

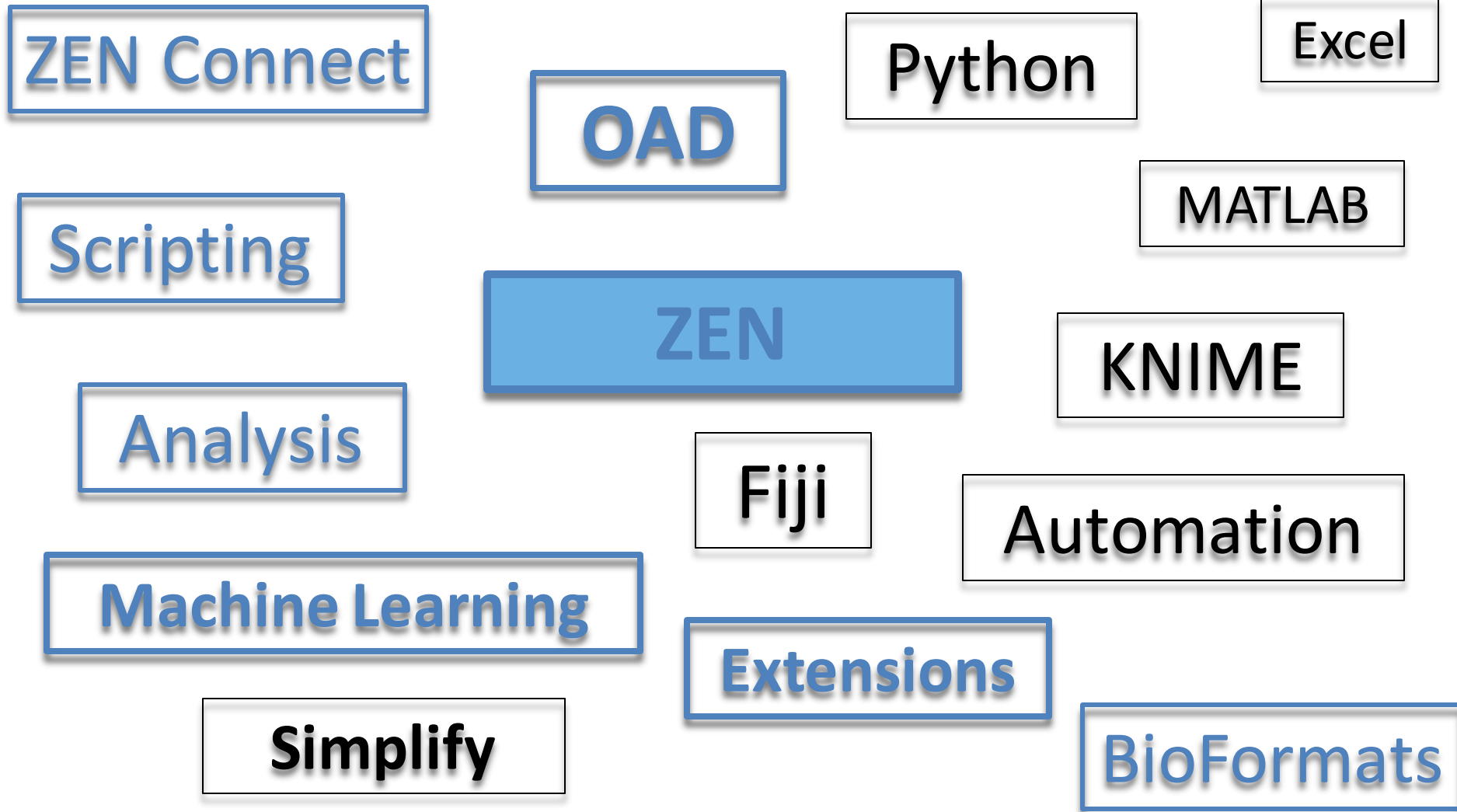
ZEN (blue edition) 3.1

Guided Acquisition



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Product Management
2019-11-04

ZEN is only part of the workflow



- **Open Application Development** (OAD) uses powerful **Python Scripts** to **simplify, customize** and **automate** your workflows.
- **Analyze** and **Exchange** data with applications like **Fiji, Python, Knime, CellProfiler, Icy, MATLAB, Excel** and ...
- The **CZI-API for .NET** (ZeissImgLib) and for **C++** (libCZI) and **BioFormats** (CZIReader) allow easy access to CZI files from many external applications. (OME-TIFF Export and Import in ZEN Blue is possible)
- **BioFormats Import** as a module inside ZEN Blue (read 3rd party file formats)
- Create “**smart**” experiments with **Experiment. Feedback** and modify the acquisition **On-the-fly** based on **Online Image Analysis** and **External Inputs**

What is Adaptive Feedback Microscopy?



There are different ways how to define **Adaptive Feedback Microscopy**, but in general one or more of the points below apply:

- **Automatically guide the system to the correct places inside a sample**
- **React on changes inside the sample during a running acquisition**
- **Optimized acquisition parameters based on the analysis of the current sample**
- **React on external signals from the “outside” and adapt the acquisition or the workflow based on those**
- **Send signal to the “outside” based on online or offline image analysis to modify the sample and continue with the workflow**

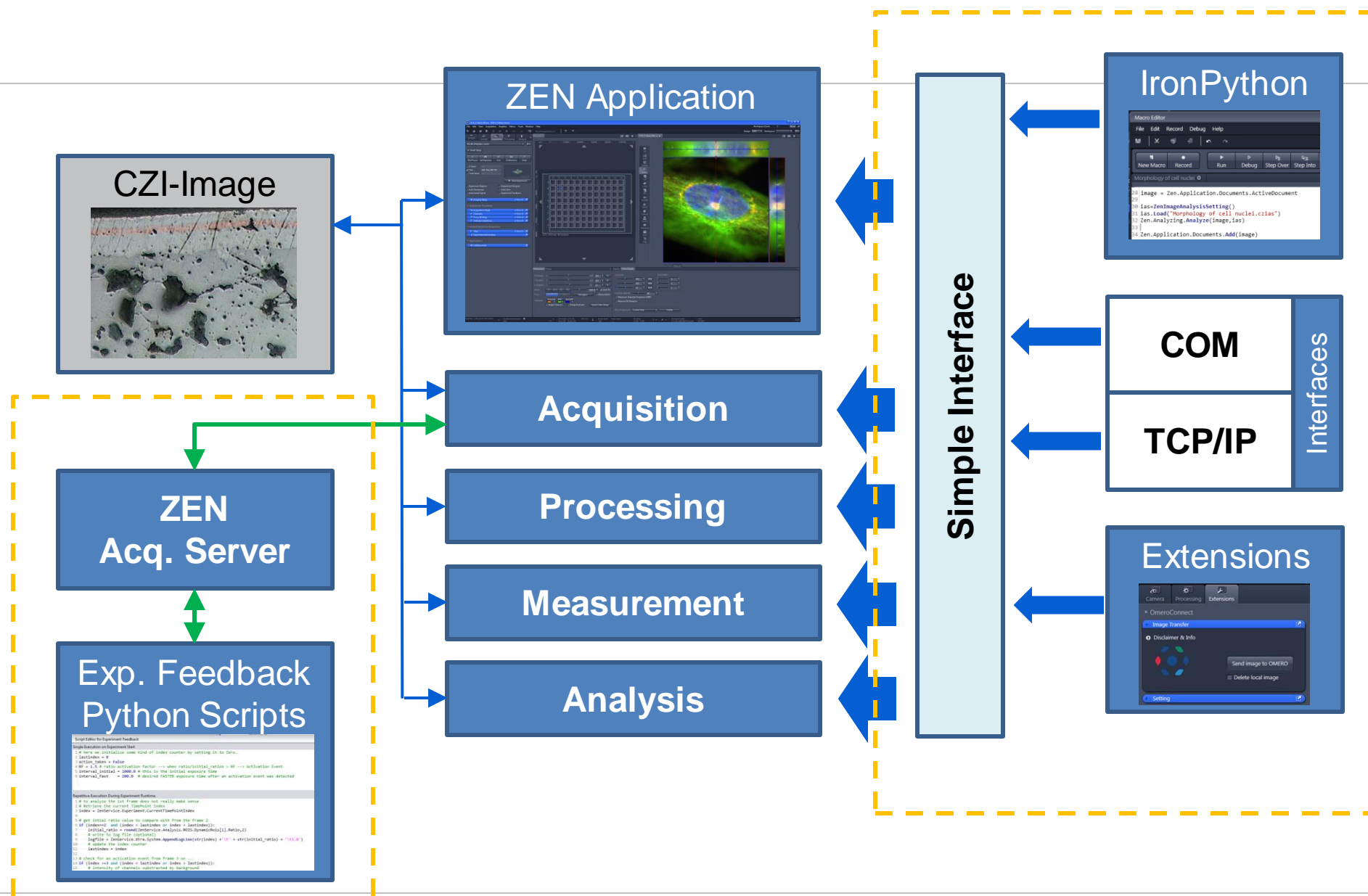
Adaptive Feedback Microscopy

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**?

