

Automating Cellular Imaging with ZEISS Celldiscoverer 7



PAA

AT THE HEART
OF LABORATORY
AUTOMATION

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Seeing beyond

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Sparked by the evolution of reliable and affordable automated microscopes, the past decade has witnessed a surge in the popularity of automated cell screening assays. The wealth of advantages afforded by automating live cell screening assays is clear. Generating high quality, reproducible data at statistically relevant numbers yields robust experimental results, while laboratory staff are freed from the burden of manual repetitive steps to focus on more dynamic tasks. Enhancing laboratory quality and efficiency, automation is a driving force behind scientific discoveries - and is a popular solution for cell screening applications throughout Biotechs and academia.

Automating imaging workflows can be as simple as running a batch of sample plates through the imager for fixed endpoint assays to more complex multi batch, multi read kinetic assays with scheduling to generate data over multiple time points and samples.

Improving accuracy, reproducibility and throughput in all steps of the imaging workflow is the biggest challenge in automated microscopy. Often the data will only be revealed through multiple runs of experiments or complex assays. Automation and parallelization of imaging can be the only way to get there. Observing live specimens in various sample carrier formats over several days or imaging lots of multi-well plates is a demanding and time-intensive task for the microscope as well as its user.

Integration of robotic solutions for sample handling into an imaging system can boost the degree of automation and thereby throughput. Carl Zeiss, one of the world's leading manufacturers of microscopes, have teamed up with Peak Analysis and Automation (PAA), a leading robot manufacturer and supplier of automated solutions to provide an offering to meet the changing demands of the research community for high throughput imaging solutions.

ZEISS Celldiscoverer 7 – a fully automated life cell imaging platform

ZEISS Celldiscoverer 7 – an automated live cell imaging system designed to make complex microscopy simple – harbors multiple automation functions like automatic sample recognition, measurements of sample carrier bottom material and bottom thickness to automatically adapt the system for optimal imaging conditions, an auto-calibration routine for micro-well-plates, an auto-immersion water objective, a variety of autofocus options as well as automated image acquisition and processing to name but a few. All these features were assembled in this machine to allow for the easy, reproducible execution of sophisticated experiments.



The instrument enables imaging of multiple sample carrier types including multi-well plates, dishes, chamber slides, etc. giving the freedom to image various sample types from fixed or living cells, tissues, organoids, spheroids, and small model organisms. Therefore, environmental conditions such as CO₂, O₂ levels and temperature are tightly controlled.

ZEISS Celldiscoverer 7 harbors a unique optical concept – 4 front lenses in combination with 3x magnification changers deliver 12 magnifications (2.5x up to 100x), suited for a large variety of applications. Three of those magnifications (25x, 50x, 100x; NA 1.2) have an auto-immersion function that supplies and removes water fully automatically.

Two variants are available, a pure widefield setup with different high-end camera options as well as a combined system including the LSM 900 for confocal 3D optical sectioning. To speed up data acquisition, both camera and confocal images can be acquired within one experiment and precisely overlaid using the unique mixed mode acquisition.

The LSM system offers ZEISS key technology – the Airyscan 2 detector providing outstanding SNR and fast scanning option, thus enabling for fast and gentle live cell imaging. In addition to that advanced photomanipulation and spectral imaging experiments are possible using the LSM.

In this way, the Celldiscoverer 7 is a fully automated and flexible live cell imaging machine providing multimodal imaging possibilities for highest applicational flexibility.

PAA Automated Solutions for Sample Loading

The PAA automated solution provides automated sample handling for both fixed end point batch assays and Kinetic assays with an incubator to enable multiple incubation / read points and multiple batches to be run using the innovative Assay Manager software interface. The overall setup allows customization according to the application needs by integrating additional hardware (e.g., bar code reader, liquid handling). Thus, two main application types are offered as standard.

Fixed endpoint assays

This process utilizes the PAA sequential stacks to enable an operator to add a batch of plates to be processed through ZEISS Celldiscoverer 7. The stacks are connected to the bench with a PAA 'click and connect' POD base for easy operator load and unload of the stacks, to provide a system that is easy to load and easy to operate.

