Guided Acquisition: Automate your microscopy - Detect rare events with ease

ZEN Guided Acquisition for Life Sciences Applications

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Seeing beyond

ZEN Module Guided Acquisition

Automate your microscopy, detect rare events with ease

Rare event detection in high demand

In life science research it is often necessary to selectively examine specific objects from a large population, e.g. to identify and selectively image a few dividing cells in a petri dish, to trace one specific neuron in a sectioned brain slice, or to acquire a 3-dimensional volume of cultured organoids with a certain size and shape. Such experiments are usually time consuming and prone to bias depending on the individual operator, especially if the events happen rarely. The ZEN Module Guided Acquisition has been designed to simplify this process by combining microscopy automation with image analysis. It can be used with multiple ZEISS imaging platforms such as Axio Observer 7 with scanning stage, Celldiscoverer 7, or LSM 980 with Airyscan 2.

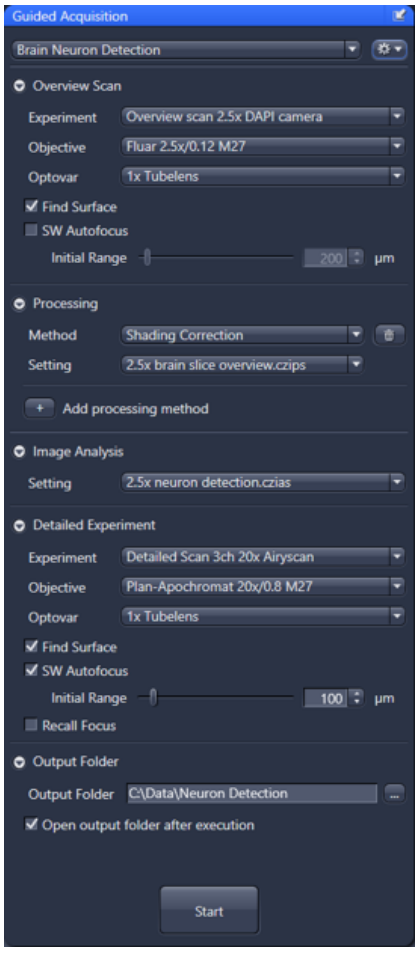
Guided Acquisition Workflow

1. Scan a large area with low magnification and fast imaging modality

2. Perform a pre-defined image analysis to detect objects of interest

3. Acquire detailed images for every detected object using specified settings

Once the Guided Acquisition workflow is optimized for a given sample, all settings can be saved and reused for another similar sample with one simple click.



- Low magnification
- Large area
- High throughput

- Image Analysis
- High specificity
- High efficiency

- High resolution
- Multi-dimension
- Full flexibility

1. Overview Scan

The purpose of the overview scan is to quickly tile-scan large areas using low magnification objectives and fast imaging settings (e.g. single DAPI channel using a camera with short exposure time and 2x2 binning). The image quality of this overview scan must be just good enough, for the following image analysis step to reliably detect the objects of interest. Imaging parameters for the overview scan can be adjusted and saved into one
"Experiment" setting. The focusing strategy can be specified as part of the "Experiment" setting complemented by additional Guided Acquisition options. Both the hardware focusing device Definite Focus 2 and Software Autofocus can combined for highest flexibility. An optional image processing step allows to perform, e.g. Airyscan processing or shading correction, of the overview scan prior to Object Detection if necessary.

2. Object Detection

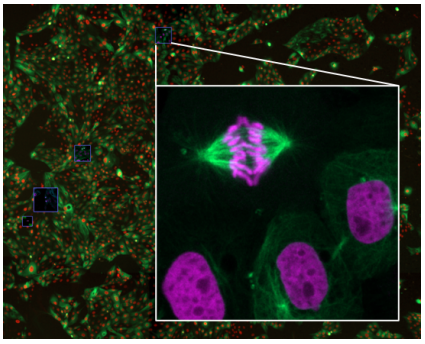
For the detection of objects of interest in the overview scan, Guided Acquisition uses the powerful and flexible ZEN Image Analysis module. Objects are isolated by image segmentation, using algorithms based on global thresholding, local variance, or Machine Learning (requires additionally the ZEN Intellesis module).

