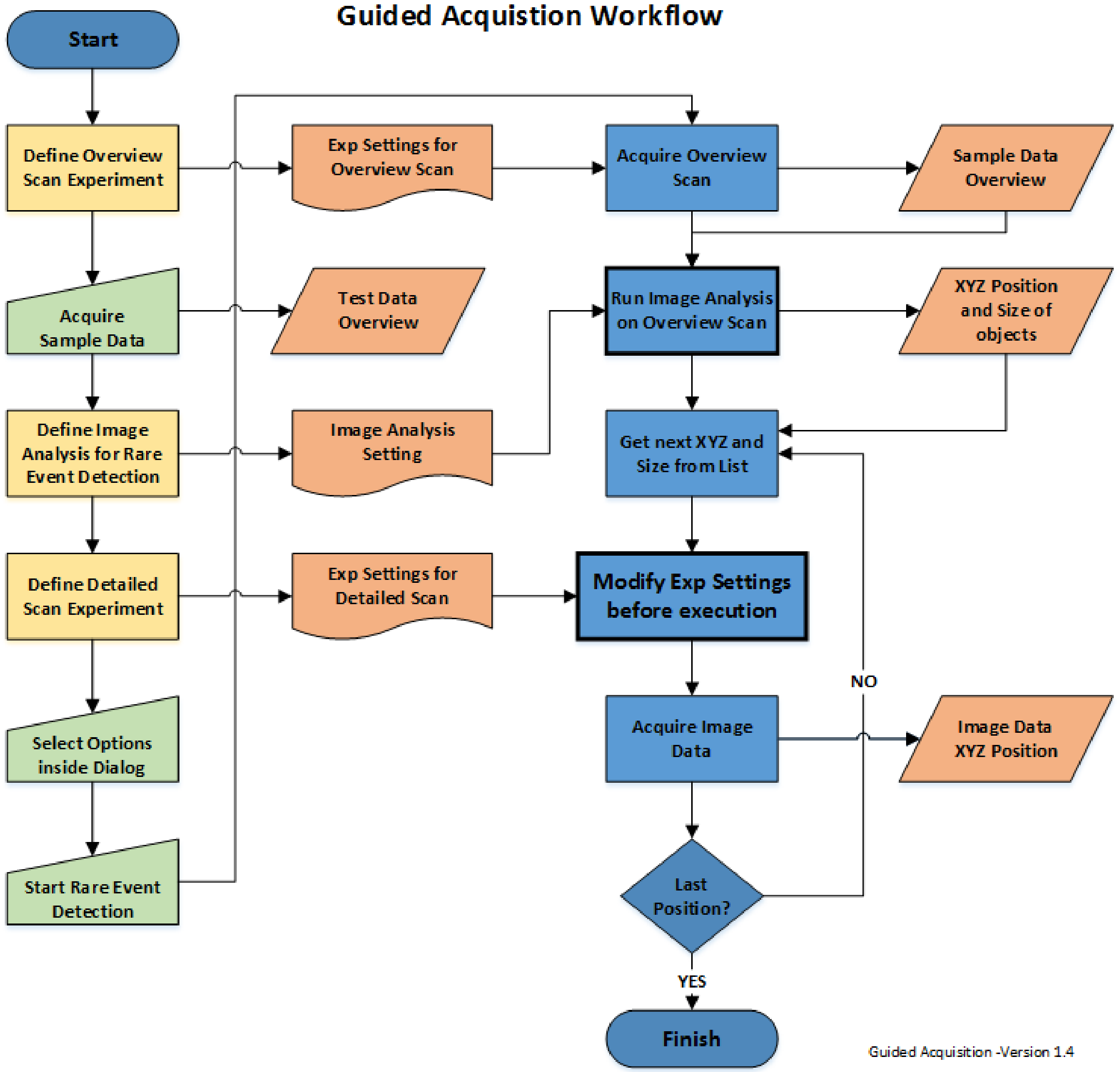
ZEN (blue edition) 3.1
Guided Acquisition