Kinetic Assays

PAA also offer an integrated incubator that can be used for multi-read incubation steps. The standard incubator has space for 42 standard multi-well plates and can control temperature

and CO₂ concentration to keep the cells at optimal conditions. Options for Hypoxia studies can also be applied to the incubator to mimic physiological changes caused by disease and the ability of cells to maintain cell and tissue homeostasis.

Larger incubator capacities can also be offered for more long-term studies with larger batch runs. The PAA Assay manager software interface enables multi-batch / multi-read studies to be easily programmed and seamlessly scheduled on the system.

Two variants for automated sample handling from PAA working seamlessly together with all other components of ZEISS Celldiscoverer 7 system are available: 1. S-LAB entry level variant
2. KX-2 high-end variant

Robotic loader	Celldiscoverer 7 Widefield	Celldiscoverer 7 + LSM 900
S-LAB	yes	no
KX-2	yes	yes

S-LAB plate handler integration with ZEISS Celldiscoverer 7

The innovative S-LAB plate handler is a low-cost plate handler that can be used to integrate ZEISS Celldiscoverer 7 Widefield version.

The S-LAB plate handler has been designed to provide an entry level automation solution with a compact plate handler that has the reliability and robustness of a robot arm. The S-LAB can handle lidded and unlidded microplates and is supplied with a de-lidding station and barcode reader. The S-LAB has optical plate sensing for reliable unattended operation, and a plate loading time as quick as 16s with a full cycle time of 32s for quick and reliable sample transfer.

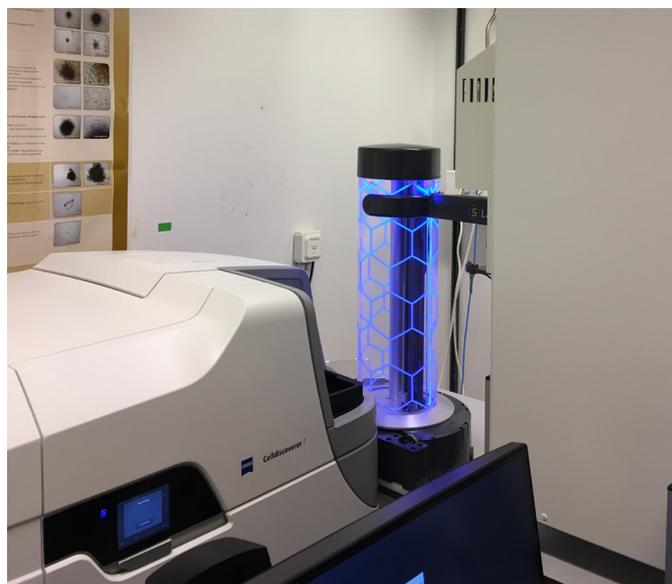


Figure 1 Installation of S-LAB plate handler, Celldiscoverer 7 and incubator at a customer site

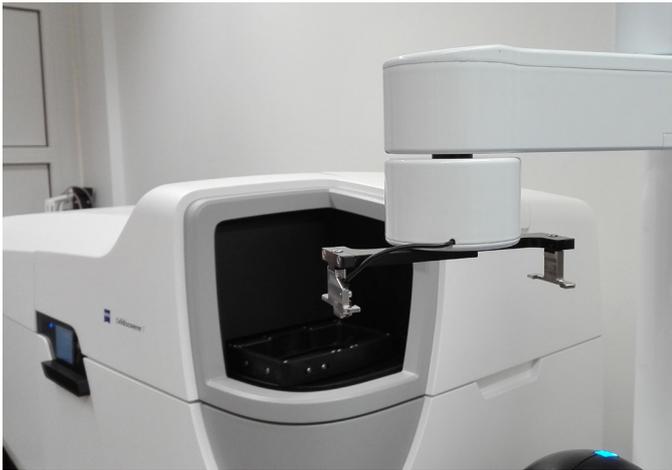


Figure 2 Installation of KX-2 robot, Celldiscoverer 7 and incubator at a customer site

The S-Lab can be supplied with stacks to provide a capacity of up to 100 standard microplates (unlidded) / 80 lidded for Fixed endpoint assays and is also compatible with an automated incubator for kinetic assays as shown in the image above.