Additional filtering refines the list of detected objects based on their intensity, size or shape. Image analysis can be performed on both multi-channel fluorescent images and RGB color images, with various bit-depths. For downstream Detailed Acquisition, the location (X/Y scanning stage coordinates) and size (X/Y bounding box) of the detected objects is automatically recorded.

3. Detailed Acquisition

The third step consists of a different set of "Experiment" settings, typically with high magnification, high resolution, and multiple dimensions, which is performed for each detected object. If the size of a detected object is larger than a single field of view, a tile scan will be automatically configured, based on its bounding box size. All objects that were previously detected by the image analysis step will be acquired sequentially based on their stage coordinates. For each object, a different focus offset can be defined to accommodate samples with differing depths.
At the end of the workflow, all images (overview scan and detailed acquisitions) and settings (experiment, processing and analysis settings, and tables of detected objects) will be stored in one folder for easy access.

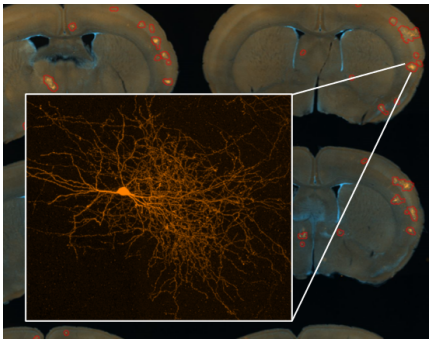
Guided Acquisition in Action



Mitotic Cell Detection from Petri Dish

In this example, porcine kidney cells (LLC-PK1) were cultured in a 35 mm glass bottom petri dish. The nuclei were labeled with Histone 2B mCherry, and microtubles with tubulin mEmerald. The goal was to detect the mitotic cells in the population. The experiment was performed using ZEISS Celldiscoverer 7. The overview scan was acquired with a Plan-Apochromat 5x/0.35 objective, 1x magnification changer, and the Axiocam 506 mono; the detailed acquisition was performed with a Plan-Apochromat 50x/1.2 water immersion objective, 0.5x magnification changer, and Airyscan MPLX HS mode. Image Analysis was performed on the nuclear channel, where mean intensity and area were used to detect the mitotic cells.

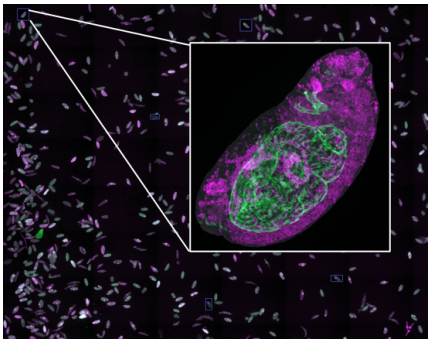
Sample obtained from ZEISS Oberkochen demo lab



Labeled neuron detection from mouse brain sections

In this example, 15 sectioned mouse brains were prepared on a standard microscope glass slide. The nuclei were labeled with DAPI, and the cells-of-interest are cortical interneurons which express membrane Tdtomato by low titre retroviral infection. The experiment was conducted using ZEISS Celldiscoverer 7. The overview scan was acquired with a Plan-Apochromat
5x/0.35 objective, 0.5x magnification changer, and the Axiocam 506 mono; the detailed acquisition was performed with a Plan-Apochromat 20x/0.95 objective, 0.5x magnification, Airyscan MPLX HS mode, and Z-stacks (figure shows maximum intensity projection of the detected neuron). Image Analysis was performed on the neuronal channel, where mean and range of intensity were used for detection.