Dr. Marion Lang
Product Management
2019-11-04

Guided Acquisition

Workflow Diagram

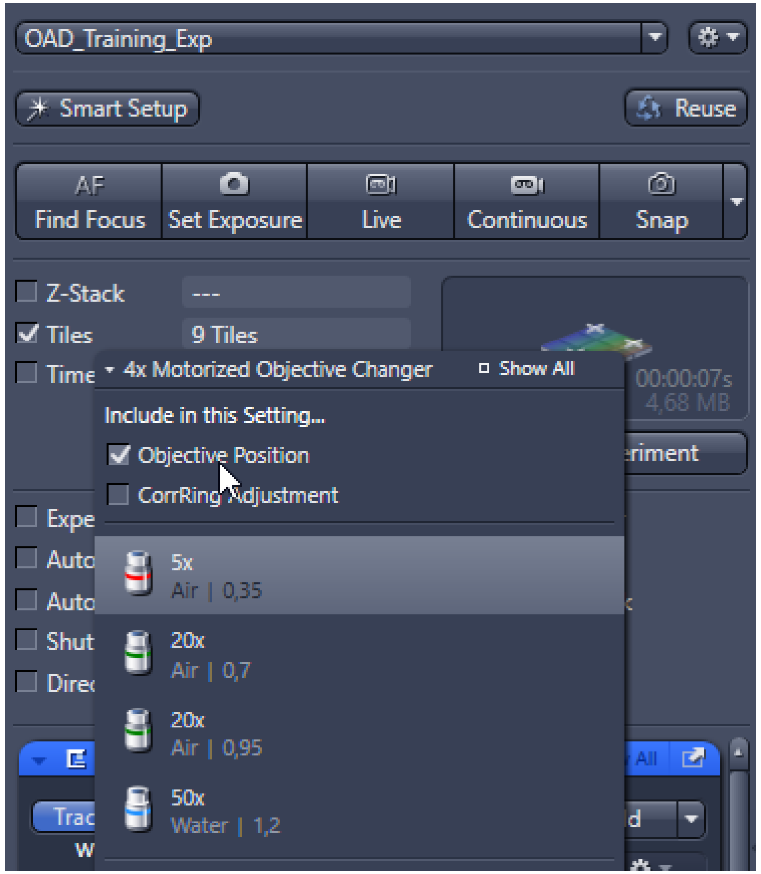

Guided Acquistion Workflow
Start
Define Overview Scan Experiment
Exp Settings for Overview Scan
Acquire Overview Scan
Sample Data Overview
Acquire Sample Data
Test Data Overview
Run Image Analysis on Overview Scan
XYZ Position and Size of objects
Define Image Analysis for Rare Event Detection
Image Analysis Setting
Get next XYZ and Size from List
Define Detailed Scan Experiment
Exp Settings for Detailed Scan
Modify Exp Settings before execution
NO
Select Options inside Dialog
Acquire Image Data
Image Data XYZ Position
Start Rare Event Detection
Last Position?
YES
Finish
Guided Acquisition -Version 1.4

Guided Acquisition – Find objects of interest

Set-up experiments

1. Overview Experiment (e.g. tiles scan, low magnification)
   Make sure to include the Objective in the Experiment setting!
   Imaging Setup -> click on the objective -> activate „Objective Position“

2. Detailed Experiment (e.g. z-stack, higher magnification)
   Make sure to include the Objective in the Experiment setting!



OAD_Training_Exp

☑ Tiles 9 Tiles

4x Motorized Objective Changer

Include in this Setting...