KX-2 robot integration with ZEISS Celldiscoverer 7

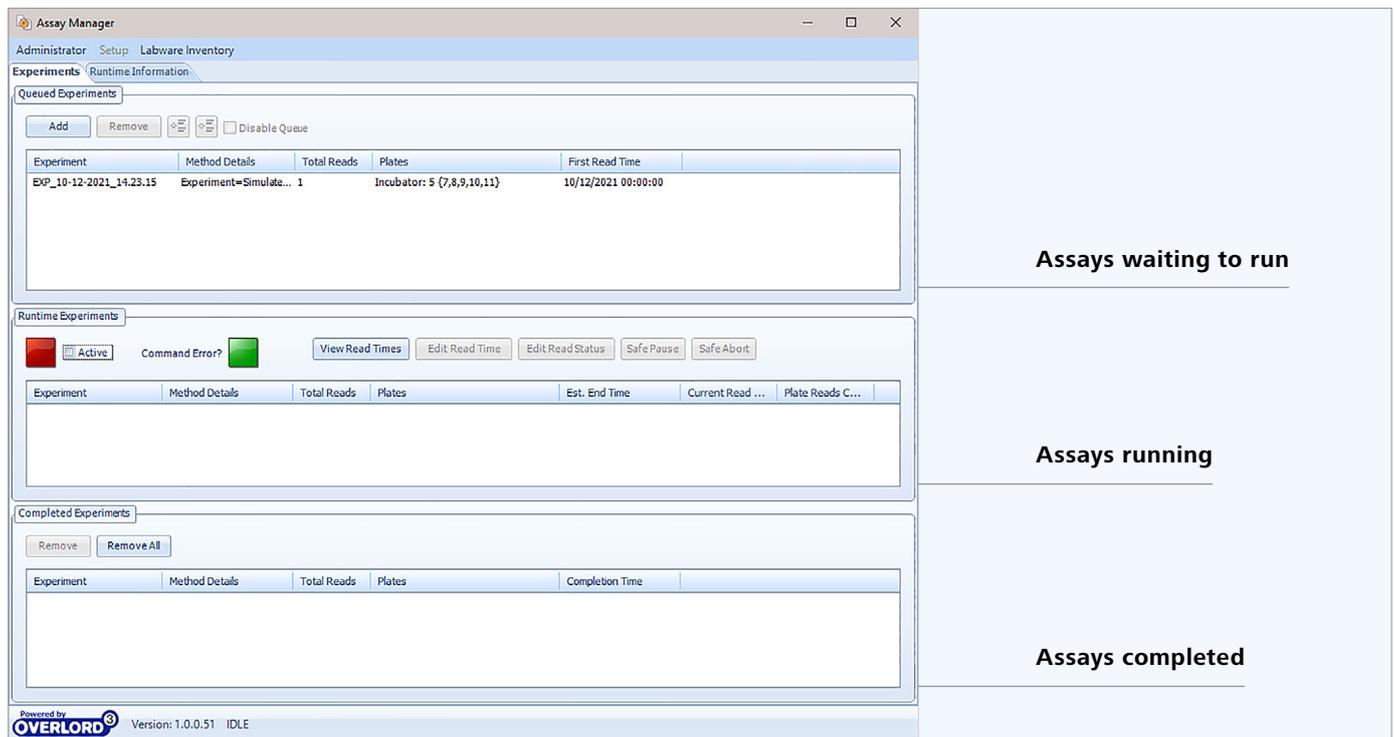
The core of the system is the PAA collaborative KX-2 laboratory robot which provides a world beating specification to enable quick and easy integration of ZEISS Celldiscoverer 7.

The KX-2 robot is completely safe to use on the open bench with full operator protection and meets the international collaborative robot standard ISO-TS-15066. The robot is dragged to teach so it is easy for the operator to re-teach positions or to add new labware without the need for a qualified robot engineer. An integrated bar code reader on the gripper enhances sample traceability and speeds up the process time between plate transfers to the imager. This is further enhanced with full 360° continuous rotation on the main access to enable the robot to find the best path between plate transfers, without the need to re-set the axis.