OAD – ZEN Interfaces

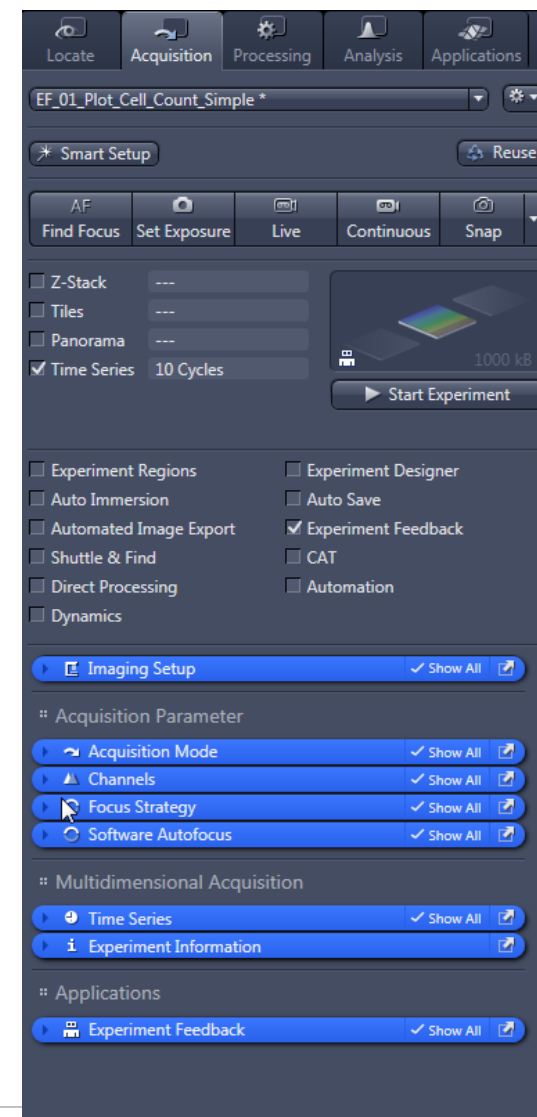


Experiment Feedback

ZEN Acquisition Server

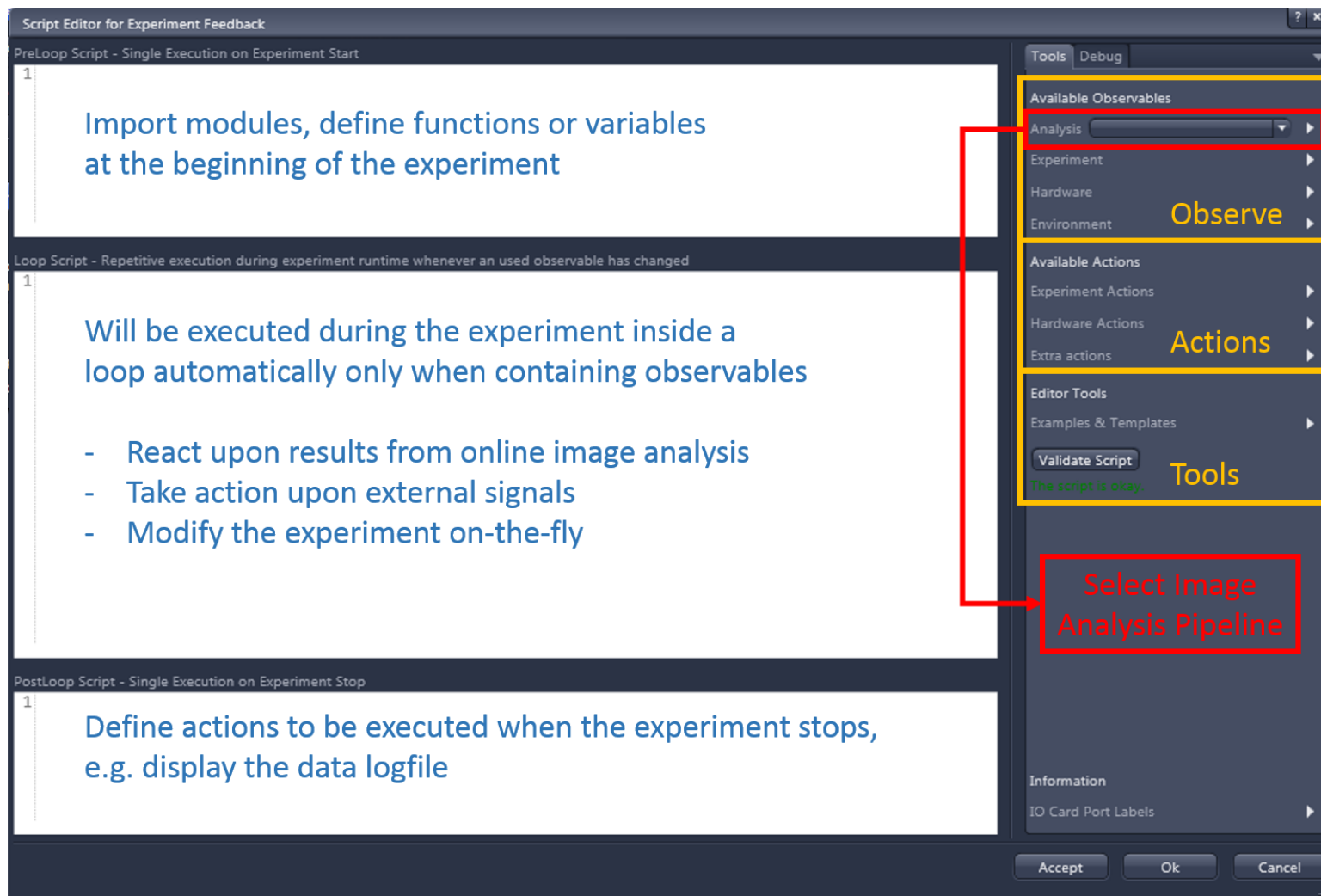


- **Adaptive Acquisition Engine:**
Modify running experiments using Python scripts
- Access the **current system status** & results from **online image analysis** on runtime during the experiment



