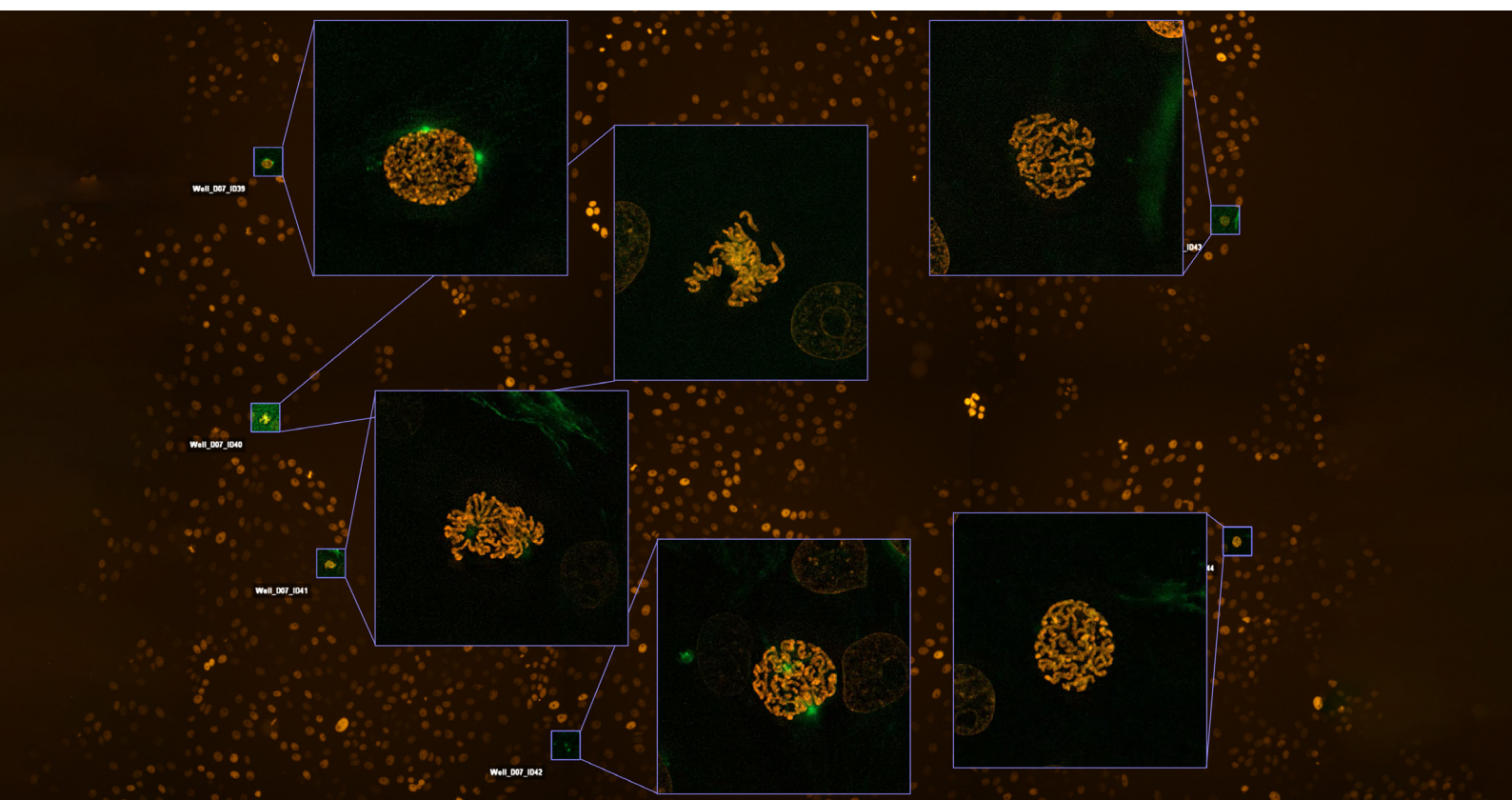


Study Mitotic Progression with Guided Acquisition Rare Event Detection



Seeing beyond

Date: March 2026

Microscopy is a cornerstone method to study the dynamics of living systems, and new optical methods continue to push the boundaries in terms of spatial and temporal resolution. With this advance in technology, more live biological events become accessible with imaging.

There are however limits of what can be achieved with reasonable effort if a traditional approach to imaging is employed. Consider an event that is occurring only occasionally, and imaging conditions that don't allow screening for a sufficiently large sample area to capture the event. This is where Smart Microscopy [1] workflows can be very helpful. They encompass the intended event acquisition, and can also automate the prior event detection.

ZEISS Microscopy has now introduced event detection into Guided Acquisition, its GUI-based Smart Microscopy tool available in ZEN, which is commonly used to control LM instruments in the Life Sciences arena. In this application note, we explore its features and capabilities in the context of cellular mitosis.

We will demonstrate how to configure these workflows easily and enhance their quality using AI-based event detection. Additionally, we'll show how to analyze the data with ZEISS arivis Pro.

Introduction

Principles of Smart Microscopy

To obtain a systematic understanding of Smart Microscopy, it can be quite instructive to understand its main experimental strategies and applications according to Figure 1. The key characteristic is the **automatic adjustment of a motorized, computer-controlled imaging system** as a result of analyzing data from this system in real-time in a perpetual adaptive feedback loop.

Depending on the intent of the feedback, Smart Microscopy can be divided into several sub-categories. In the simplest case, **quality-driven** feedback aims at adjusting imaging parameters on-the-fly, such that optimal image quality or sample preservation can be achieved. Often, such feedback mechanisms are built-in in modern imaging systems and often operate unnoticed by the user. They may control advanced technologies such as adaptive optics for deep tissue imaging but also include commonly available features like autoexposure or autofocus.

Next in line would be **event-driven** feedback which already brings unique applicative benefits. The intent is identifying sample regions or events of scientific interest and generating high-resolution image data for these, with imaging parameters that couldn't be easily applied to the entire sample space. Such approaches help to generate more specific data faster, reduce data sizes, prevent photodamage or excessive manual workload. Within ZEN, Guided Acquisition and AI Sample Finder would fall into this category.