☑ Objective Position

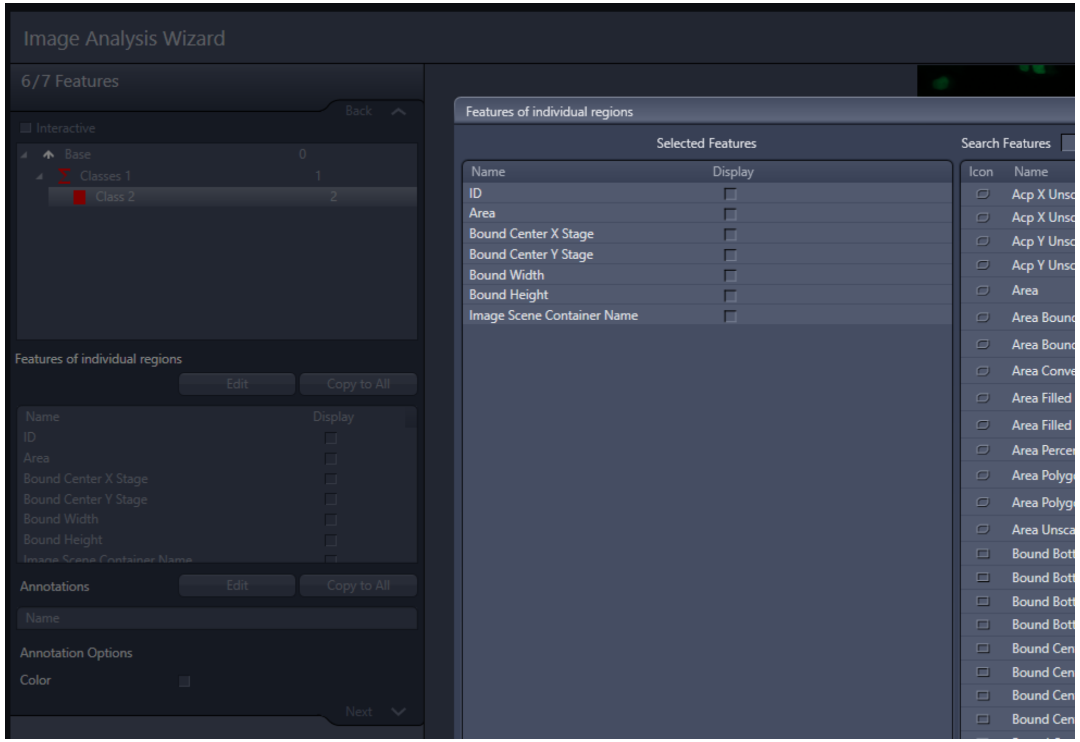
☐ CorrRing Adjustment

Guided Acquisition – Find objects of interest

Set-up image analysis

Execute the overview experiment manually (or a few tiles) to set up the image analysis:

- Detect the objects of interest
- Make sure not to detect too many objects for testing purposes
- Define measurement parameters for the objects (necessary for the script!):
  - Object ID
  - Bound Center Stage X
  - Bound Center Stage Y
  - Bound Width
  - Bound Height
  - ImageSceneContainerName



Name	Display
ID	□
Area	□
Bound Center X Stage	□
Bound Center Y Stage	□
Bound Width	□
Bound Height	□
Image Scene Container Name	□

Icon	Name
	Acp X Unsc
	Acp X Unsc
	Acp Y Unsc
	Acp Y Unsc
	Area
	Area Bound
	Area Bound
	Area Conve
	Area Filled
	Area Filled
	Area Percer
	Area Polyg
	Area Polyg
	Area Unsca
	Bound Bott
	Bound Bott
	Bound Bott
	Bound Bott
	Bound Cen
	Bound Cen
	Bound Cen
	Bound Cen

Guided Acquisition – Find objects of interest

Create a User Interface

```
# Initialize Dialog
window = ZenWindow()
window.Title = 'MyDialog'
window.Initialize('Guided Acquition - Version : 1')

# add components to dialog
window.AddLabel('Image')
window.AddImage2dView(image)
window.AddDimension(image)
window.AddFolderBrowser('destfolder','Destination folder','C:\\OAD\\Output\\CZI Images')
window.AddDropDown('ddval','Dropdown',['first','second','third'],1)

# show the window
result = window.Show()
```