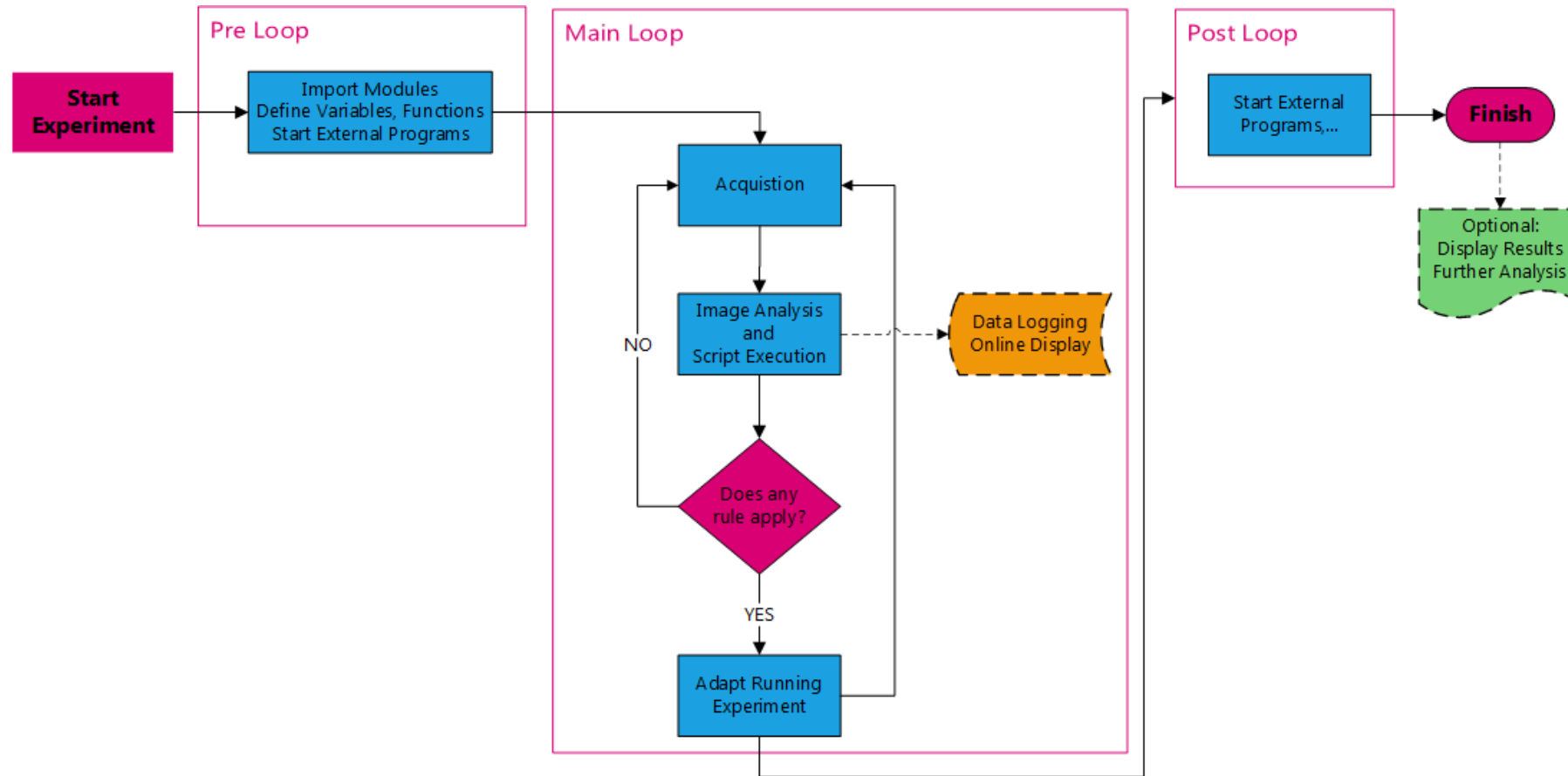
Experiment Feedback

Adaptive Acquisition Engine



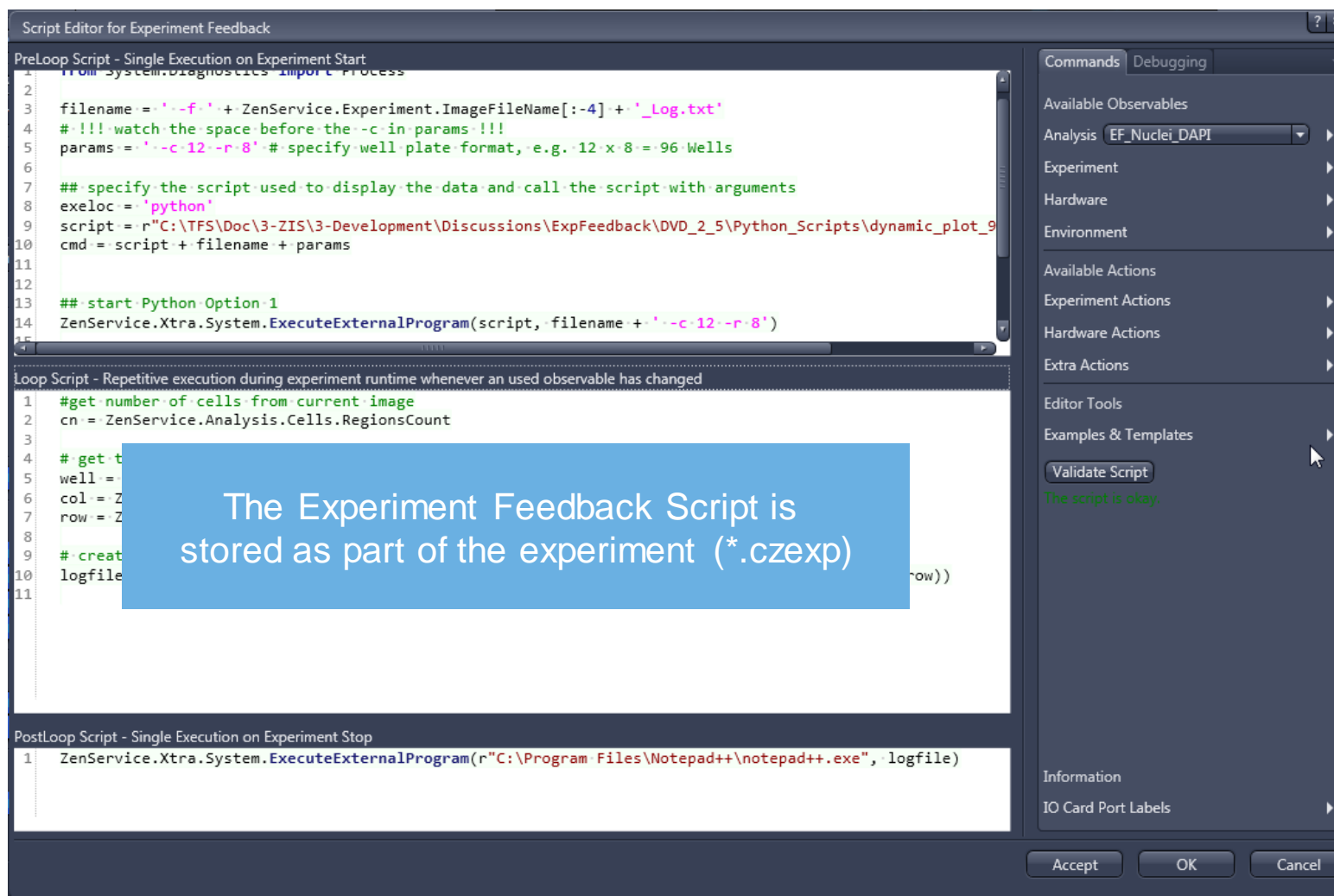
Experiment Feedback

Running the Experiment



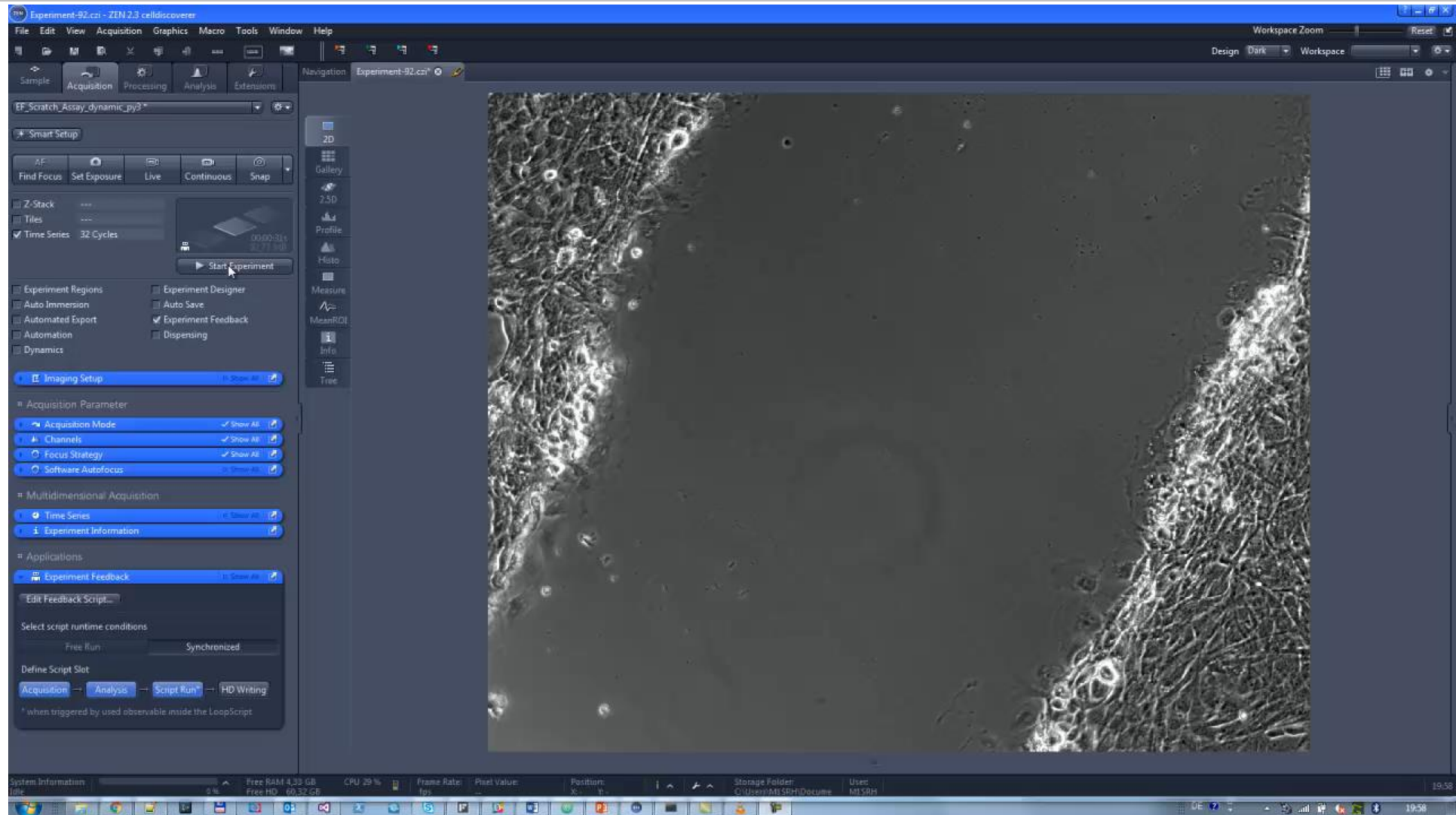
Experiment Feedback

Adaptive Acquisition Engine



Experiment Feedback

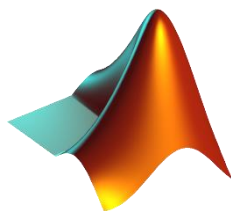
Adaptive Acquisition Engine



Sample Macros (ZEN blue DVD)

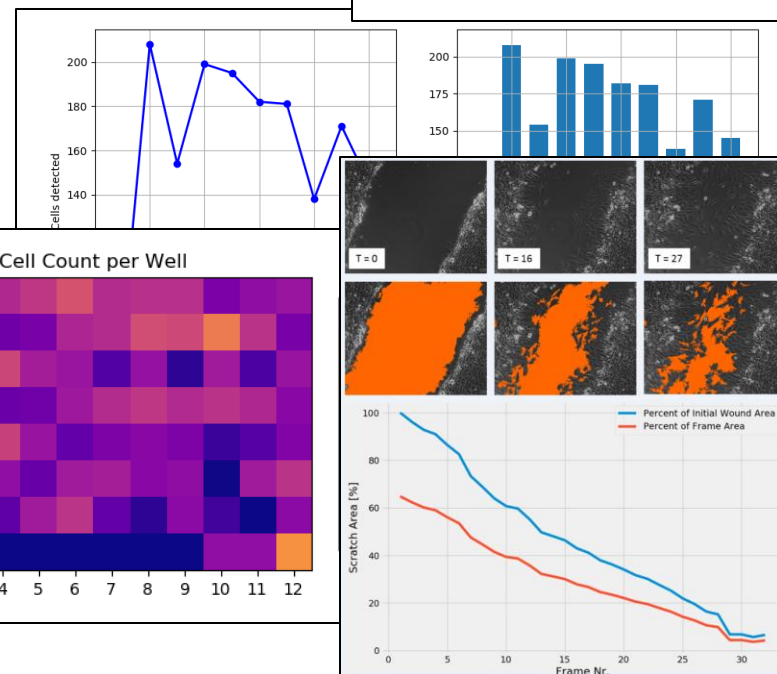


- Count cells, write logfile and start external data display
- Acquire tile images until a Total Number of Objects is Reached
- Online data display
- Acquire Image Data, Open in Fiji and Apply a Macro
- Jump to next well
- Adapt exposure time during acquisition
- Modify the blocks of an experiment
- Time-lapse per Z-Plane
- Automatic event detection
- Online dynamics
- Online scratch assay
- Online tracking



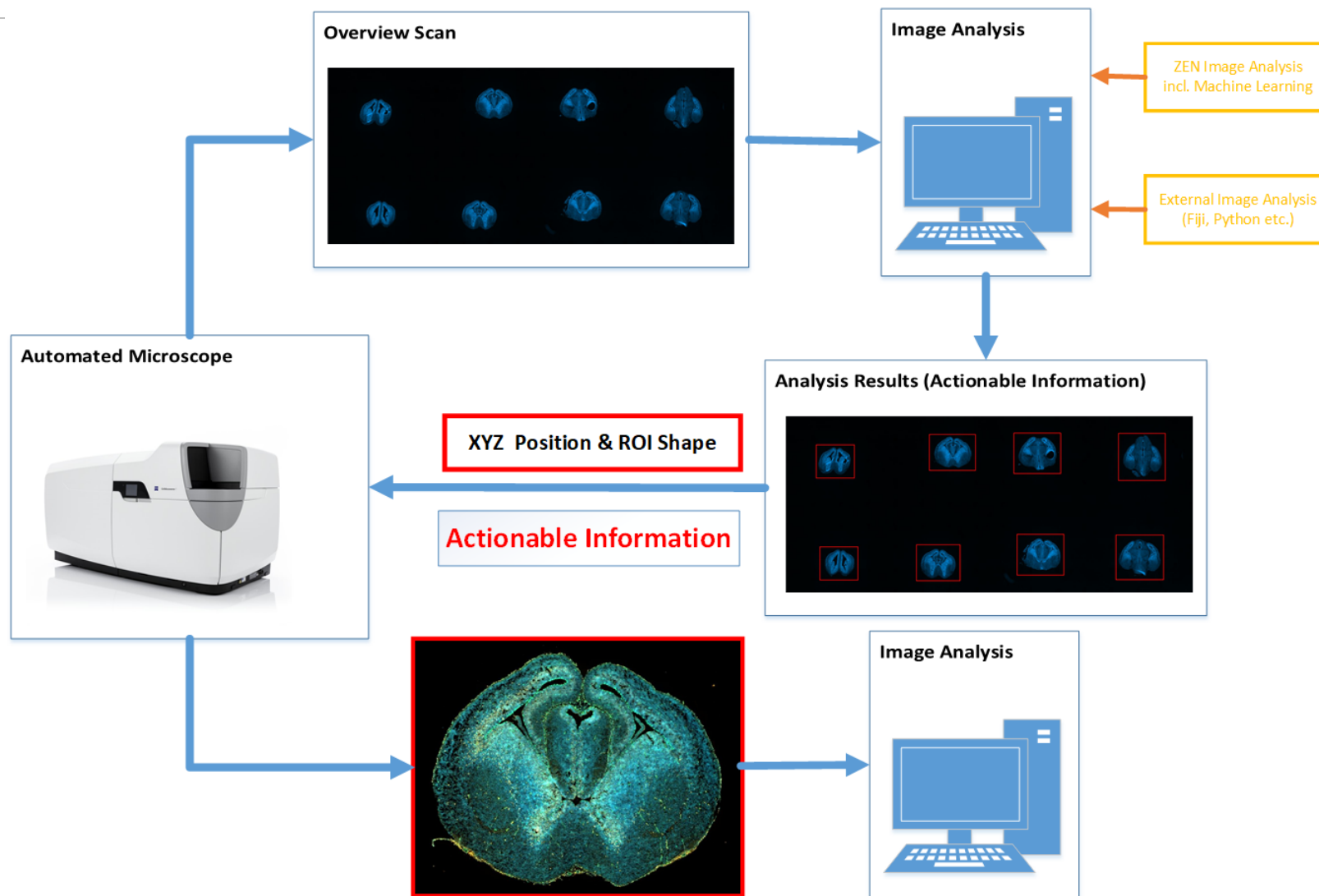
Experiment-59_Log.txt - Editor

Datei	Bearbeiten	Format	Ansicht	?
Tile	Cells	Total	PosX	PosY
1	282.0	282.0	49324.0	35324.0
2	231.0	513.0	49381.6	35324.0
3	206.0	719.0	49439.2	35324.0
4	174.0	893.0	49496.8	35324.0
5	188.0	1081.0	49554.4	35324.0
6	201.0	1282.0	49612.0	35324.0
7	180.0	1462.0	49612.0	35381.6
8	175.0	1637.0	49554.4	35381.6
9	178.0	1815.0	49496.8	35381.6
10	151.0	1966.0	49439.2	35381.6
11	156.0	2122.0	49381.6	35381.6
12	170.0	2292.0	49324.0	35381.6
13	144.0	2436.0	49324.0	35439.2
14	176.0	2612.0	49381.6	35439.2
15	220.0	2832.0	49439.2	35439.2
16	193.0	3025.0	49496.8	35439.2