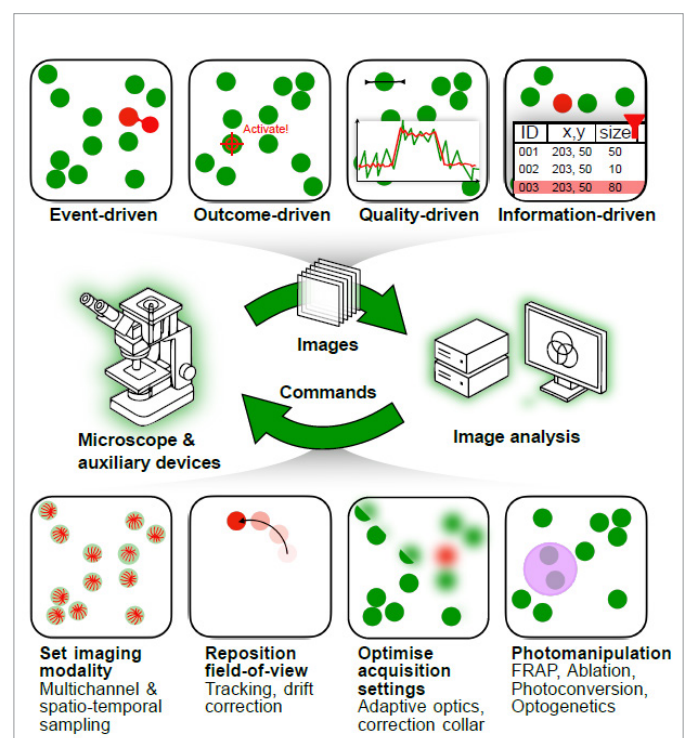


Figure 1 Smart Microscopy in a nutshell. Smart Imaging approaches are characterized by continuous feedback between the instrument and an analysis module that evaluates image data and guides subsequent imaging. Smart Imaging can be divided in subcategories based on either experimental intent (upper panel) or available experimental manipulations (lower panel). Figure from [1].

Outcome-driven feedback describes procedures where light is directly and automatically used to manipulate the biological sample. For example, workflows employing photo-bleaching (FRAP, FLIP, etc.) or locally-applied fluorescence correlation, where target area identification occurs adaptively by image analysis. A particularly exciting novel methodology in this category is opto-genetics, where cellular properties such as protein expression and migratory patterns are controlled by light [3].

All the above categories have in common that the workflow is supervised and target properties (e.g. cells in mitosis) are known beforehand. This is not the case for **information-driven** feedback, which employs statistical or machine learning models to guide imaging to relevant new information instead of redundant data.

For instance, an unsupervised high-content screen for detection of cellular phenotypes can be utilized, where every discernible phenotype within a feature distribution is imaged with high resolution. Phenotypes might be determined based on protein expression profiles, migratory patterns or any other spatio-temporal property [4].

Implementation Strategies & Guided Acquisition

In the emerging field of Smart Microscopy most studies have been conducted by academic groups, with custom software and hardware solutions supporting their experimental workflow. This allowed fast progress towards the individual research goal but limited the re-usability and adaptation of code and protocols.

ZEISS has therefore designed its Guided Acquisition (GA) tool with much more flexibility in mind. While relying on a fixed sequence (overview scan, analysis, detailed scans), it can integrate most ZEISS instruments running on ZEN and any experimental setup possible on these instruments (Figure 2). Data analysis is based on the Image Analysis Wizard in ZEN, which is quite flexible in terms of segmentation methods and feature filters. Together, these cover a broad range of imaging modalities and biological models.

Since ZEN 3.12 Guided Acquisition has now been updated to also run looped workflows. Most importantly, this enabled GA workflows to include biological events in time. Furthermore, employing repeated overview scans can be used to conduct long-term monitoring, e.g. of growing organoids.

Mitosis detection as „prototype“ rare event

To test the new GA tool on a sufficiently common phenomenon, we decided to focus on mitotic events. These are interesting for several reasons. First, only 1% – 4% of cells in a culture undergo mitosis at any given time. Hence, it is sufficiently rare that a guided approach to mitosis detection saves time and disk space compared to imaging them randomly. Second, mitosis is a key biological event that many researchers are interested in for various reasons, e.g. to study its mechanisms or genomic instability. And third, based on mitotic time-lapse data we can also nicely showcase some image analysis schemes in our image analysis software arivis Pro.

We will demonstrate the capabilities of Guided Acquisition incrementally, starting with a simple experimental setup and progressively improving the workflow.