→ OAD_Training_User dialog with automatic arrangement

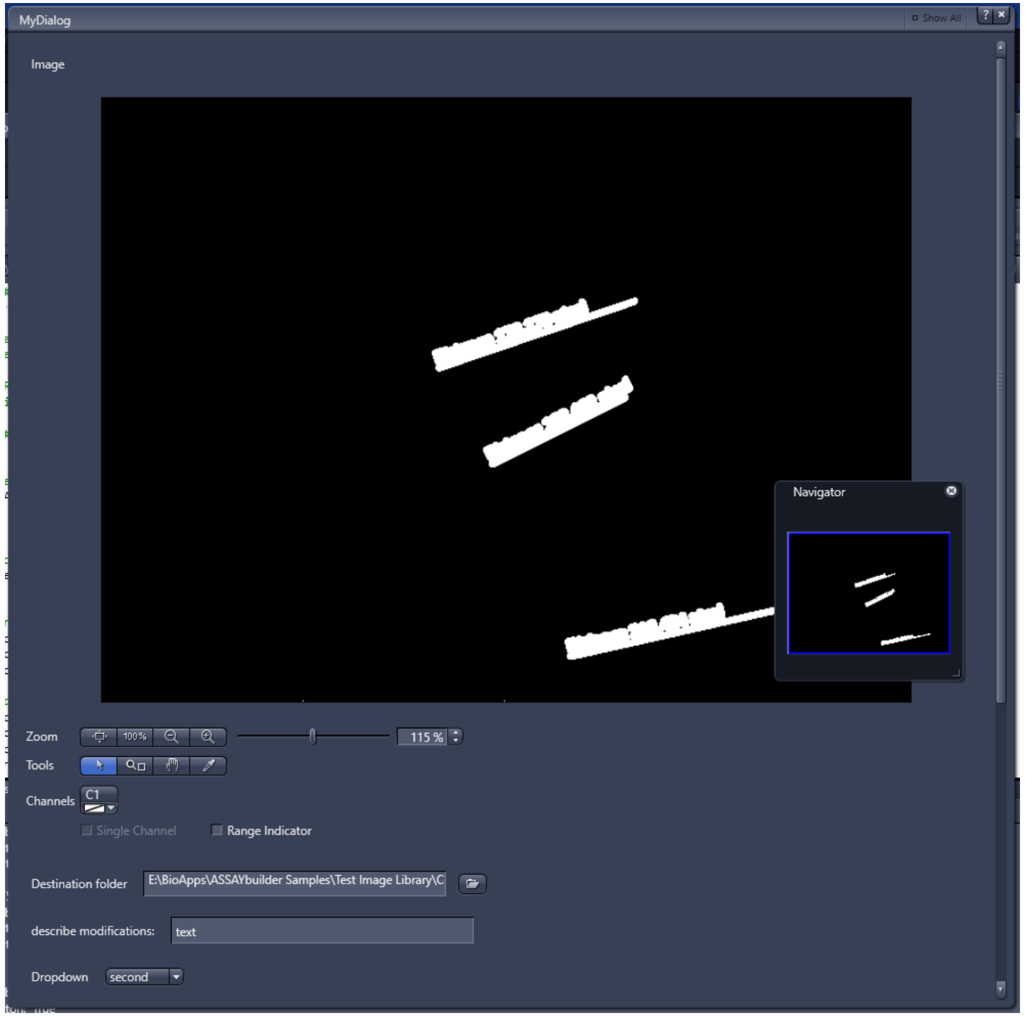
Guided Acquisition – Find objects of interest

Read out the Info from the User Interface

```
## get and check results
result=window.Show()

nosamples = str(result.GetValue('nosamples'))
concentration = str(result.GetValue('concentr‘))

print(nosamples)
print(concentration)
```



Zoom 115 %

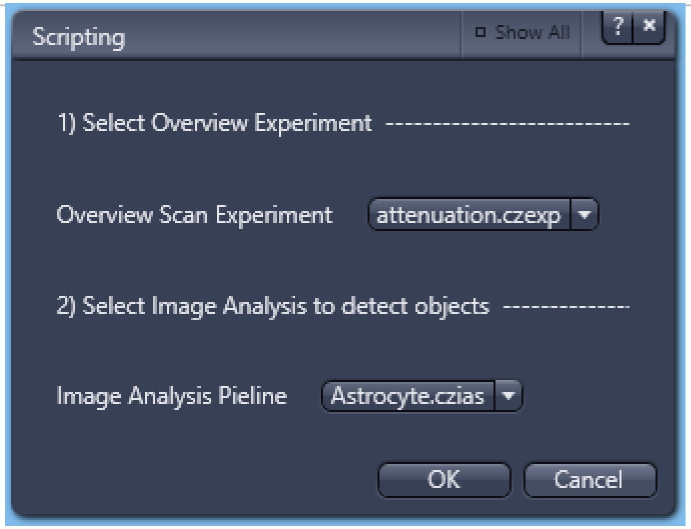
Destination folder: E:\BioApps\ASSAYbuilder Samples\Test Image Library\C