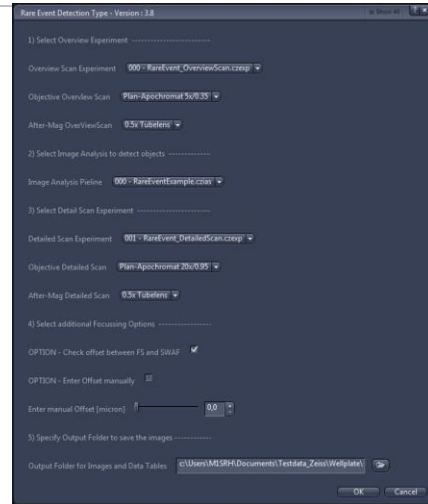
Guided Acquisition

Automate the workflow

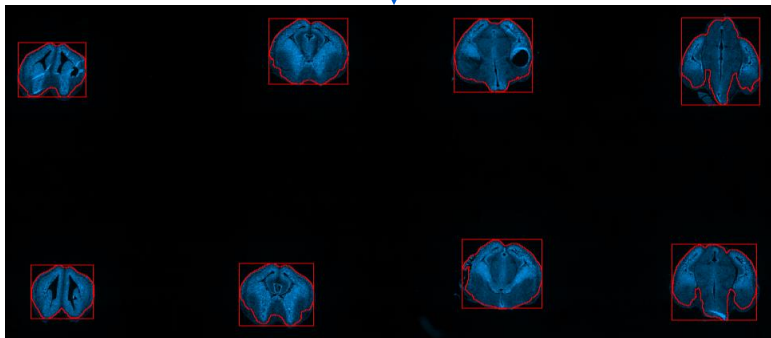


Guided Acquisition

Workflow Scheme

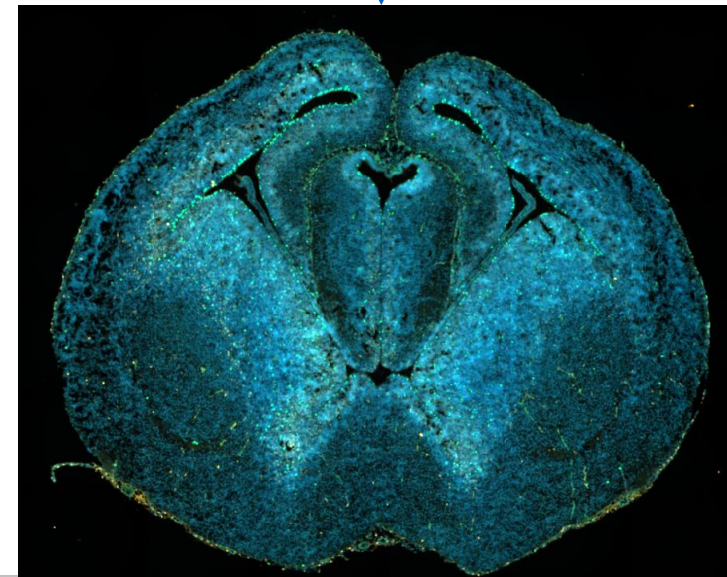


OAD - Automated Acquisition
and Image Analysis



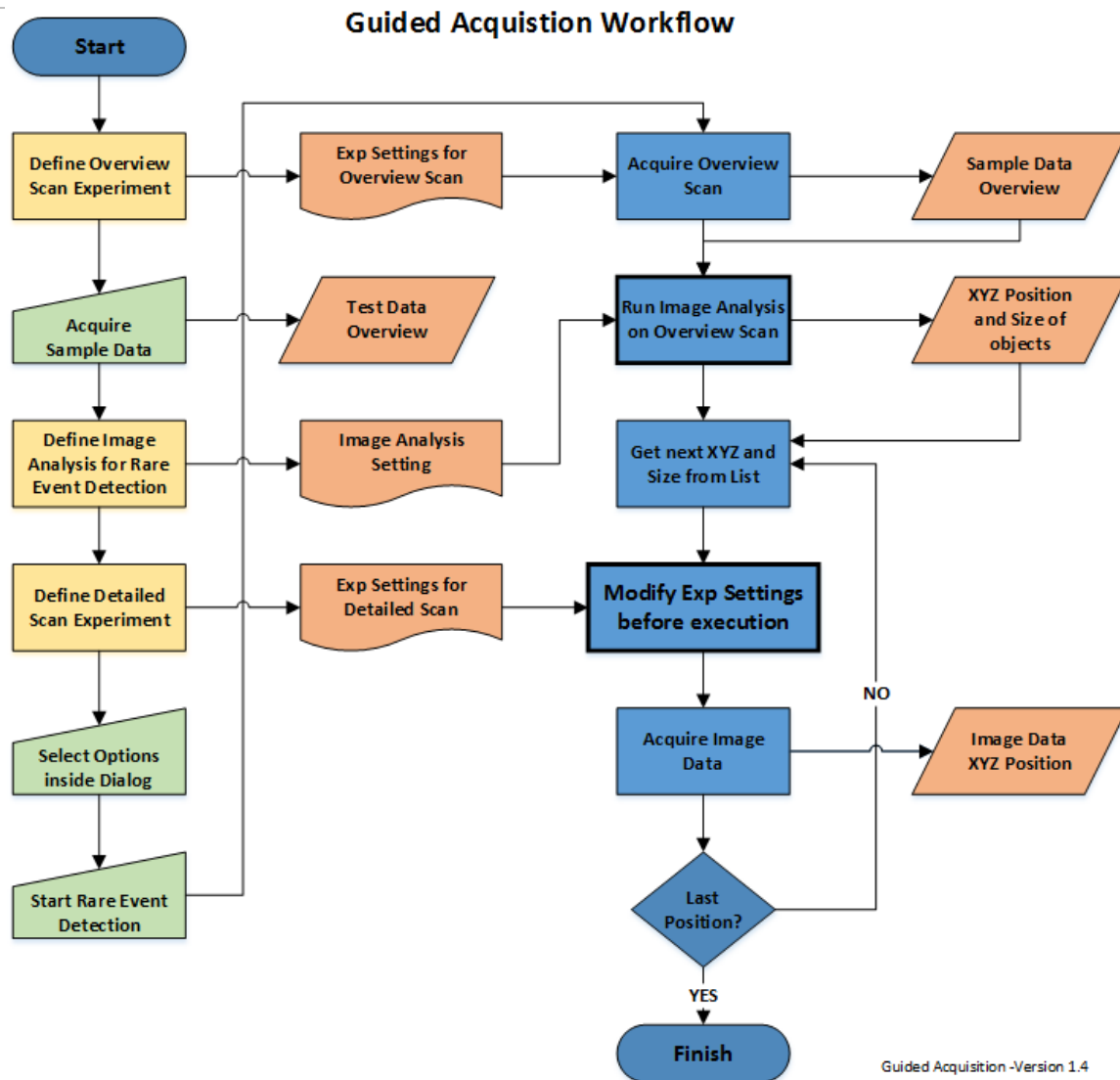
	ID	Bound Center X Stage[μm]	Bound Center Y Stage[μm]	Bound Width[μm]	Bound Height[μm]
	A	B	C	D	E
1	2	40.786,876	10.236,734	3.014,505	2.511,325
2	3	47.835,969	10.371,678	2.996,208	2.799,510
3	4	30.956,571	10.934,324	2.584,515	2.085,909
4	5	56.540,981	10.604,970	2.996,208	3.320,987
5	6	31.372,838	19.403,757	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