The KX-2 offers a best-in-class radial reach of 641 mm to enable additional instrumentation such as liquid handlers to be added to the system in the future, coupled with a radial compactness of 183 mm allows a large work area to be populated all of which is accessible to the robot thereby enabling a smaller footprint to save on valuable laboratory space.

Managing your data flow with Assay Manager software providing controlled scheduling for multi batch applications, data transfer to servers and auto LIMS applications

The PAA Assay Manager software interface has been developed for kinetic assays and enables the operator to easily run sample batches with multiple reads and incubation times. The scope of Assay Manager has been designed to enable multi batch runs from different operators to be loaded and run on the system with the requested processes seamlessly interleaved by the PAA scheduling engine to ensure that plates are read at precisely the correct time in the schedule.



Assays waiting to run

Assays running

Assays completed

A typical Assay Manager process for a Kinetic assay with ZEISS Celldiscoverer 7 could include the following steps:

1. Operator 1 will load the initial batch of sample plates to be processed into the incubator.
2. Operator 1 selects read (= imaging) protocols, number of reads and read interval.
3. The operator 1 will then start the run.
4. At any stage, Operator 2 can load their batch of plates into the incubator, select their read intervals and imager read settings and queue their samples on the Assay Manager interface.
5. Assay Manager will schedule the sample batch from Operator 2 and will interleave the processes to ensure the plates are read at the right times.
6. Operator 3 can then load their batch of plates which will then be interleaved into the schedule.
7. Additional operators can add additional sample batches at any stage of the process and as long as there is sufficient duration in read intervals to avoid conflict, they will be interleaved into the process.
8. If interleaving of the operators' sample batch is not possible due to a scheduling conflict, then they will be informed at the time and provided with a start time for their batch that would enable interleaving without conflict.
9. As each operator batch is completed, the operator will be informed that their assay is finished and instructed to remove their plates from the incubator.

The information generated by the process can be passed to a LIMS system either by a flat file transfer into a shared folder or by full 2-way integration that will enable the LIMS system to pass a workflow to the system and receive the image data by return.

