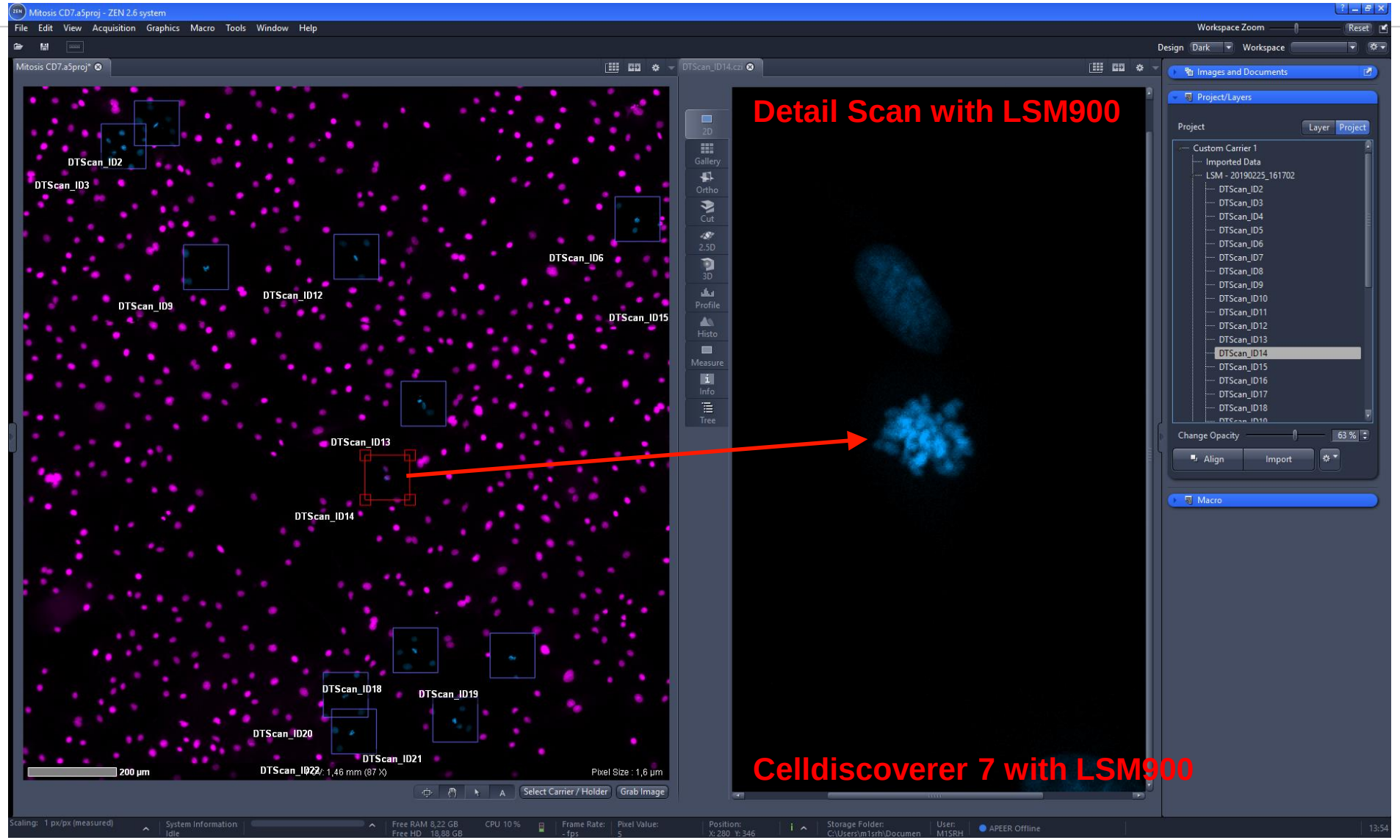
Use ZEN Connect for sample-centric data storage