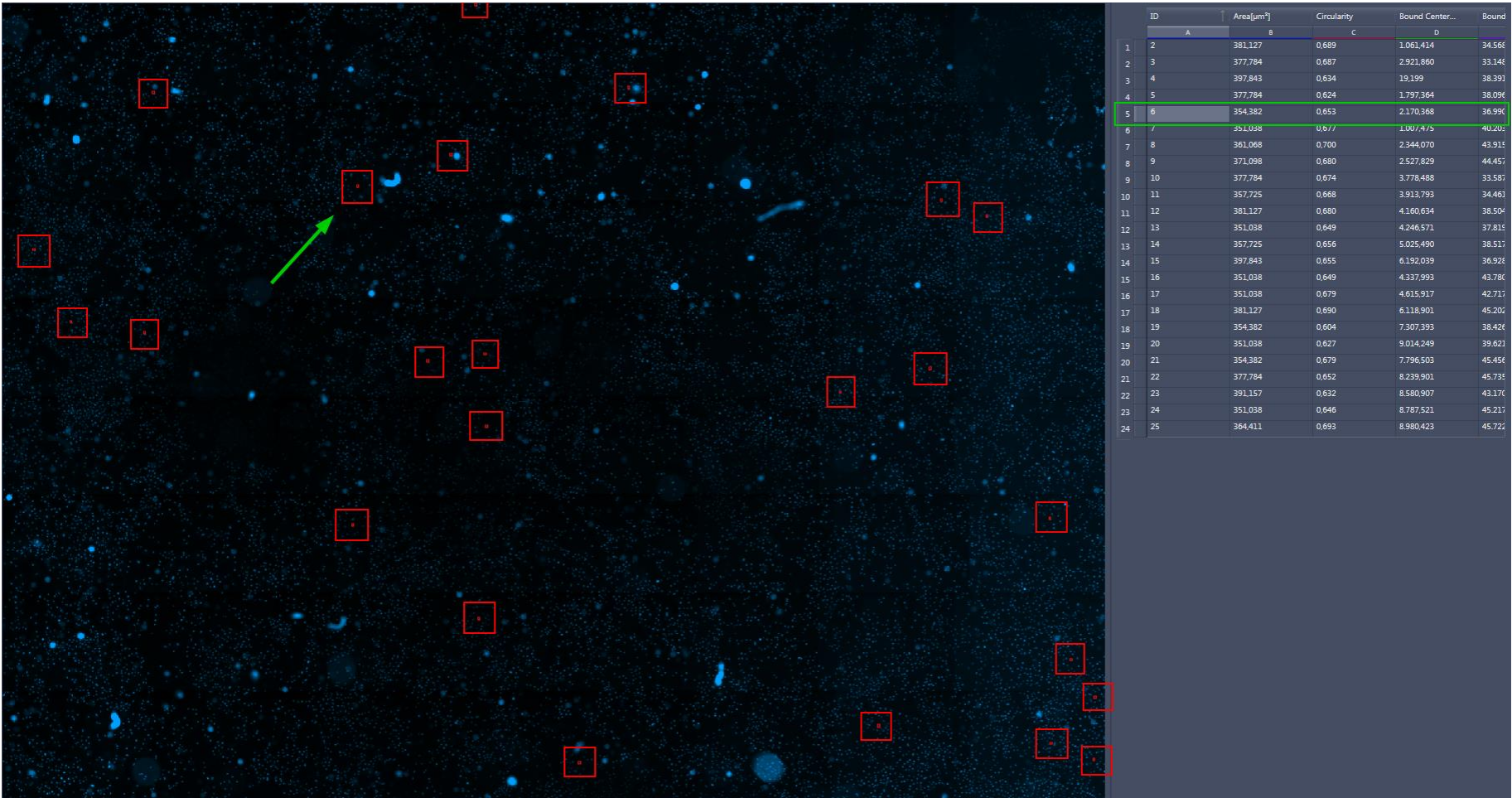
Workflow Diagram



Guided Acquisition -Version 1.4

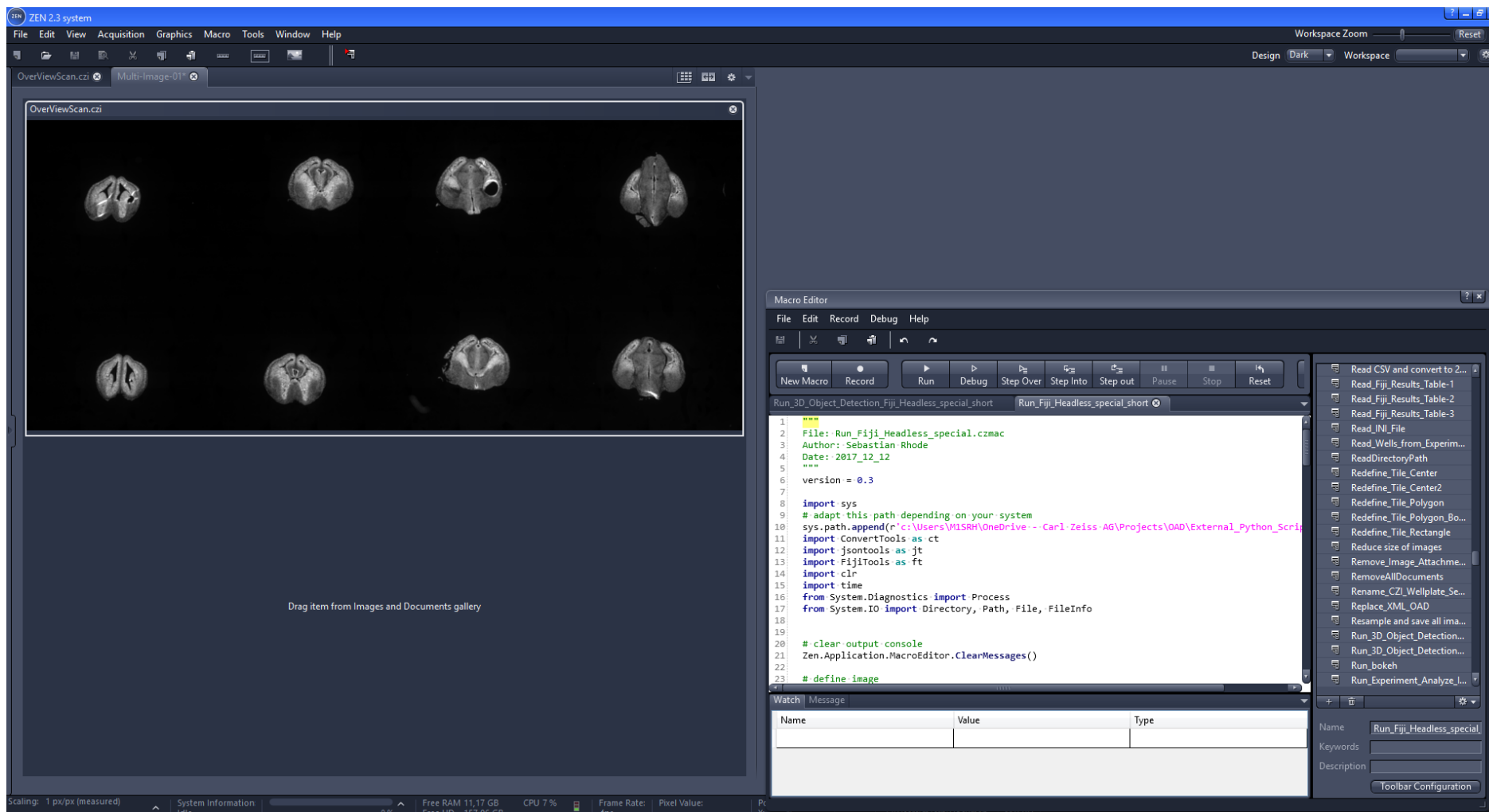
Guided Acquisition

Detect rare objects automatically



Adaptive Feedback Microscopy

Run Experiment



Adaptive Feedback Microscopy

Run External Image Analysis



The screenshot shows the ZEN 2.3 system interface. A Java command window is open, displaying the execution of a script. A yellow box highlights the following text:

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

The Java command window output includes the following lines:

```
09:10:23,336 |-INFO in ch.qos.logback.classic.joran.JoranConfigurator@245a060f - Registering current configuration as sa  
fe 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:///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-2.99.0.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(...) r  
eturned 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.exe  
[INFO] BinFactor : 1  
[INFO] RankFilter : MEDIAN  
[INFO] Filter Radius : 3  
[INFO] Threshold Method : Triangle  
[INFO] Threshold Background : black  
[INFO] Min. Particle 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 -----
```

Adaptive Feedback Microscopy

Read Results and take Action



The screenshot displays the Zen 2.3 system interface. The main window shows a grid of microscopy images. Below the main window, there is a section labeled 'OverViewScan_PA.ome.tif' showing a grid of processed images. To the right, a table displays the results of the processing, including labels, areas, and dimensions. Below the table, a macro editor window is open, showing a list of macros and a code editor with Python code for running a macro. The code includes imports for system paths, time, and file handling, and defines a function to run a macro.

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	19.595.569	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	

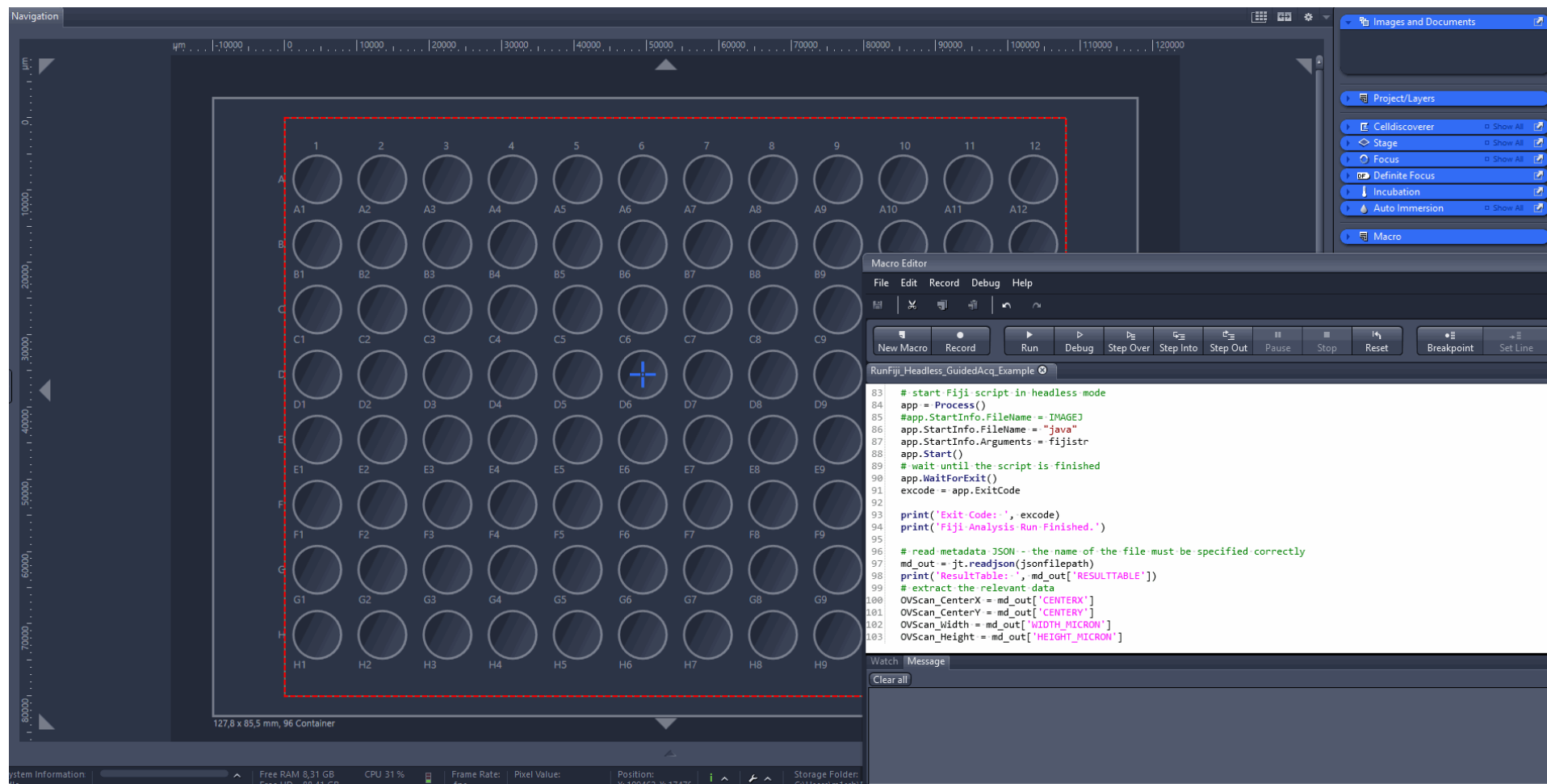
```

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\MI5RN\OneDrive -- Carl Zeiss AG\Projects\OAD\External_Python_Scripts')
11 import ConvertTools as ct
12 import json 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
23