System configuration for automated plate handling

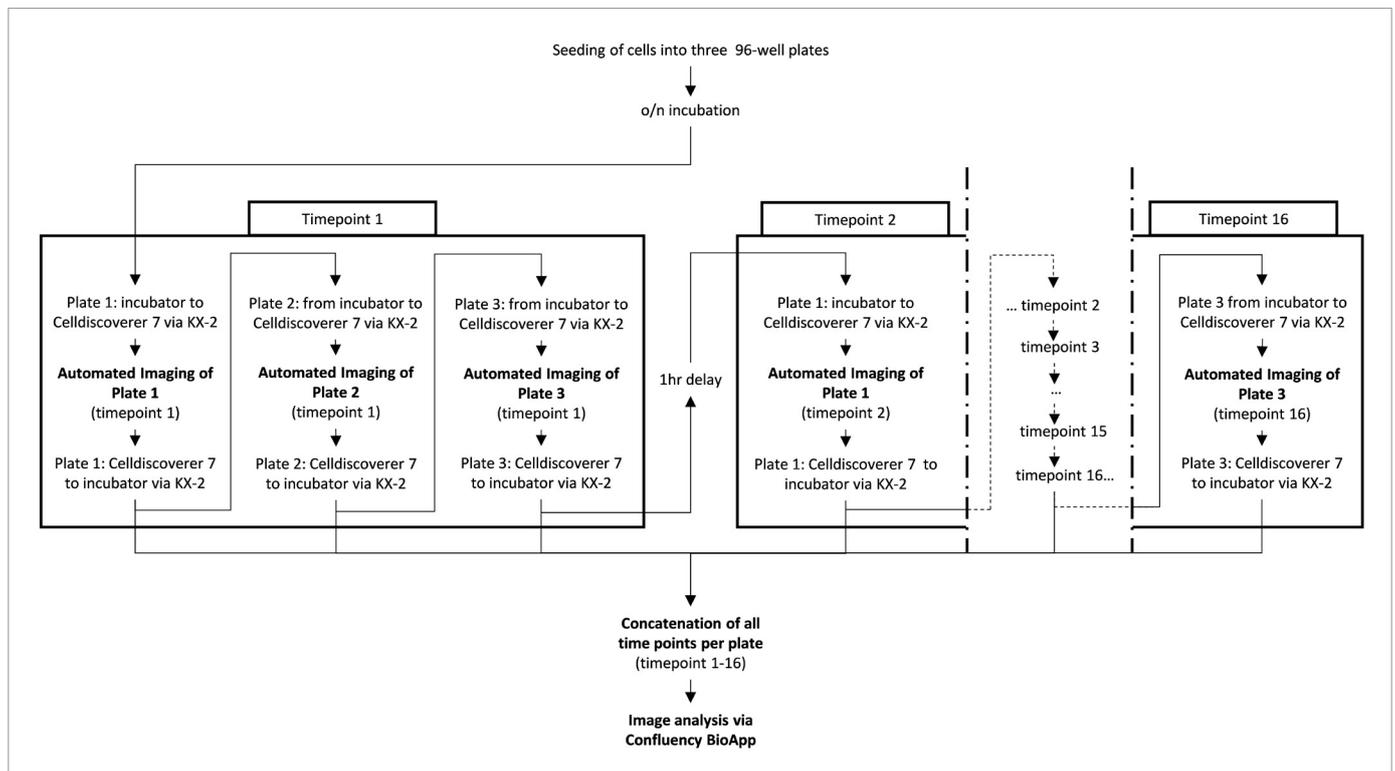
The option to equip ZEISS Celldiscoverer 7 with a robotic loader requires a special system configuration. Celldiscoverer 7 requires a modified sample chamber to enable the handling of the sample by the robotic loader as well as the ZEN module Macro Environment along with dedicated macros.

The automated solution for sample handling from PAA includes the following parts:

- Plate loader (S-LAB™ plate handler or KX-2™ robot)
- S-RUN™ control software
- Assay Manager software (User interface)
- Barcode reader
- System bench
- Optional: incubator (e.g. Thermo Fisher Cytomat C2)
- Optional: liquid handling solution



Figure 3 Schematic illustrating a standard system design



Application Example: Automatic generation of growth curves for multiple plates

In a typical workflow using ZEISS Celldiscoverer 7 with a robotic loader, sequential imaging of multiple samples, such as multi-well plates, is executed over a long period up to several days. This enables to monitor e.g., cell viability and to automatically generate growth curves over time. All ZEISS Celldiscoverer 7 imaging modalities can be employed seamlessly within such an automated workflow. In the following diagram, such an example workflow is being outlined schematically.

In this example, three 96-well plates with cells seeded in various densities were imaged over a time course of 15 hours. The plates were kept under ideal conditions inside the incubator and transported quickly via the KX-2 robot into the incubated ZEISS Celldiscoverer 7 and back once every hour.

Imaging was performed using the unique Phase Gradient Contrast (PGC) – especially designed for multi-well plates filled with liquids to guarantee optimal image quality of the complete well not disturbed by liquid meniscus. Images were acquired at 10x magnification using the 5x/2x objective (NA 0.35).

For subsequent and automatic image analysis, the image data of individual timepoints per well were concatenated.

Several out of the box image analysis solutions for common assays are available along with ZEISS Celldiscoverer 7 to automate data analysis and tailor result presentation with interactive measurement tables, heatmap and plots. These ZEN BioApp modules are available to provide simple automated image analysis workflows customized for:

- Cell counting
- Quantifying the cell confluency
- Quantifying gene expression
- Spot detection

The imaging data of the plates have been analyzed using the ZEN module “Confluency” to automatically identify and segment the cells and measure the covered area and the area percentage. Applications addressed by this module include cell confluency assays as a measure of quality control in cell-based assays as well as wound healing assays to follow cell migration and cell-cell interaction. Figure 4 shows the analysis results of one of those plates.