Guided Acquisition
ZEN Connect for sample-centric data storage

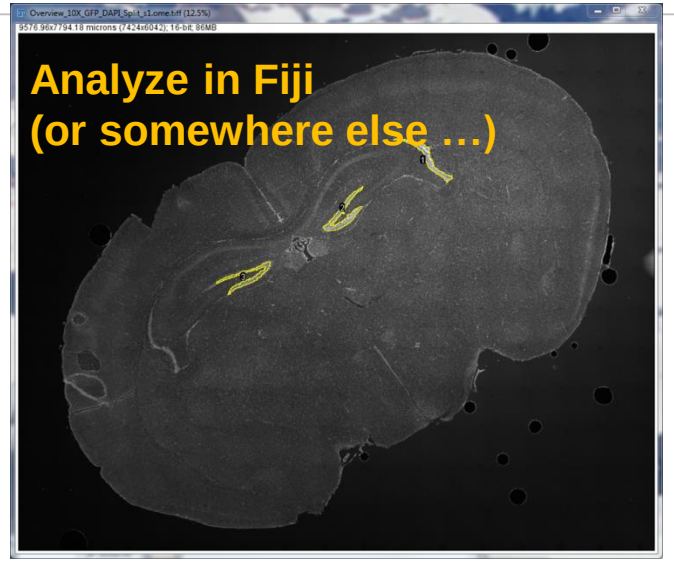


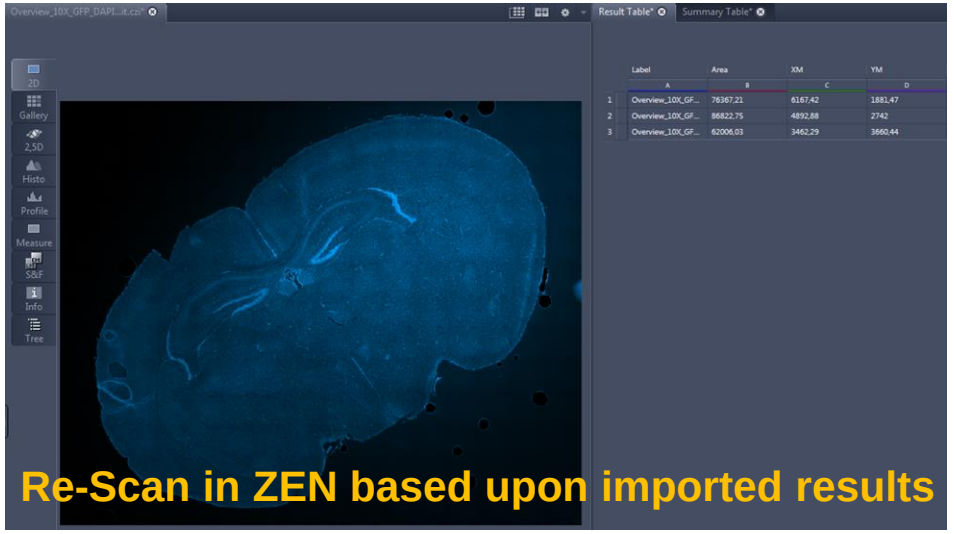
Guided Acquisition
ZEN Connect for sample-centric data storage



Guided Acquisition - Advanced
Incorporating external image analysis (Fiji)