```

Adaptive Feedback Microscopy

Read Results and take Action

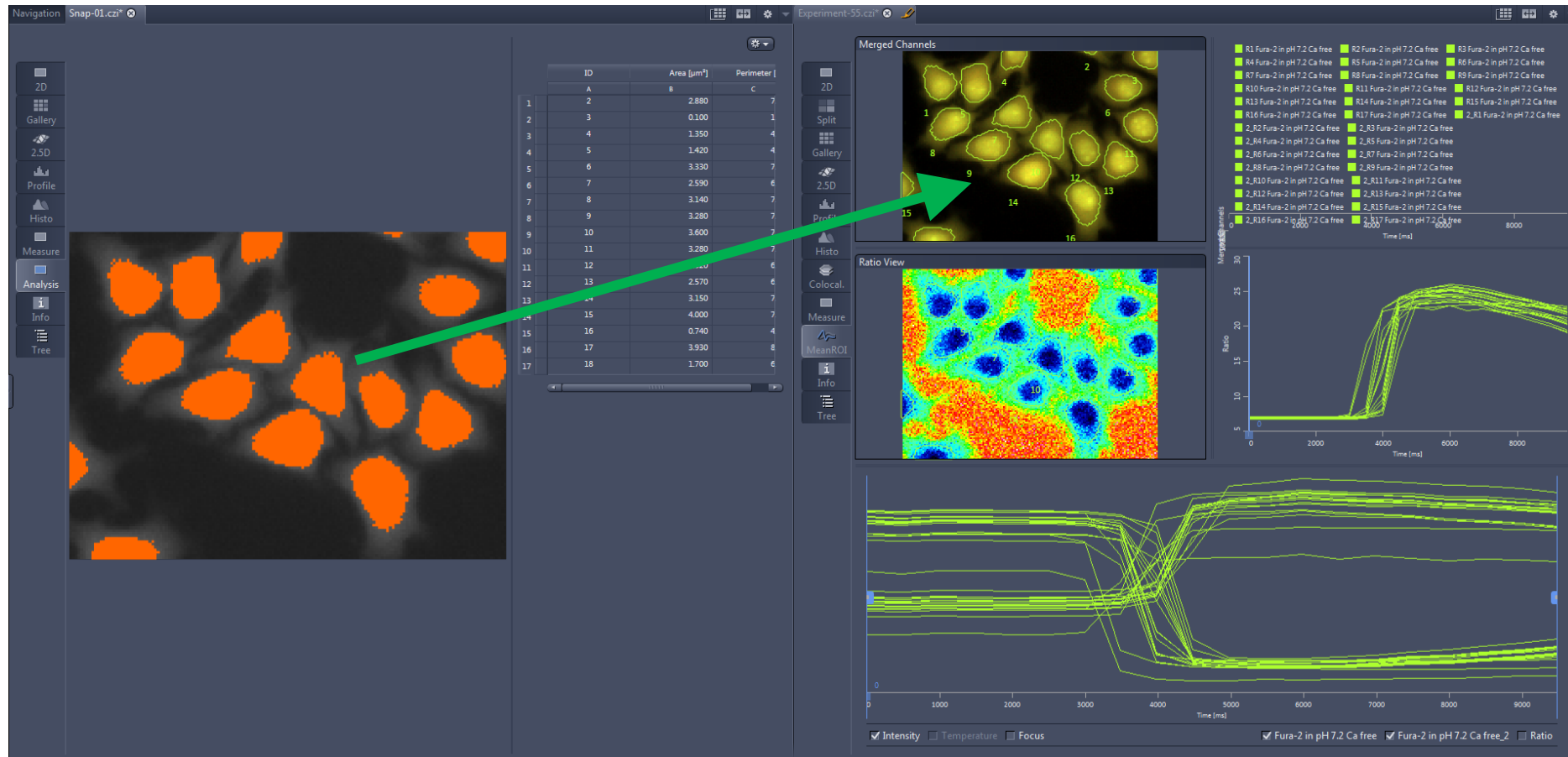


Adaptive Feedback Microscopy

Convert Image Analysis Results into Actionable Information

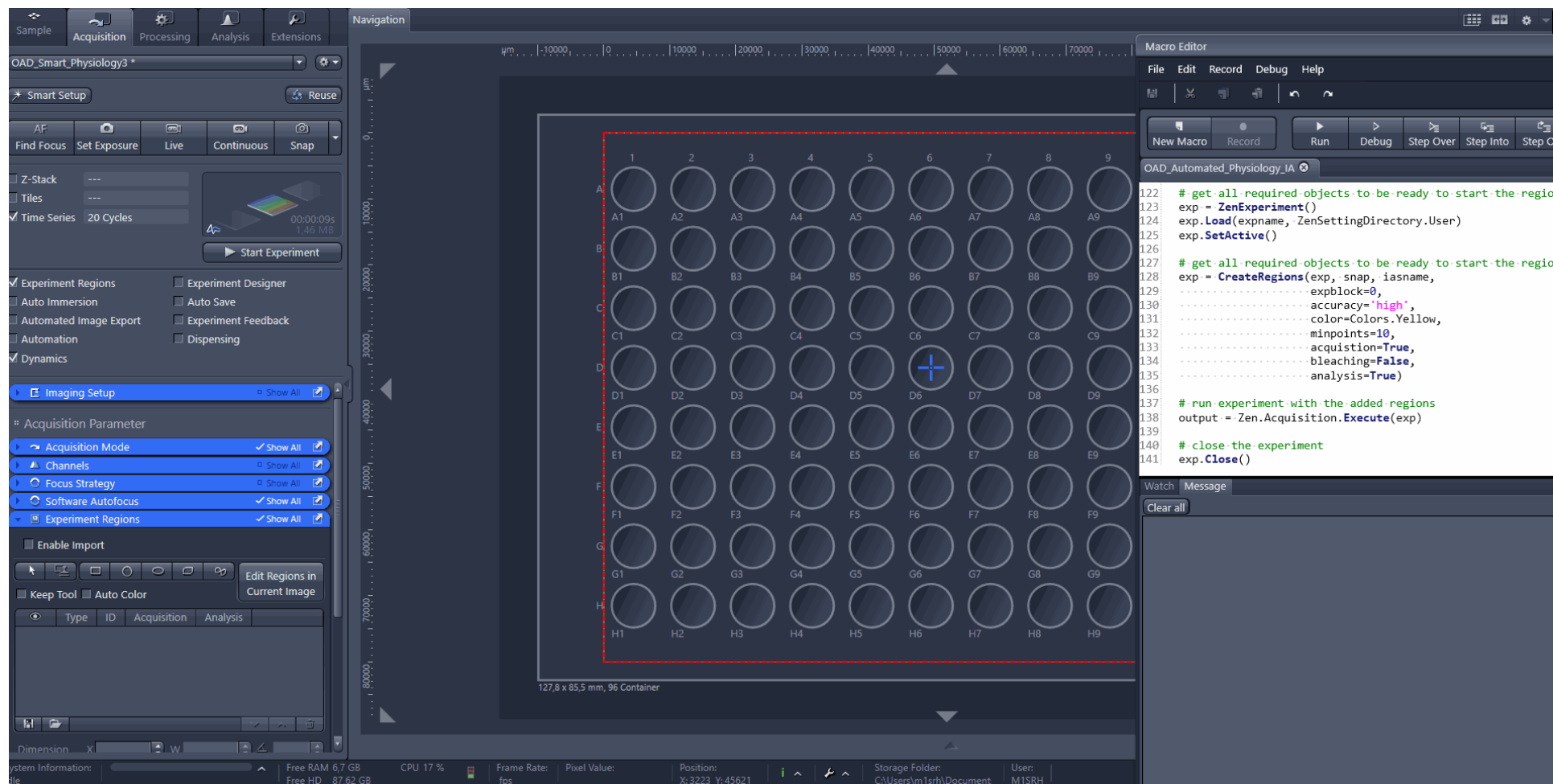


- Create regions for Dynamics automatically based on IA results



Adaptive Feedback Microscopy

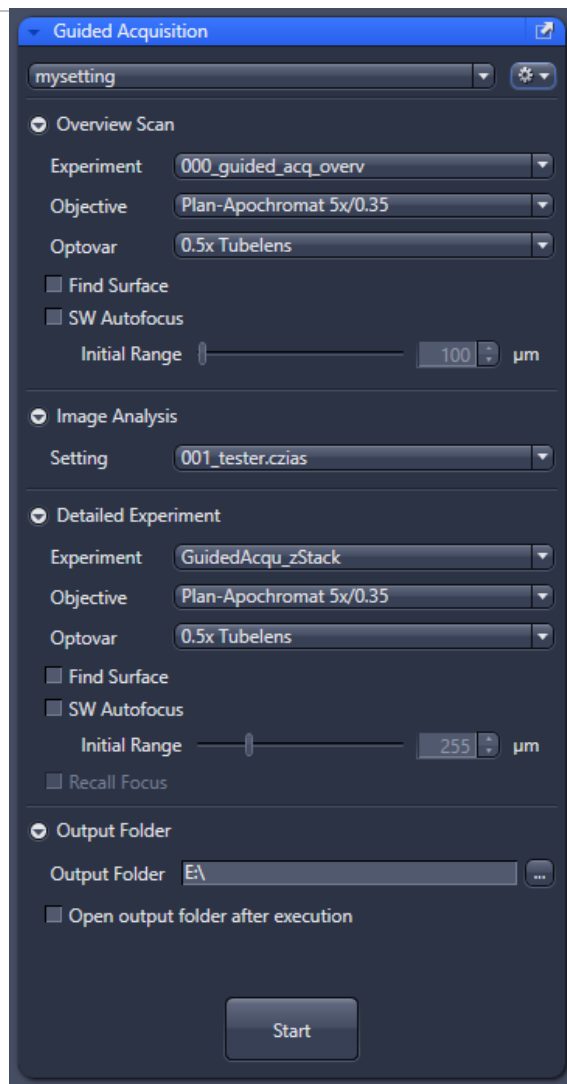
Convert Image Analysis Results into Actionable Information