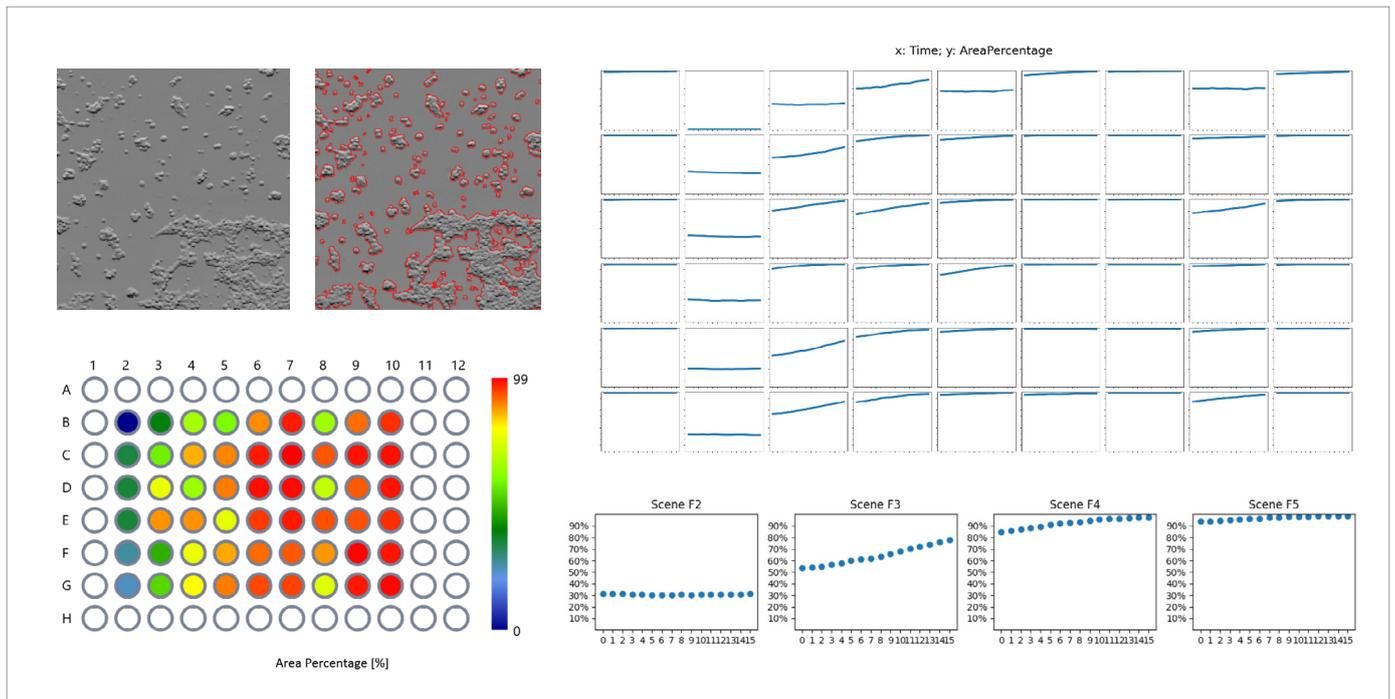


Figure 4 Results of one plate of a time lapse experiment. Top left: segmentation results (ZEN module Confluency). Bottom left: plate layout and heatmap display of cell confluency for the first timepoint (% area coverage). Right: Growth curves of individual wells (layout as shown in depiction). Bottom right: Growth curve enlargements of wells F2 to F5 (x: time point (h), y: confluency (%))

When looking at the individual results per well in a heatmap format for the first timepoint, the difference in densities is color-coded. While some wells started with a relatively low number of cells indicated in blue, others were already completely overgrown (100% confluency, red color code). The individual growth curves per well show a constant cell growth over time for some wells.

Expand your automation options for advanced ZEISS Celldiscoverer 7 applications

Some applications might require automatic interaction with multiple samples on a specific time point or on a regular basis, e.g., by adding or exchanging liquids. This includes complex cell-based assays, studies of the circadian rhythm, developmental biology over long time periods, compound research, or repetitive staining procedures. In such a case, the ZEISS Celldiscoverer 7 with automated plate loader solution from PAA can be extended towards a liquid handling device. Thanks to the flexibility of the Assay Manager, multiple devices are already integrated and ready to use.