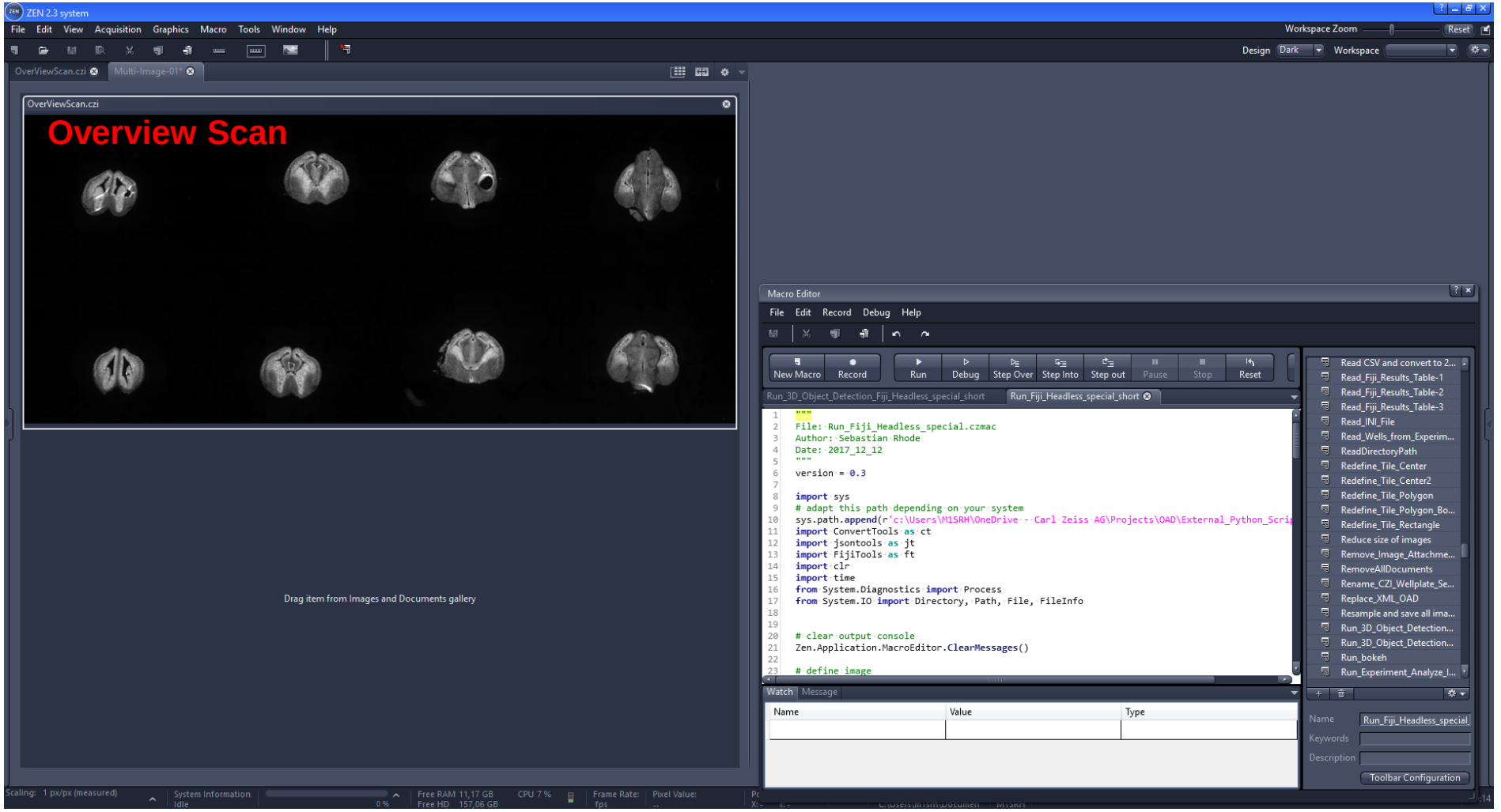
Open in Fiji