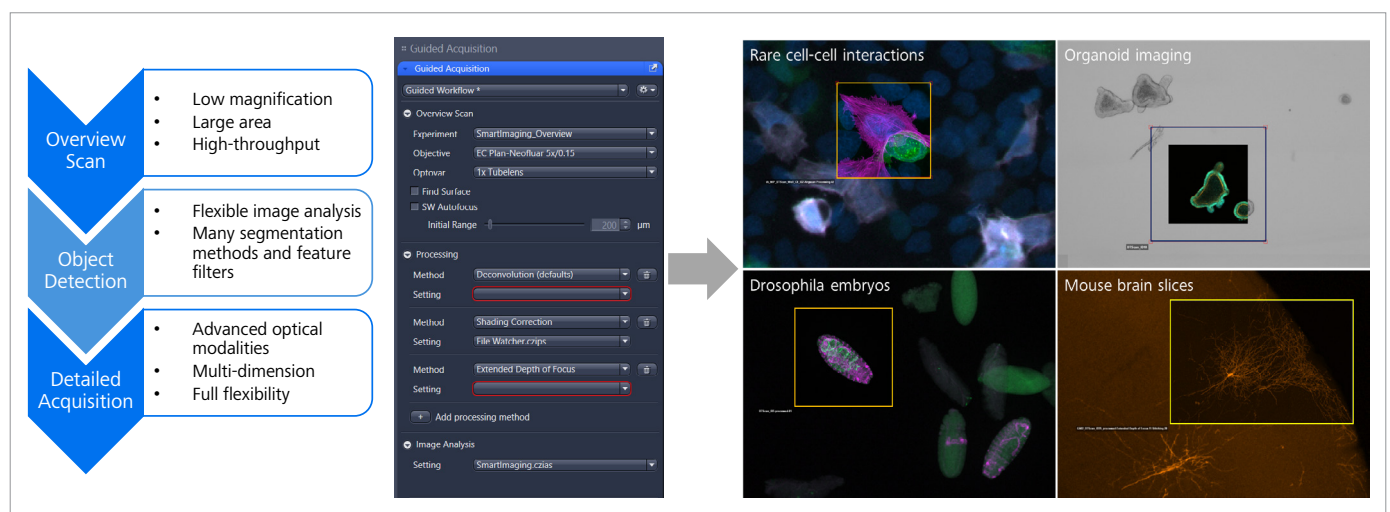


Figure 2 Basics of Guided Acquisition. The workflow follows a fixed sequence with an overview scan, subsequent object detection and one or more detailed scans that create high-resolution scans of identified targets (left panel). Configuration of imaging and analysis is implemented very flexibly (central panel), such that GA workflows can run across different optical modalities and biological scales.

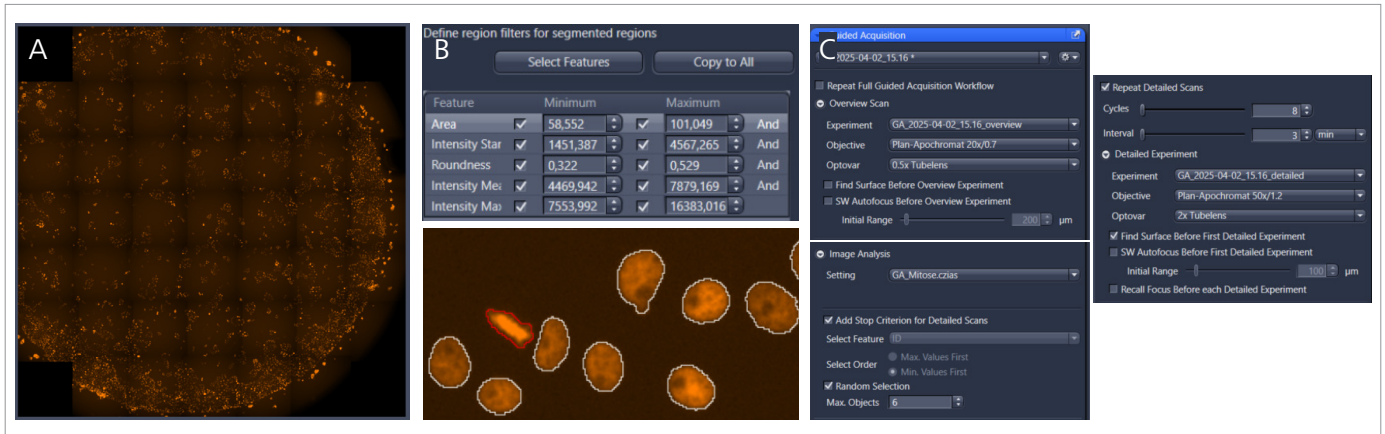


Figure 3 Setting up Guided Acquisition for mitotic cells. (A) Overview low-resolution tile scan to be used in subsequent mitosis detection. (B) Filtering segmented nuclei for metaphase cell detection. Metaphase cells can be automatically identified based on their elongated shape and higher fluorescence intensity (cell with red outline). (C) Final GA configuration based on pre-configured acquisition and image analysis setups. 6 individual cells were tracked in parallel, for a time-lapse with 8 cycles and 3 minutes per cycle.

Experimental procedures

Instrument setup

Experiments were performed on a Celldiscoverer 7 with incubation at 37 °C and 5 % CO₂ using Guided Acquisition. Overview scans were acquired in widefield mode with LED555 and Axiocam 820m at 10× magnification with a 20× 0.7 air objective and a 0.5× tube lens, stabilizing the focus position with hardware autofocus. The resulting multiple tiles were segmented in ZEN to detect mitotic cells.

Detailed scans were acquired at the automatically identified positions with Axiocam 820m at 100× magnification with a 50× 1.2 water-immersion objective and 2× tube lens, using 480 and 555 nm excitation. Therefore, detailed scans were done at 10-fold higher magnification than overview scans.

Cell culture

The cell line used was pig kidney LLC-PK1 expressing H2B-mCherry and αTubulin-GFP. Cells were maintained in a 96-well plate in MEM-alpha supplemented with 10 % FCS, 100 U/mL streptomycin, and 100 μg/mL penicillin under standard culture conditions (37 °C, 5 % CO₂, 95 % humidity).

Results

Establishing a basic Smart Microscopy setup

To configure a GA workflow, the three components (overview scan, image analysis and detailed scan) were adjusted individually. First, we created a tiles experiment for the overview scan. Only the H2B-mCherry signal is required for subsequent detection of mitosis. Therefore, only the red channel was acquired in the overview scan. Definite focus was used on every second tile for focus stabilization (Fig. 3A).

For image analysis, we aimed at detecting metaphase cells first, because they have a very recognizable phenotype. Nuclei were segmented first with simple threshold-based segmentation. We then used the filtering tool to detect metaphase cells, based on their elongated shape and highly compacted chromatin (small area, larger intensities and smaller roundness) (Fig 3B). Within Image Analysis Wizard this can be done interactively (by clicking positive nuclei in the viewer), without the need to manually determine feature parameters.