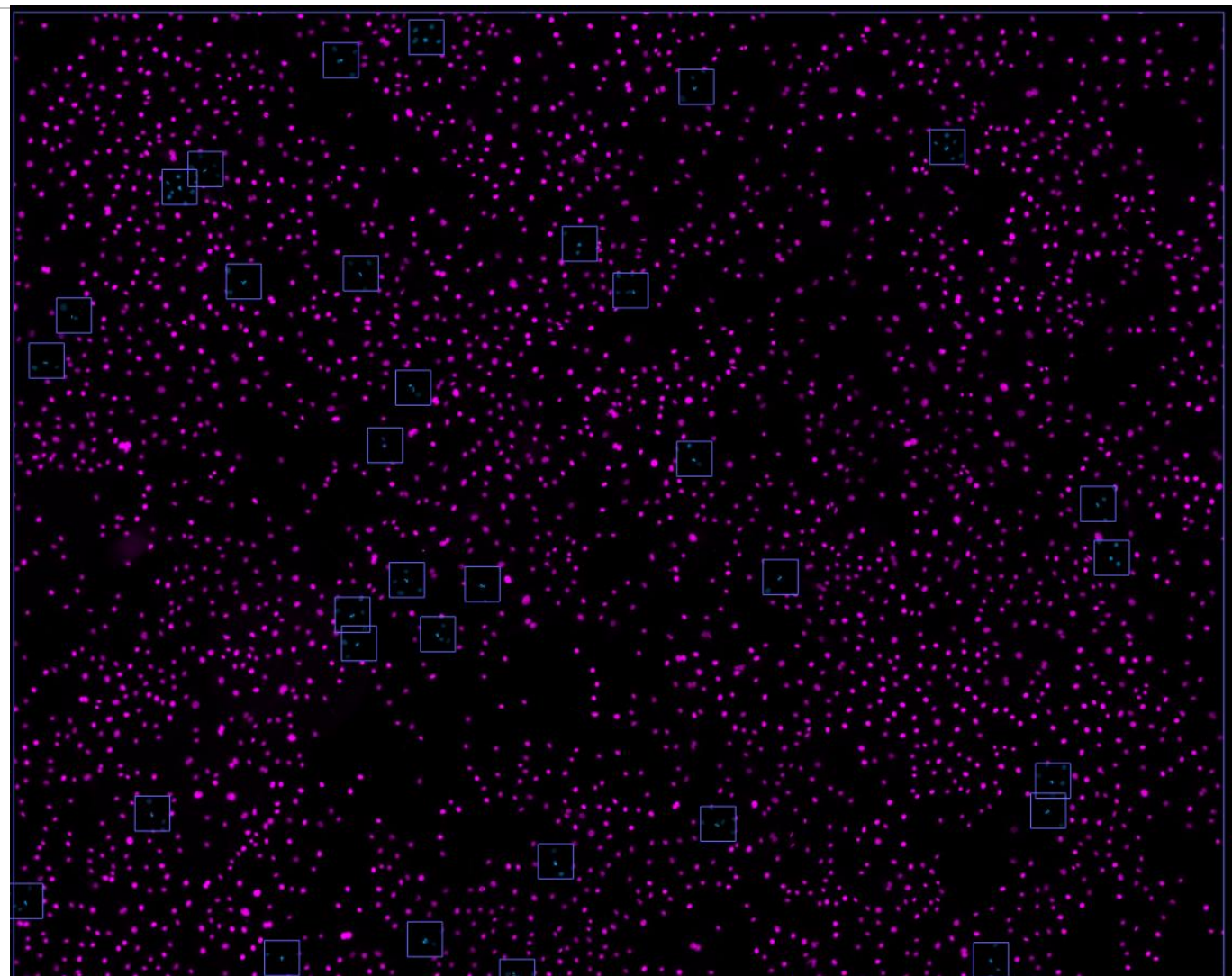
- Commands to modify experiments based on IA results interactively or using python scripting
- Create regions for Dynamics automatically
- Use Image Analysis results (detected regions) and convert them to ...
 - ... Experiment Regions
 - ... FRAP Regions
 - ... New Tile Regions

Guided Acquisition – Find objects of interest

New in ZEN blue 3.1: Guided Acquisition Module

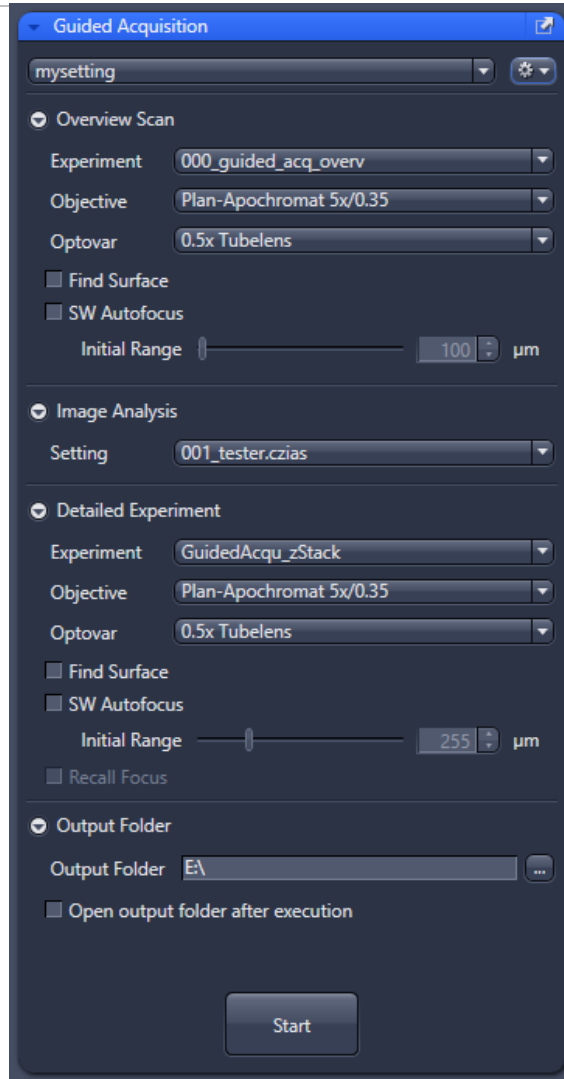


Overview Scan
(e.g. low-res widefield)

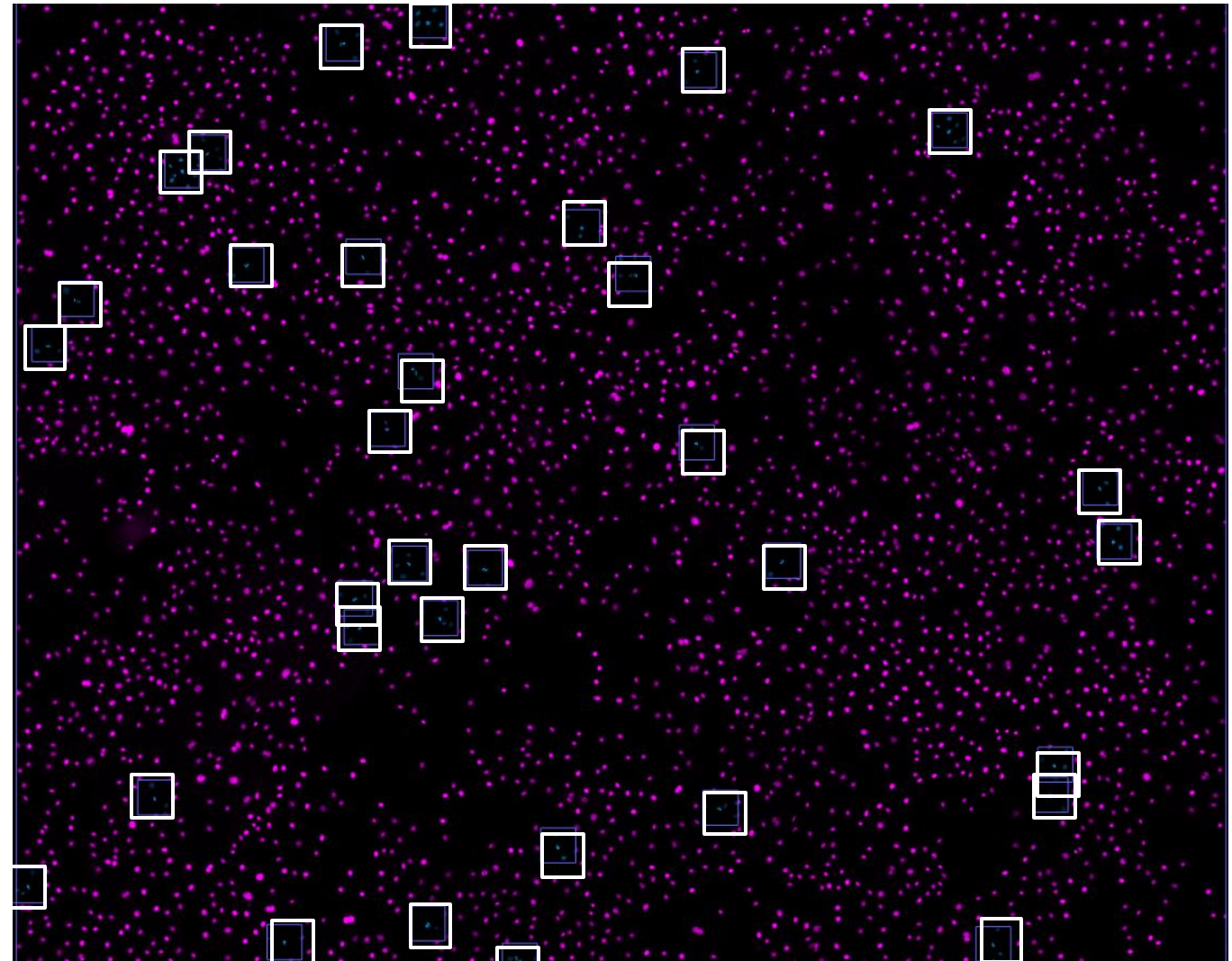


Guided Acquisition – Find objects of interest

New in ZEN blue 3.1: Guided Acquisition Module

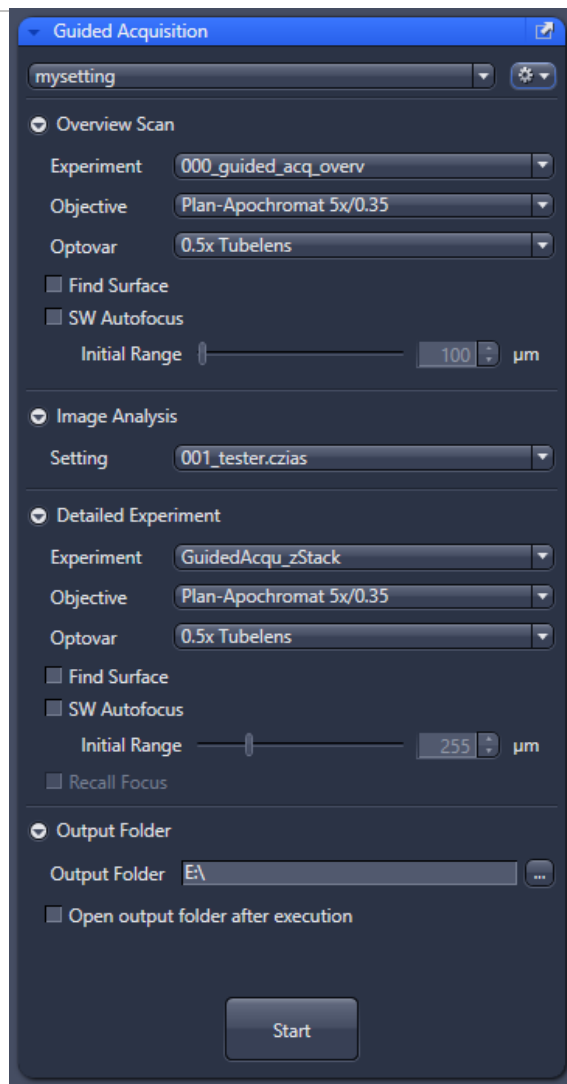


} **Image Analysis**
(e.g. find mitotic cells)