describe modifications: text

Dropdown: second

Guided Acquisition – Find objects of interest

Generate a Drop-Down with Experiments and IAs



Scripting

1) Select Overview Experiment ------------------------

Overview Scan Experiment attenuation.czexp

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

Image Analysis Pieline Astrocyte.czias

```
from System.IO import Directory, Path

def getshortfiles(filelist):
    files_short = []
    for short in filelist:
        files_short.append(Path.GetFileName(short))

    return files_short

# check the location of experiment setups and image analysis settings are stored
docfolder = Zen.Application.Environment.GetFolderPath(ZenSpecialFolder.UserDocuments)
imgfolder = Zen.Application.Environment.GetFolderPath(ZenSpecialFolder.ImageAutoSave)


# get list with all existing experiments and image analysis setup and a short version of that list
expfiles = Directory.GetFiles(Path.Combine(docfolder, 'Experiment Setups'), '*.czexp')
ipfiles = Directory.GetFiles(Path.Combine(docfolder, 'Image Analysis Settings'), '*.czias')
expfiles_short = getshortfiles(expfiles)
ipfiles_short = getshortfiles(ipfiles)
```

→ OAD_Training_Load and show experiments and IA in dropdown

Guided Acquisition – Find objects of interest

Execute an Experiment

Set up an experiment, and test it in the GUI first.
Then execute via Script.

```
## Load experiment
exp.Load("OAD_Training_Exp",ZenSettingDirectory.User)
## Execute experiment, display acquired image
## Image is saved automatically in temp folder of AutoSavePath of Saving tab of Tools/Options menu
image = Zen.Acquisition.Execute(exp)
```

→ OAD_Training_Load and execute a tiles experiment

Guided Acquisition – Find objects of interest

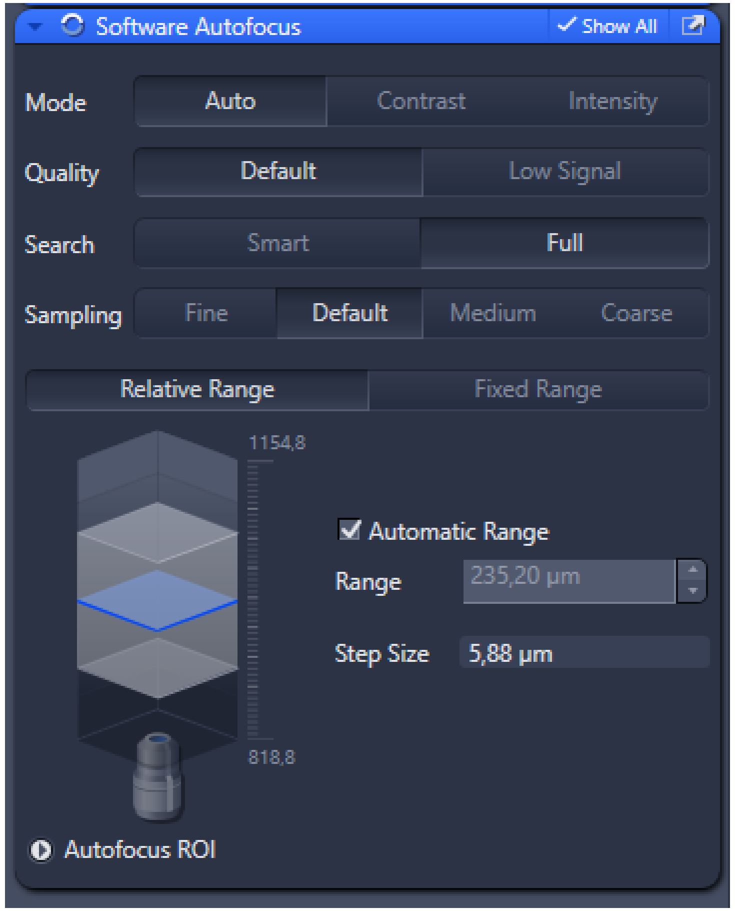
Perform Autofocus - Simple

```
exp = ZenExperiment()
exp = Zen.Acquisition.Experiments.ActiveExperiment
#exp = Zen.Acquisition.Experiments.GetByName("testexp24.czexp")

# get actual position
old_pos = Zen.Devices.Focus.ActualPosition
print(str(old_pos))

# Run Autofocus based on the settings defined in the Experiment
try:
    Zen.Acquisition.FindAutofocus(exp, 30)
except:
    print("SWAF failed, the original position will be used")
```