For detailed scans, a z-stack acquisition with 13 slices and 0.5 μm slice intervals was configured, now detecting both green and red channels, and using the software autofocus for focus stability. With all acquisition and analysis setups in place, the GA setup was created (Fig. 3C). A timelapse was configured with 8 cycles and 3 minutes / per cycle. Given that a single stack requires about 20 seconds to acquire, we set the number of detailed scans to 6, thereby ensuring that the 3 minutes cycle interval stays in time.

Image processing

After successful completion of a time-lapse GA run, data exists as [CZI files from single time points and] must be concatenated as the current implementation does not perform this automatically. Therefore, we created an Open Application Development (OAD) script that takes the output folder of Guided Acquisition, identifies the detailed scans and merges them into time-lapse data sets: <https://github.com/zeiss-microscopy/OAD/tree/master/SmartMicroscopy/Guided%20Acquisition>.

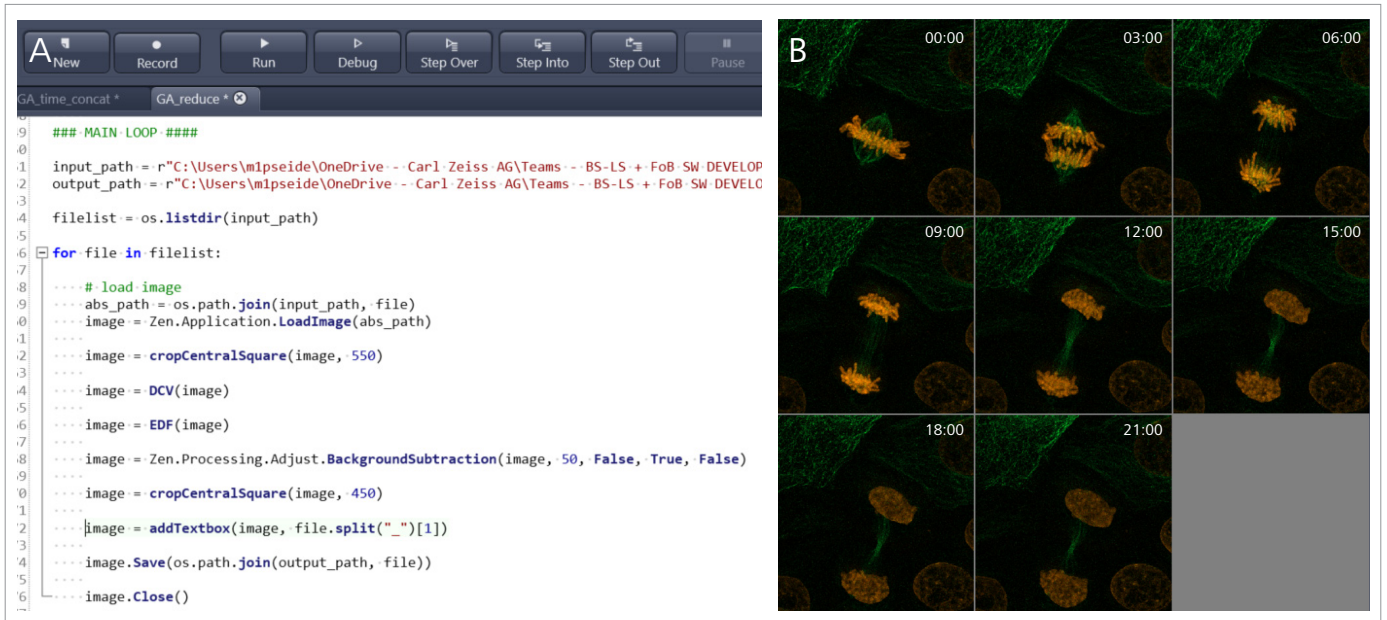


Figure 4 Image post-processing for mitosis data. (A) To optimize this data set, deconvolution, Extended-Depth-of-Focus (EDF), background subtraction and image cropping was used. This results in a projected, time-lapse data set of sufficient size to show mitotic progression. (B) Final image sequence for one detailed scan.

To enhance the raw data for image visualization and subsequent analysis, we employed an OAD script again to perform deconvolution, Extended-depth-of-Focus (EDF), background subtraction and image cropping in an automated fashion (Fig. 4). The final result is a highly-resolved data set specifically for mitotic events.

Extending the workflow – Part 1: Well-plate imaging

Studying mitotic progression can be a valuable read-out for example in (genotoxic) drug screening experiments. Hence, rare-event detection with Guided Acquisition must be compatible with high-throughput well plate setups.

To adapt our current workflow, we simply extended the overview scan to scan a complete multi-well plate (Fig. 5).

However, in this case it must be considered, that acquiring an overview of all wells first and then proceeding to detailed scans, will cause most of the detected mitoses to be acquired too late.

To mitigate this problem, Guided Acquisition can detect the well-plate setup and automatically offers the option „Acquire and Process Wells Sequentially“ (Fig. 5). In this mode overview and detailed scans were done for every well individually before proceeding to the next well. As a result, all detected mitoses were scanned in a timely fashion.

To ensure stable focus in this extended setup, we adjusted the autofocus strategy: Definite Focus was used for overview scans, and the tiles setup focus was re-used for detailed scans.