Sample courtesy of Dr. L. Lim, Katholieke Universiteit Leuven/VIB Center for Brain & Disease Research, Belgium

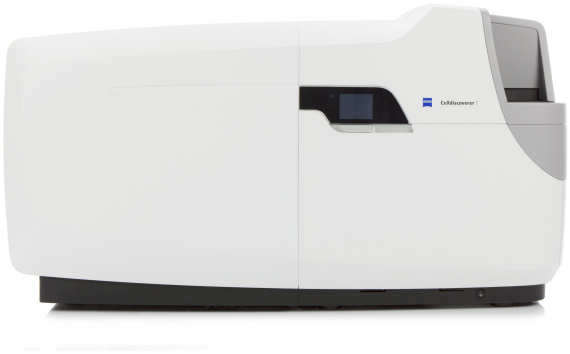


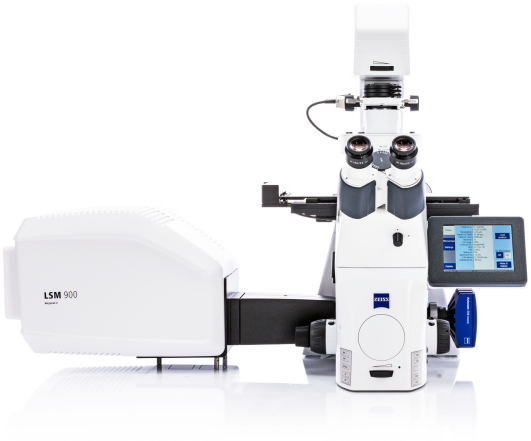
Drosophila embryo detection with lateral oriented gut structure from a prepared slide

In this example, a group of fixed drosophila embryos were prepared on a standard microscope glass slide. Longitudinal visceral muscles (one type of gut muscles) were labeled with Alexa 488, and Cut (one type of homeodomain transcription factor) with Cy3. The experiment was performed using ZEISS Celldiscoverer 7. The overview scan was acquired with a Plan-Apochromat 5x/0.35 objective, 0.5x magnification changer, and the Axiocam 506 mono; the detailed acquisition was performed with a Plan-Apochromat 20x/0.95 objective, 0.5x magnification changer, Airyscan MPLX HS mode, and Z-stacks (figure shows maximum intensity projection of the detected embryo). Image Analysis was performed on the gut structure, where green positive embryos were detected first by mean intensity, then filtered by geometric features to identify those with preferred lateral orientation.

Sample courtesy of Dr. G. Wolfstetter, University of Gothenburg, Germany

Guided Acquisition is available for multiple platforms





Hardware Requirements:
Axio Observer Z1/7
Axio Imager M1/M2/Z1/Z2
Axio Examiner
Axioscope 7
Axio Zoom.V16
Celldiscoverer 7 (with LSM 900)
LSM 800 (with Airyscan)
LSM 800 MAT
LSM 900 (with Airyscan 2)
LSM 900 MAT
LSM 980 (with Airyscan 2)
Scanning stage is required for all stands
Motorozed objective nosepiece is recommended
Definite Focus 2 is recommended for Axio Observer 7

Software Requirements:
ZEN blue 3.1 and above
ZEN blue 3.2 is required for overview image processing and detector parcentricity correction
ZEN module Image Analysis is required
ZEN module Tile & Position is recommended
ZEN module autofocus is recommended for software autofocus
ZEN module Intellesis is recommended for machine learning based image segmentation
Additional automation possible via the ZEN module Macro Environment
Seamless integration with ZEN Connect and Direct Processing modules

Definite Focus 2 is recommended for Axio Observer 7

*Front page image shows Guided Acquisition for detection of cell-cell interaction between mammalian U2OS cells expressing late endosome (Rab5-mEmerald) or actin (lifeAct-tdTomato). Sample from ZEISS Oberkochen demo lab

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