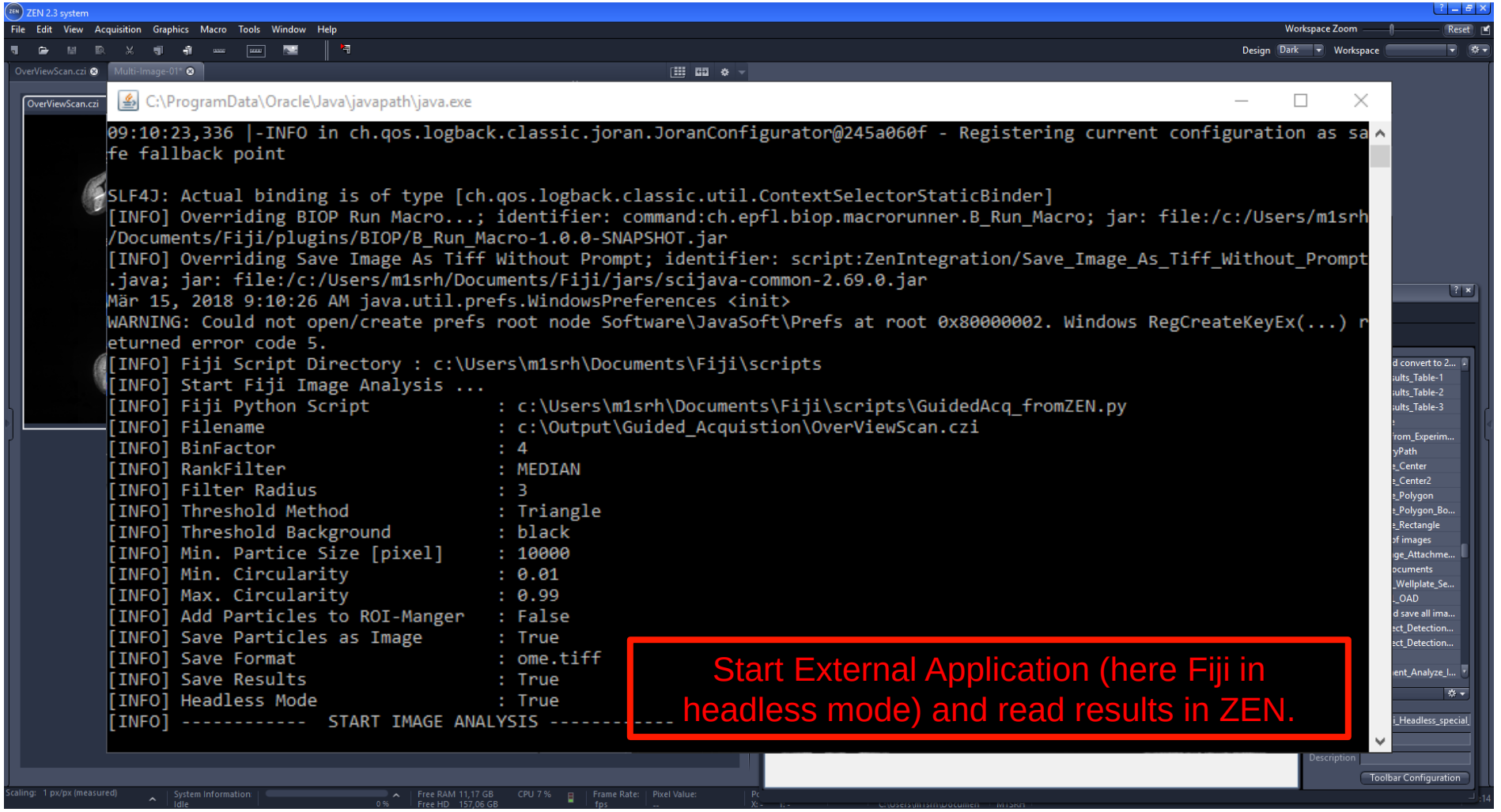


Import Results

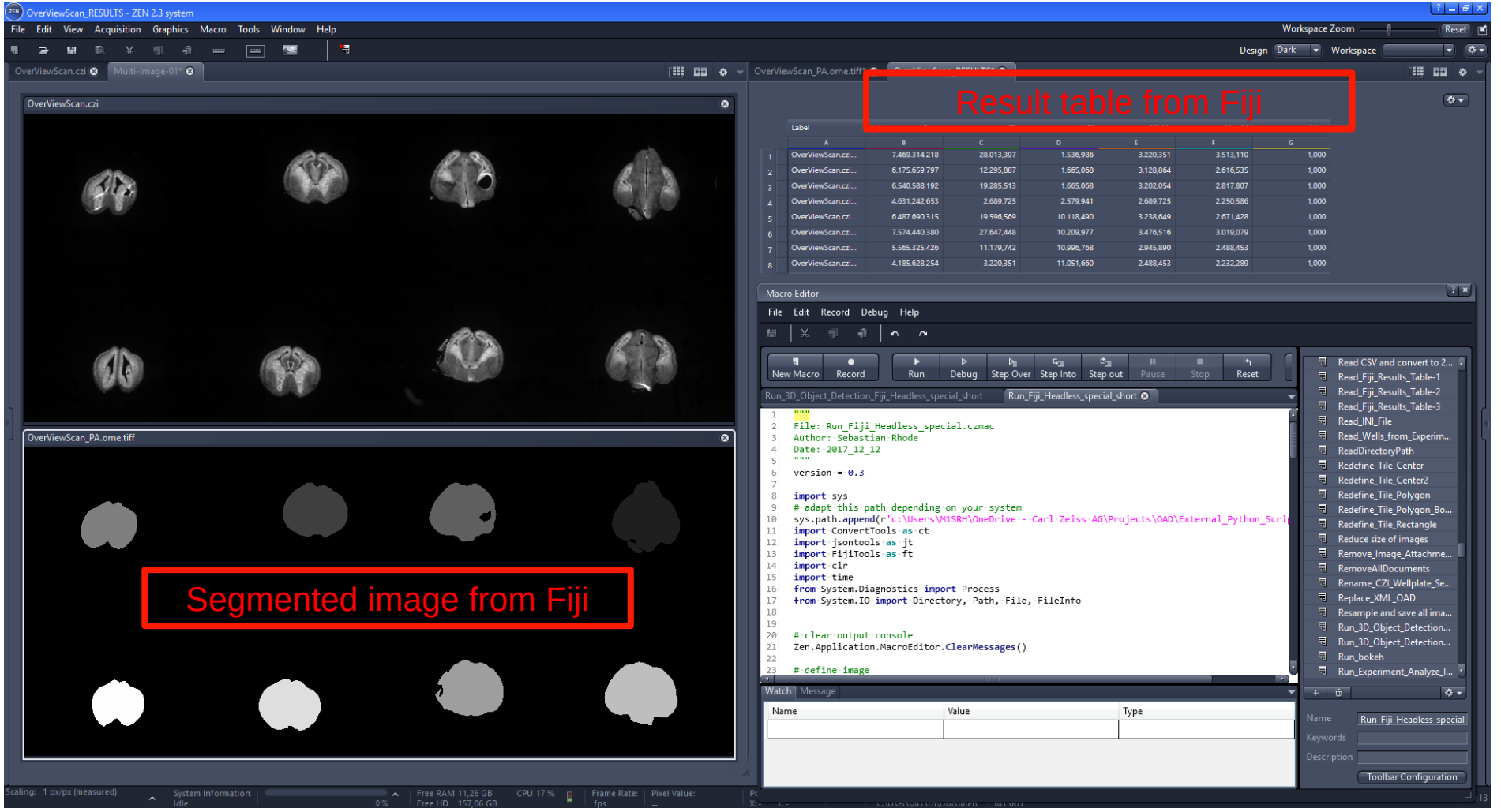
Guided Acquisition Advanced
Run overview scan