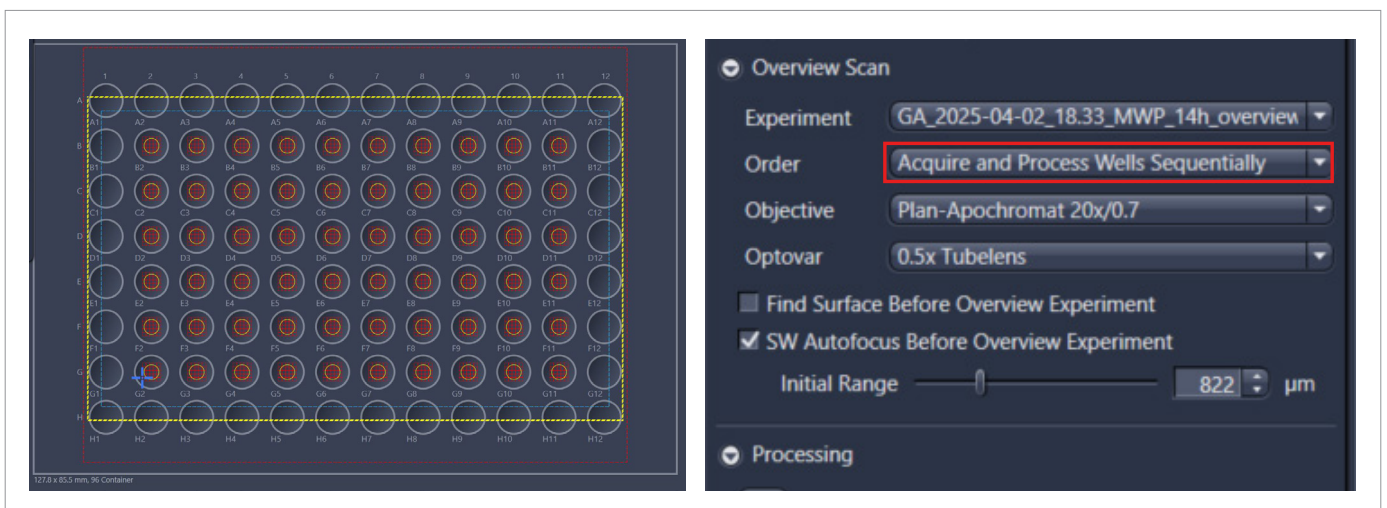


Figure 5 Extending mitosis detection to high-throughput conditions. GA well-plate imaging basically requires two simple steps of protocol adaptation. First, the overview scan's tiling is changed to a complete well-plate setup (left panel). The GA user interface then offers the option "Acquire and Process Wells sequentially" (right panel).

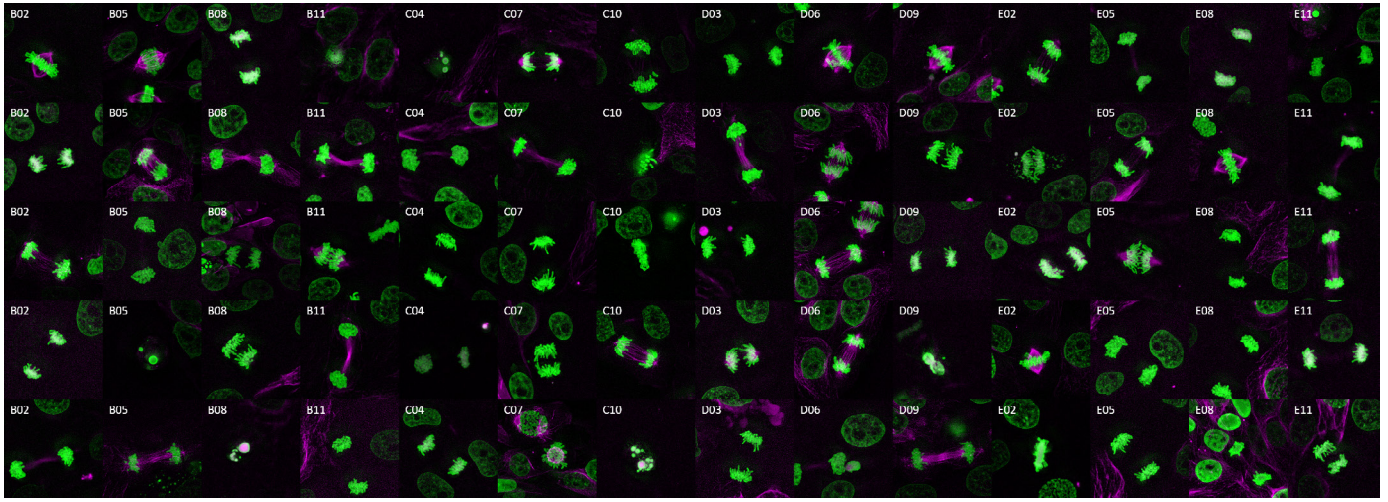


Figure 6 Mitosis detection to high-throughput conditions. Mosaic image was created by post-processing the raw time-lapse data in ZEN and then doing a montage from single cropped images in ZEISS arivis Pro.

Results are displayed in Figure 6, which shows the power of this approach. In 18 hours of a fully automated overnight experimental run, data from 300 positions were acquired, from which 266 were bona-fide mitotic events while 34 were wrongly detected (apoptotic) cells. Doing this manually or by brute-force scanning would have been virtually impossible.

Extending the workflow – Part 2: Deep Learning

Despite the impressive throughput in the well-plate run, the results were not yet completely satisfying. With the current workflow, apoptotic cells were falsely detected in ~15 % of cases (Fig. 7A). We also wondered whether it would be possible to detect cells in prophase, the earliest stage of mitosis. Figure 7B shows that prophase cells have a distinct amorphous texture (caused by chromatin starting to condense) that can be detected by eye. However, we failed to access this phenotype with a reliable conventional analysis.

We therefore aimed at integrating a more potent Deep Learning model into the workflow. To this end, representative data from

overview scans was first uploaded into ZEISS arivis Cloud, our web platform for annotation and training of Deep Learning models. 2 hours were spent to annotate 60 prophase and 90 metaphase cells (as control) (Fig. 7C), which was sufficient to generate a semantic segmentation model reliably detecting prophase cells.

This model was then imported to ZEN and directly incorporated in the object detection workflow. To further improve the robustness, feature filtering was used to eliminate any residual false-positives (Fig. 8).

Analysis of mitotic delay with ZEISS arivis Pro

In the last part of this app note we want to showcase how to analyze mitotic data employing Zeiss' dedicated image analysis platform arivis Pro.