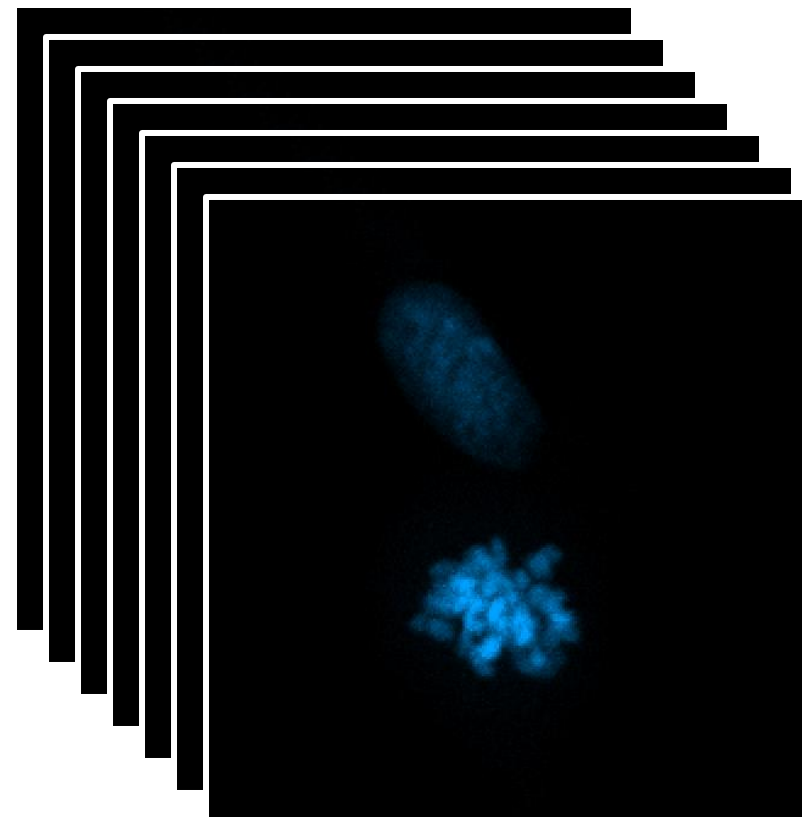


Guided Acquisition – Find objects of interest

New in ZEN blue 3.1: Guided Acquisition Module



Detailed Scans
(e.g. high-res confocal)

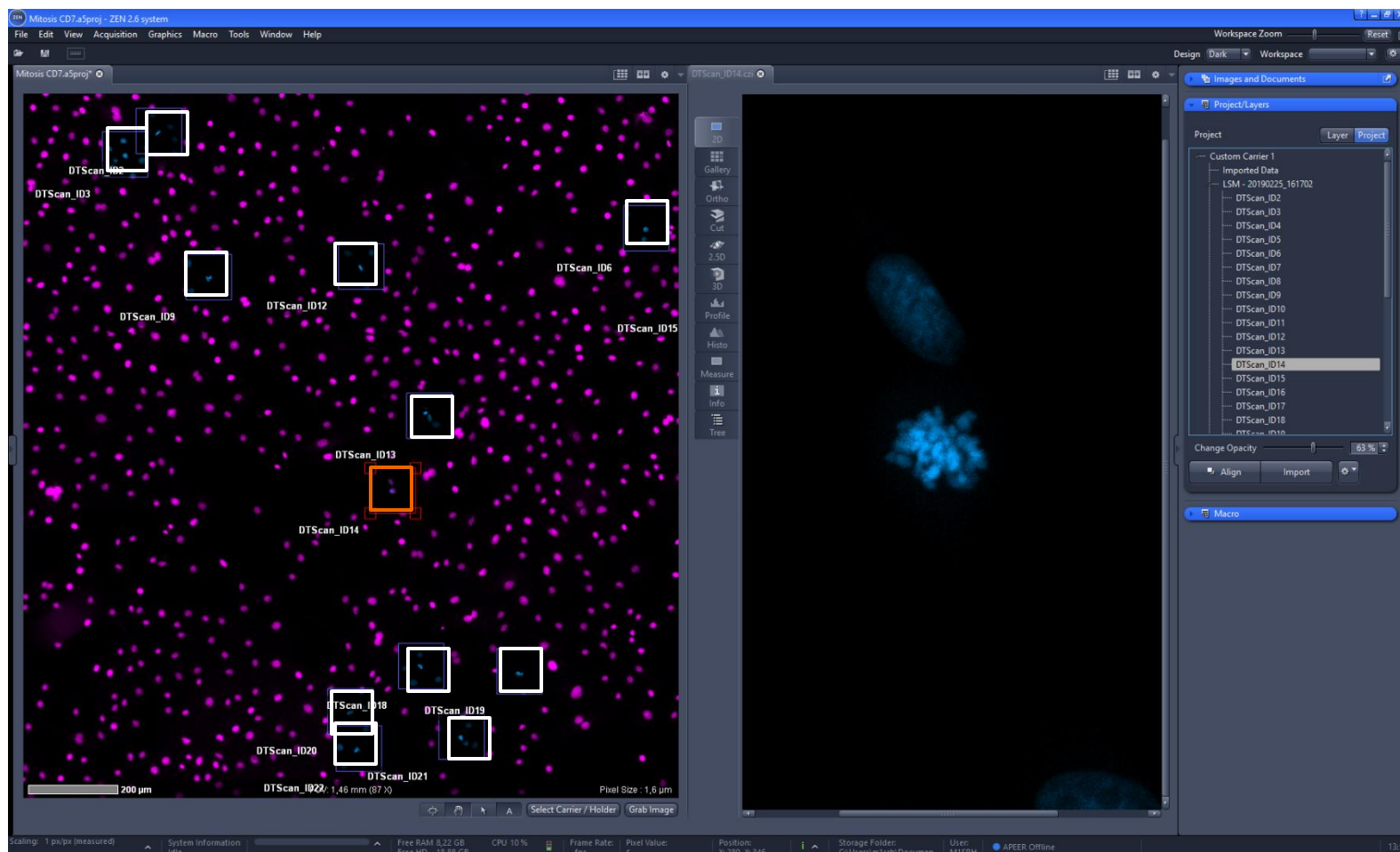


Guided Acquisition – Find objects of interest

New in ZEN blue 3.1: Guided Acquisition Module



- Add Images automatically to an open ZEN connect project



What is the right system for Adaptive Feedback?



Well, it depends from your application ...



... but all motorized ZEN Blue systems can be used

Adaptive Feedback Microscopy

When things go wrong ...

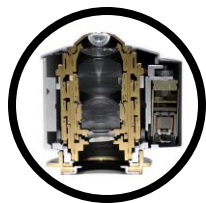


Celldiscoverer 7

Ready for Adaptive Feedback Microscopy



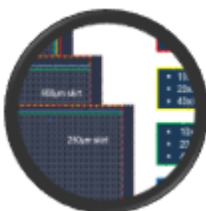
Unique Optical Concept
Unparalleled Sensitivity



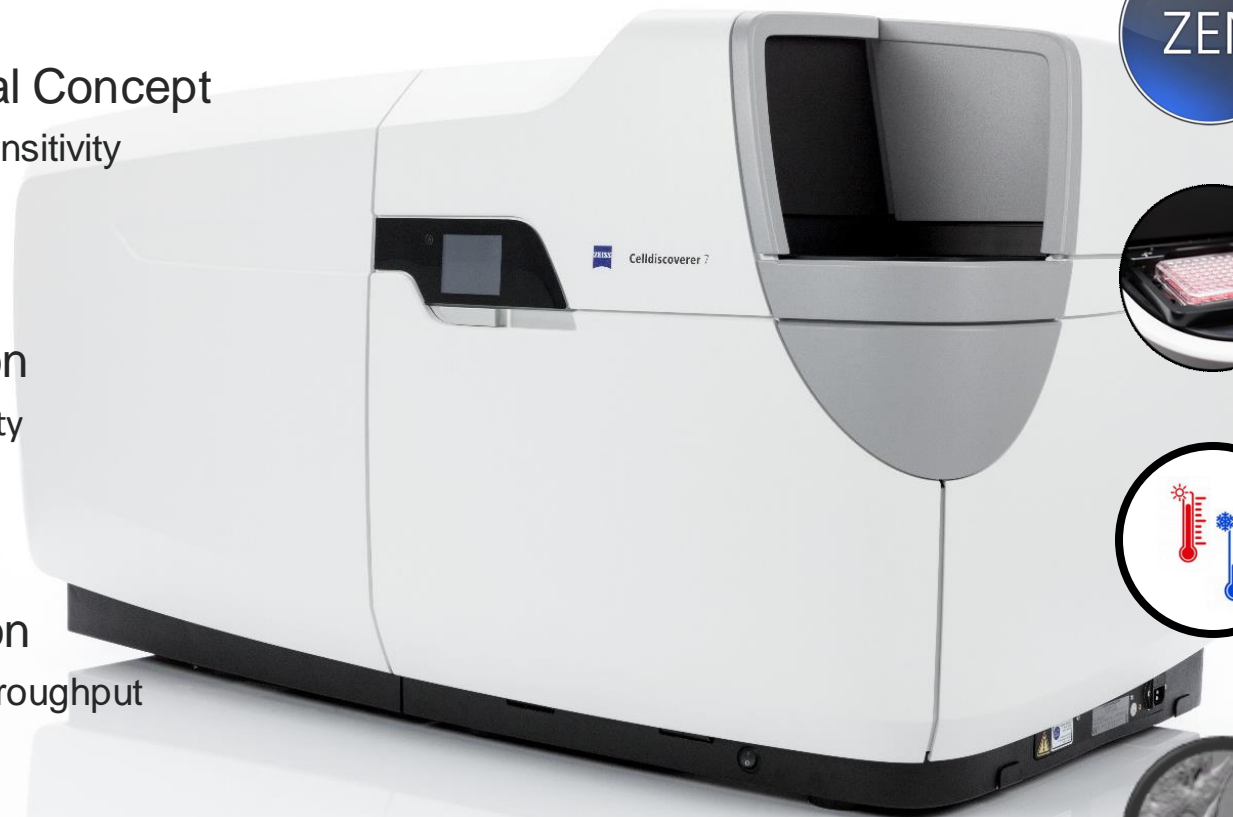
AutoCorrection
Ideal Image Quality



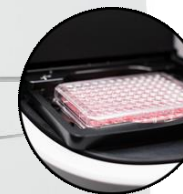
AutoImmersion
Enables High throughput



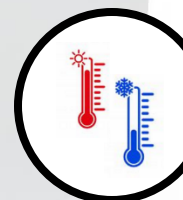
Adaptive LensGuard
Protect Your Precious Lenses



Open ZEN blue SW
Celldiscoverer 7 is Part of
Your Workflow



Auto-Carrier Recognition
Optimized Imaging Conditions



Rock-solid Incubation
Reliable long-term Timelapse
Experiments.



Phase Gradient Contrast
Adaptive Label-free Imaging

Adaptive Feedback Microscopy – Potential Issues

Adaptive Lens Guard