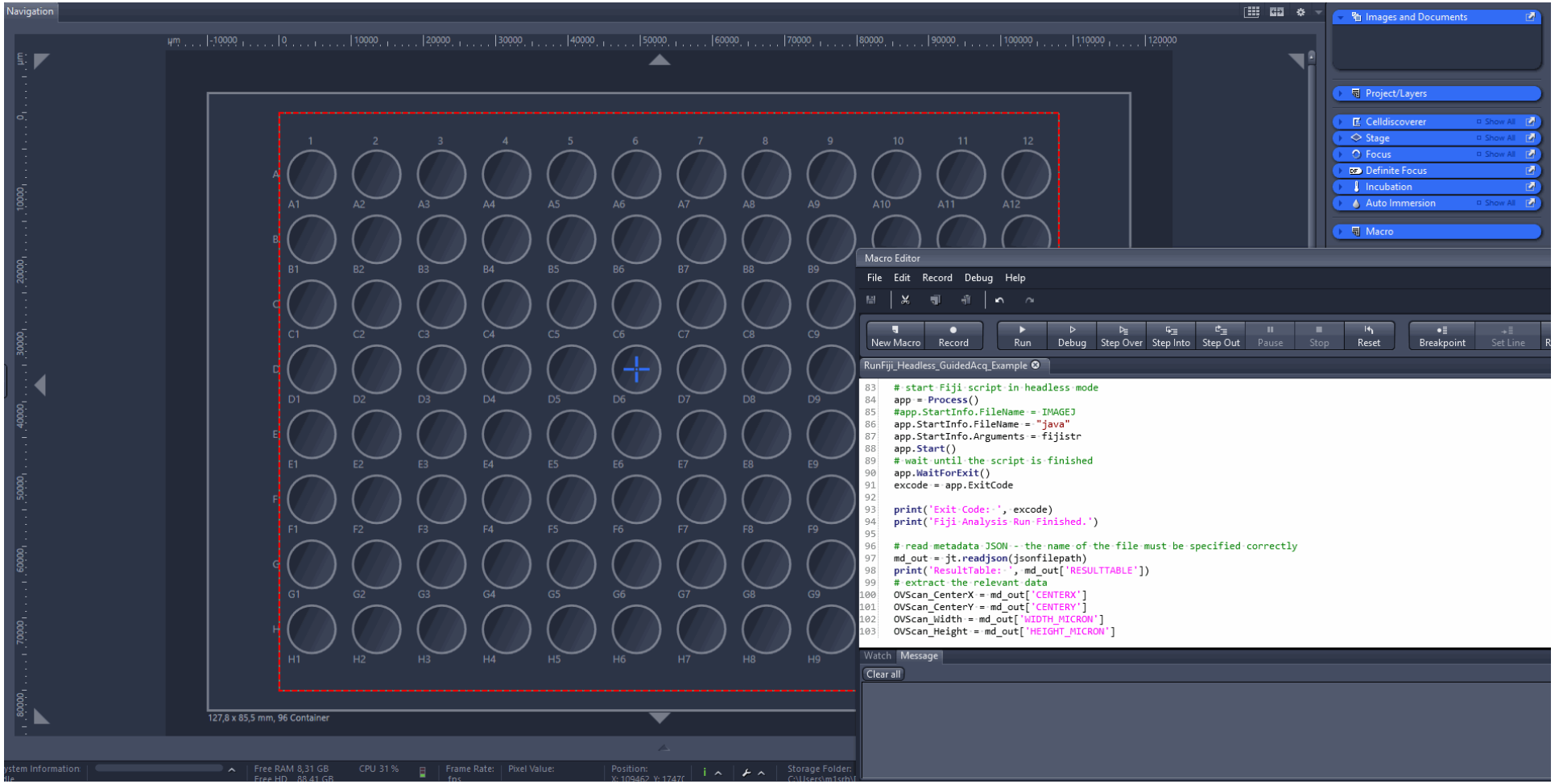
Guided Acquisition Advanced
Start external image analysis



Guided Acquisition Advanced
Read the results and take action



Guided Acquisition Advanced
Read the results and take action



Useful Links

- **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**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