One typical read-out for our mitosis data would be measuring the time until a cell undergoing mitosis fully divides into two daughter chromosome sets (= enters anaphase). A failure to

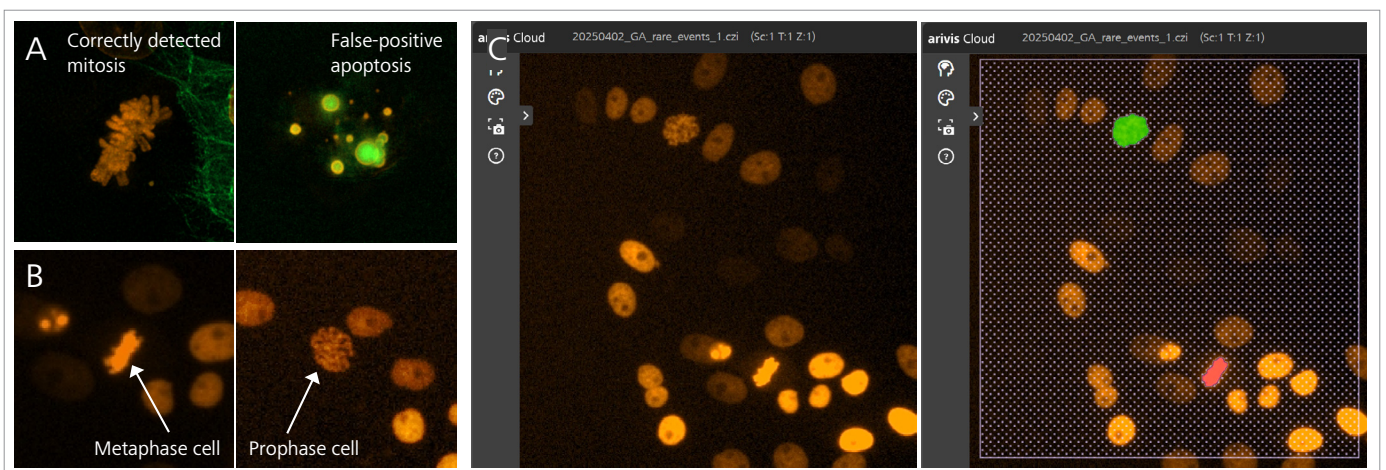


Figure 7 Improving object detection by integrating Deep Learning. (A) The current procedure wrongly detects apoptotic cells in ~15 % of cases. (B) Furthermore, the threshold-based segmentation does not detect Prophase cells (the earliest stage of mitosis). (C) Employing ZEISS arivis Cloud, prophase and metaphase were annotated and a Deep Learning model was trained. An example image is shown without (left panel) and with (right panel) object masks.

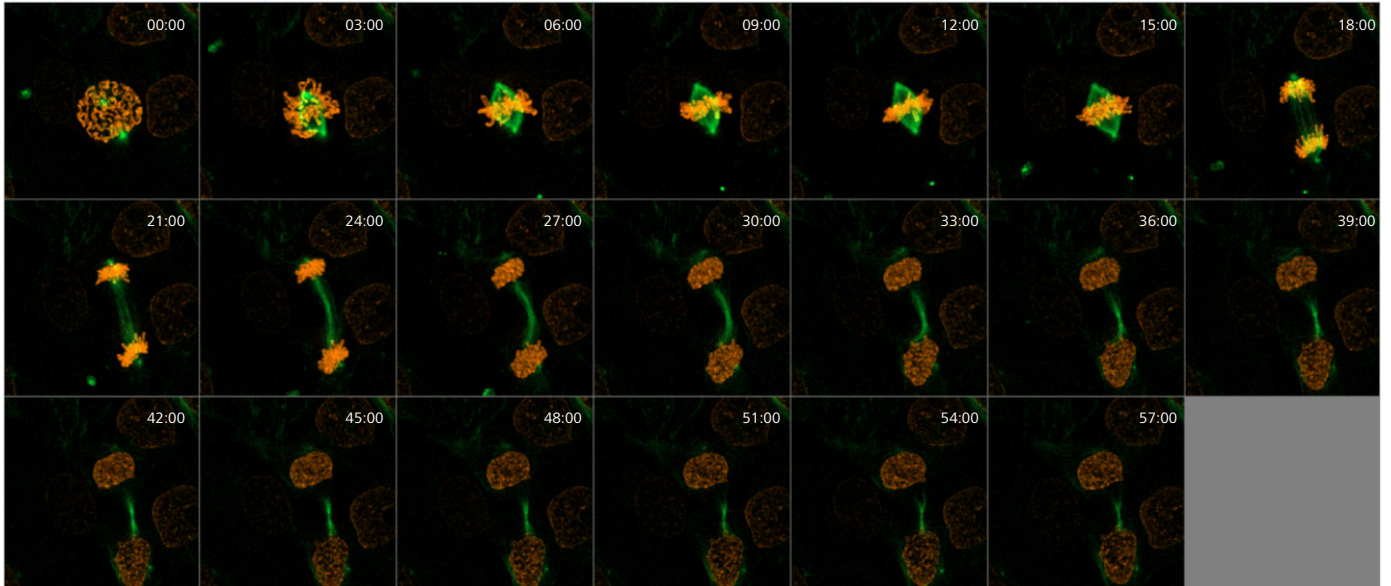


Figure 8 Full mitosis detection with semantic segmentation. This example data shows a time-lapse montage over 20 cycles and 3 minutes per cycle. Notice how the Deep Learning model reliably detects prophase cells early in mitosis, therefore generating to best possible mitotic event data.

divide in a timely manner, due to DNA-damage or activated arrest checkpoints is defined as „mitotic delay“ [5]. This may occur sporadically or because of drug treatments.

To this end, we imported a data set of ~140 mitoses to arivis Pro, with the aim to segment the dividing nuclei in every time frame and to measure the distance between both dividing chromosome sets (Fig. 9).

Segmentation of dividing cells is challenging due to the presence of bystander cells within the field-of-view, which need to be distinguished by the analysis. The best approach for this challenge consisted of (i) segmenting all cells based on simple threshold-based segmentation (+ applying watershed) and (ii)

using arivis Pro's tracking functionality to obtain full tracks of identified nuclei (Fig. 9). Then, in a manual process, (iii) removing all unwanted tracks from the data set and (iv) calculating distances between dividing nuclei.