Going forward, the flexibility of the PAA S-RUN control software enables larger systems to be designed and developed to expand the assay capabilities.

One application that is frequently being requested is to add a liquid handling system onto the setup to enable compound dosing of the cell plates to determine changes of cell viability.

The schematic below shows a proposed customer system where a liquid handler and labware carousel is included to enable both dosing of the cells and cell maintenance during long incubation cycles with multiple reads on ZEISS Celldiscoverer 7 to enable the assays to be adjusted dependent on the imaging data.

PAA work with all main liquid handler suppliers and can integrate the customer choice of liquid handler onto the system to enable the best instrument for the process to be selected by the user.





The work cell can include a HEPA filtered downflow to protect the cells while they are being processed. PAA offer the only full deck laminar air flow system to provide ultimate sample protection. Fully controlled centrifugal impellers are used to deliver an even downflow across all of the instrumentation and the full deck. Sensors on the doors increase air flow over the door to protect the internal environment from contamination when the door is opened to enable an operator to safely access the internal instrumentation without the fear of introducing contamination.

PAA also provide antimicrobial surfaces on the system benching to further eliminate potential sources of contamination and all surfaces where liquids are handled are bonded to contain spillages with sensors to alert the operator in the event of a liquid spillage in the system.

The containment alarm system is fully integrated into the S-RUN control software to enable a run to be paused and the operator alerted in the event of an issue to enable problems to be quickly resolved and the process to continue.

The Future

The revolutionary Millipede scheduling engine contained within S-RUN uses artificial intelligence to monitor process times on repeated procedures to optimize the scheduling algorithm and find the best fit for the process to increase process speed and sample throughput.

Summary

Certainly, automation does not just begin at the imaging system and does also not stop after images have been acquired. With the integration of the PAA automated solution, ZEISS Celldiscoverer 7 gains a large boost in automation performance. Experiments with high complexity and multiple batches can be executed automatically. The unique combination of widefield and confocal imaging (even with Airyscan 2) adds another dimension for automated 3D data acquisition and sample manipulation.

Complete and automated workflows can be realized using Guided Acquisition, ZEN connect, ZEN analysis options, as well as APEER or third-party options.

The proposed setup for automating live cell imaging significantly increases the sample throughput, data read-out and system utilization leading to faster time to results, increased statistics and reliable data output, independent of the number of system users. A maximum of automation in every aspect is desirable for many researchers. The increasing complexity of their experiments and results requires more simplicity when it comes to instrumentation. Thus, they don't need to think about the experiment execution and can leave it all to the imaging device. In addition, ease of use in automation minimizes costly mistakes. ZEISS Celldiscoverer 7 with the automated plate handling solution from PAA is now prepared to serve the demands in this application field.

Especially, imaging facilities benefit from the overall system throughput and ease of use reducing the manual interaction at the system for highly repetitive tasks. Systems could be run at full capacity for 24/7 operation.

With increasing standards in documentation and reproducibility, even more interdisciplinary research projects and continued short-term researcher contracts, automation also enters the realm of smaller labs. With ZEISS Celldiscoverer 7, the researchers especially benefit from the high sample throughput and flexibility covering multiple imaging modalities as well as guaranteed data reproducibility that is realized due to the high degree of automation.

In this way, ZEISS Celldiscoverer 7 automated solution accounts for possible needs for throughput, adding value, ease of use and flexibility to your research.

Acknowledgments

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Appendix

Supported barcode formats by PAA barcode reader:

- Code 11
- Code 39
- Code 93
- Code 128
- EAN-13
- EAN-13 add-on
- EAN-8
- EAN-8 add-on
- GS1 DataBar
- IATA
- Industrial 2 of 5
- Interleaved 2 of 5
- Korean Postal Authority code
- Matrix 2 of 5
- MSI / Plessey
- 2 of 5
- Telepen
- UK / Plessey
- UPC-A
- UPC-A add-on
- UPC-E



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