→ OAD_Training_SWAF simple



Software Autofocus ✓ Show All

Mode: [✓ Auto] Contrast Intensity

Quality: [✓ Default] Low Signal

Search: Smart [✓ Full]

Sampling: Fine [✓ Default] Medium Coarse

[✓ Relative Range] Fixed Range

☑ Automatic Range

Range: 235,20 µm

Step Size: 5,88 µm

Autofocus ROI

Guided Acquisition – Find objects of interest

Perform Autofocus - Advanced

→ OAD_Training_SWAF_programmable

```
def runSWAF_special(SWAF_exp, delay=5, searchStrategy='Full‘,
   sampling=ZenSoftwareAutofocusSampling.Coarse, relativeRangeIsAutomatic=False,
   relativeRangeSize=500, timeout=0):

       # get current z-Position
       zSWAF = Zen.Devices.Focus.ActualPosition
       # set DetailScan active
       SWAF_exp.SetActive()
       # set SWAF parameters
       SWAF_exp.SetAutofocusParameters(searchStrategy=searchStrategy, sampling=sampling,
       relativeRangeIsAutomatic=relativeRangeIsAutomatic, relativeRangeSize=relativeRangeSize)
       SWAF_exp.Close()

       return zSWAF

exp = ZenExperiment()
exp = Zen.Acquisition.Experiments.GetByName("OAD_Training_Exp.czexp")
zSWAF_new = runSWAF_special(exp)
```

Guided Acquisition – Find objects of interest

Modify Tiles Region of an Experiment

```
exp = ZenExperiment()
# Load a Tiles experiment!
exp.Load("OAD_Training_exp.czexp")

# get focus position
zpos = Zen.Devices.Focus.ActualPosition

# Perform Image Analysis and Create table with Bound and Stage information for each object
SingleObj = Zen.Analyzing.CreateRegionTable(img)
# get Bounds info from IA
soi = SingleObj.GetBoundsColumnInfoFromImageAnalysis(True)

# check the number of objects = rows inside image analysis table
num_POI = SingleObj.RowCount
```

→ OAD_Training_Modify tile regions

Guided Acquisition – Find objects of interest

Modify Tiles Region of an Experiment

```
# execute experiment at the position of every detected object
for i in range(0, num_POI, 1):

    # get XY-stage position from center position of object
    xpos = SingleObj.GetValue(i, soi.CenterXColumnIndex)
    ypos = SingleObj.GetValue(i, soi.CenterYColumnIndex)

    # get height and width of bounding box of object
    bcwidth = SingleObj.GetValue(i, soi.WidthColumnIndex)
    bcheight = SingleObj.GetValue(i, soi.HeightColumnIndex)

    # Modify the XYZ position and size of the TileRegion on-the-fly
    exp.ClearTileRegionsAndPositions(0)
    exp.AddRectangleTileRegion(0, xpos, ypos, bcwidth, bcheight, zpos)

    # execute the tiles experiment
    output = Zen.Acquisition.Execute(exp)
```

Guided Acquisition – Find objects of interest

OAD Script

Get **Guided_Acquisition_shortUI.py** from https://github.com/zeiss-microscopy/OAD/tree/master/Guided_Acquisition

Try Guided Acquisition Macro and adapt (e.g. perform Image processing steps,…)

→ Guided_Acquisition_shortUI

ZEISS