Finally, based on the measured distances between daughter chromosome sets, the time point of anaphase initiation was determined for every mitosis and aggregated into a histogram (Figure 10). Of note, even in the absence of any drug treatment, we find a natural variation concerning mitotic progression: A subpopulation of cells peak at around 20 minutes after mitosis initiation (=normal mitotic progression), while ~34 % of cells require 30 minutes or more.

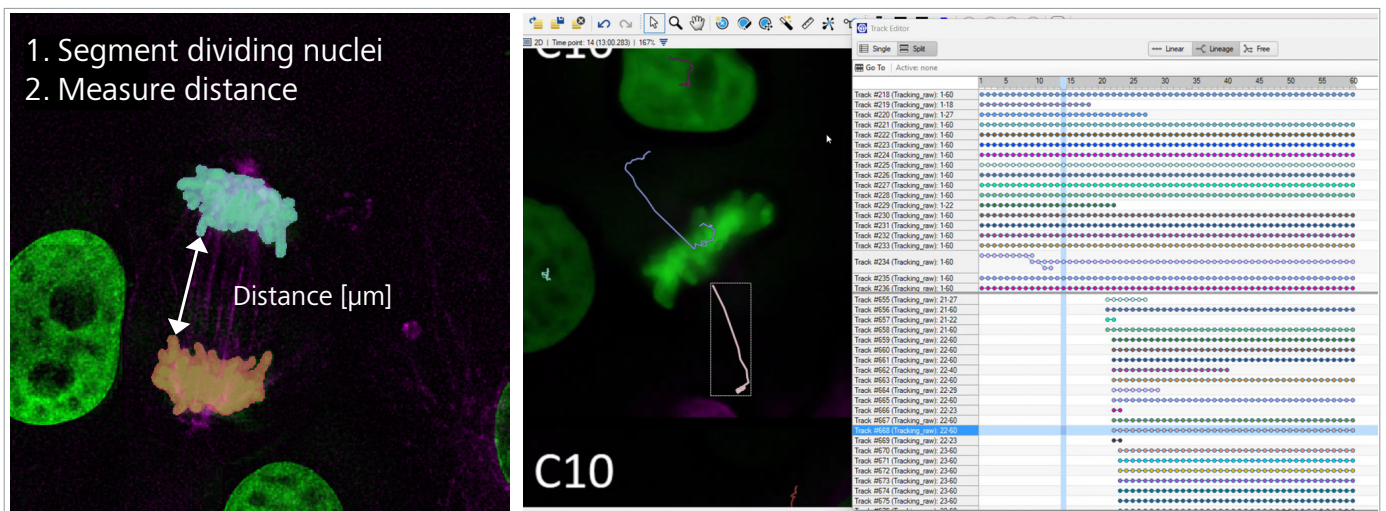


Figure 9 Image analysis scheme for quantifying mitotic delay. Dividing cells were segmented and the distance between daughter chromosome sets was measured for every time frame (left panel). To guarantee detection of only dividing cells, and no bystanders, cell tracking was employed together with manual track editing.

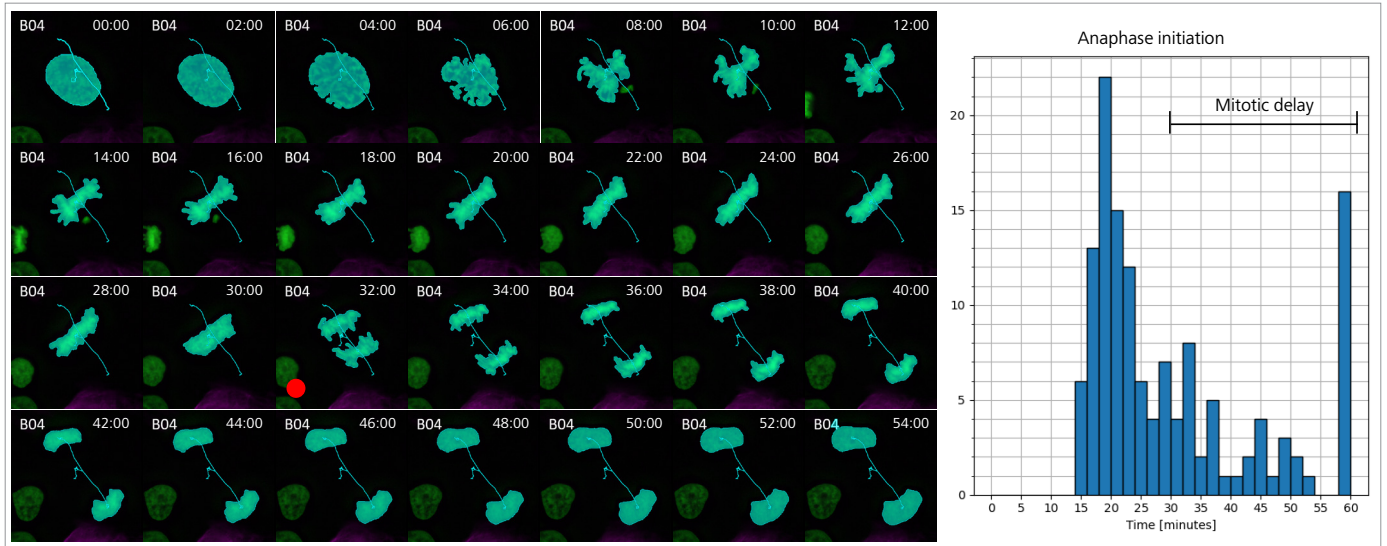


Figure 10 Analysis results for mitotic delay. (Left panel) Time-lapse sequence shows identified nuclei and tracks for one example mitosis data set. The red spot represents the initiation of anaphase, that is, the splitting of the two daughter chromosome sets. **(Right panel)** Distribution plot for anaphase initiation over all 140 recorded mitoses. Notice the peak at ~20 minutes after mitosis initiation, which is the group of cells with normal mitotic progression. Cells with more than ~30 minutes after mitosis initiation display mitotic delay of different severity.

Discussion

We hope that we could convincingly demonstrate how to employ Guided Acquisition for rare event detection. We have presented, how to perform a basic setup and incrementally improve it by introducing well-plate acquisitions and Deep Learning-based object detection. Using this method, we automatically generated high-quality mitosis data with impressive throughput (e.g. 300 mitoses in 14 hours).

Of note, with Guided Acquisition being an integral part of ZEN, this workflow could be easily transferred to other ZEISS imaging systems, depending on the experimental aim. For example, if super-resolved data is required, the user could resort to the ELYRA 7, if optical sectioning is required, the choice would be LSM 910/990.

However, the Guided Acquisition rare event detection also has its limitations. First, it follows a hard-wired sequence of steps (overview → analysis → detailed scan) that cannot be altered. Second, because the workflow depends on ZEN-intrinsic image analysis and communication, which generates some computational overhead, ultra-fast events (scale of milliseconds), e.g. mitochondria fission and fusion [6], cannot be imaged. For such events, as for any customization of GA workflows, experiments may be done employing OAD scripting (or ZEN API), which enable the use of (potentially faster) external components.

For processing, we have shown how to handle a large dataset consisting of multiple files by employing ZEN's macro editor and scripting. With this, sequences of processing steps can be fully automated further reducing hands-on time. For the data analysis, we resorted to a relatively simple read-out (mitotic delay), which was fine to showcase ZEISS arivis Pro as a viable option for this task. It should however be noted that many, more sophisticated read-outs do exist for the analysis of mitosis (e.g. kinetochore movement, metaphase plate orientation, or kinetochore misalignment), as demonstrated by Shi et al. [7].

References:

1. Lucien Hinderling, Hannah S. Heil, Alfredo Rates, Philipp Seidel, Manuel Gunkel, Benedict Diederich, Thomas Guilbert, Rémy Torro, Otmame Bouchareb, Claire Demeautis, Célia Martin, Scott Brooks, Evangelos Sisamakakis, Erwan Grandgirard, Jerome Mutterer, Harrison Oatman, Jared Toettcher, Andrii Rogov, Nelda Antonovaite, Karl Johansson, Johannes K. Ahnlind, Oscar André, Philip Nordenfelt, Pontus Nordenfelt, Claudia Pfander, Jürgen Reymann, Talley Lambert, Marco R. Cosenza, Jan O. Korbel, Rainer Pepperkok, Lukas C. Kapitein, Olivier Pertz, Nils Norlin, Aliaksandr Halavatyi, Rafael Camacho. Smart Microscopy: Current Implementations and a Roadmap for Interoperability. bioRxiv 2025.08.18.670881; doi: <https://doi.org/10.1101/2025.08.18.670881>
2. Leonor Morgado, Estibaliz Gómez-de-Mariscal, Hannah S. Heil, and Ricardo Henriques. The rise of data-driven microscopy powered by machine learning. Journal of Microscopy, 945 295(2):85–92, March 2024. <https://doi.org/10.1111/jmi.13282>
3. Josiah B. Passmore, Alfredo Rates, Jakob Schröder, Menno T. P. van Laarhoven, Vincent J. W. Hellebrekers, Henrik G. van Hoef, Antonius J. M. Geurts, Wendy van Straaten, Wilco Nijenhuis, Florian Berger, Carlos S. Smith, Ihor Smal, Lukas C. Kapitein. Outcome-Driven Microscopy: Closed-Loop Optogenetic Control of Cell Biology. bioRxiv 2024.12.12.628240; <https://doi.org/10.1101/2024.12.12.628240>
4. André O, Kumra Ahnlind J, Norlin N, Swaminathan V, Nordenfelt P. Data-driven microscopy allows for automated context-specific acquisition of high-fidelity image data. Cell Rep Methods. 2023 Mar 6;3(3):100419. <https://doi.org/10.1016/j.crmeth.2023.100419>. PMID: 37056378; PMCID: PMC10088093.
5. Farrell KC, Wang JT, Stearns T. Spindle assembly checkpoint-dependent mitotic delay is required for cell division in absence of centrosomes. Elife. 2024 Aug 2;12:RP84875. doi: 10.7554/eLife.84875. PMID: 39092485; PMCID: PMC11296703.
6. Mahecic D, Stepp WL, Zhang C, Griffié J, Weigert M, Manley S. Event-driven acquisition for content-enriched microscopy. Nat Methods. 2022 Oct;19(10):1262-1267. doi: 10.1038/s41592-022-01589-x. Epub 2022 Sep 8. PMID: 36076039; PMCID: PMC7613693.
7. Shi Y, Tabet JS, Milkie DE, Daugird TA, Yang CQ, Ritter AT, Giovannucci A, Legant WR. Smart lattice light-sheet microscopy for imaging rare and complex cellular events. Nat Methods. 2024 Feb;21(2):301-310. doi: 10.1038/s41592-023-02126-0. Epub 2024 Jan 2. PMID: 38167656; PMCID: PMC11216155.

Useful links:

Smart Acquisition Toolkit webpage (toolkit contains Guided Acquisition) – [Smart Acquisition Toolkit | ZEISS](#)
Developer Toolkit webpage (toolkit enables creating OAD scripts) – [ZEN Developer Toolkit | ZEISS](#)
ZEISS arivis Cloud homepage – [arivis Cloud - automated image analysis](#)
ZEISS arivis Pro homepage – [ZEISS arivis Pro Software](#)
ZEISS OAD github page – <https://github.com/zeiss-microscopy/OAD>



Carl Zeiss Microscopy GmbH
07745 Jena, Germany
microscopy@zeiss.com
zeiss.com/arivis-cloud

Follow us on social media:

