

Good User Handbook

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Chapter 1 – Introduction

The Stem Cell Hotel (SCH) is a collaborative phenotyping space configured to support and enable scientists from King's College London, other universities and industry. It also provides specialist CRO-type services for cell therapy and drug discovery. The SCH is staffed by a team of stem cell scientists and equipped with the latest cell imaging devices from major manufacturers. It is a centre of expertise for cell characterisation using high content analysis and artificial environments. The SCH is at the leading edge of the next-generation technologies that will transform drug discovery and cell therapy. Our data integration capability and expertise apply to both 'data-driven' and 'hypothesis-driven' research projects.

It is based on the 28th Floor of Guy's Hospital, London, within King's Centre for Stem Cells & Regenerative Medicine (CSCRM).

Website: <https://www.kclstemcellhotel.org/>



Figure 1: Stem Cell Hotel at Guy's

The SCH hosts five major instruments:

- Operetta Mk1 (PerkinElmer),
- Operetta CLS (PerkinElmer),
- Livecyte (PhaseFocus),
- IncuCyte ZOOM (Essen BioScience), and
- JuLI Stage (NanoEntek).

The Analysis is facilitated by:

- Analysis WorkStation PC, located in the Main Lab,
- Synology NAS network, for storing, backing-up, and archiving users' images.

Chapter 2 – Guidelines

Here are general guidelines that ALL users must follow once they enter the SCH.

I) Access to the SCH

All users (including the internal users of CSCRM, King's users, and the external users) are allowed to enter the SCH and use the SCH instruments and services on the condition that they have been approved by the CSCRM and the SCH.

For different user groups:

- Internal users must have their badges to unlock the door.
- External users and visitors need to contact the concierge to arrange access to the SCH, e.g., by agreeing via email dates and hours.
- In the event that prolonged access to the SCH is required, external users and visitors may ask for a temporary visitor's card from CSCRM executive assistant/laboratory managers.

II) Safety measurements

All users must have had safety induction (including fire safety and lab safety) of CSCRM.

users' coats and personal belongings should not be brought into the SCH room.

When in the SCH laboratory, users need to:

- Wear a lab coat.
- Wear lab gloves, when performing experiments.

When leaving the SCH room, users must:

- Check they are leaving a tidy area and remove their belongings.
- Wash their hands at the exit.

III) Permission to work with cells

If users would like to introduce any new cell lines into the SCH, this must be approved by the CSCRM in advance.

For such permission, please contact:

- The floor manager Dr Francesco Nicolini (francesco.nicolini@kcl.ac.uk),
- The lab manager Eamonn Morrison (eamonn.morrison@kcl.ac.uk).

IV) Good practice

After experiments, it is obligatory that users clean and tidy up their experiment space. Users must either clear away their samples as soon as possible or properly dispose of them (e.g., into the yellow waste bin in the SCH) after experiments. Details of proper disposal practices are included in the induction.

Samples (or any tissue, aluminium foils, plates, Eppendorf tubes, glass slides, etc.) must NOT be left on the benches of SCH after experiments.

Users should also not leave any of their notebooks, papers, markers, protocols, personal property, etc. on the benches of SCH.

Any food or drink is forbidden in the SCH.

Failure to comply with the rules results in a suspension of access at the discretion of the SCH.

V) Working days & hours

During normal working days (Monday – Friday of each working week), users can access and use the SCH instruments between 9:00 am and 6:00 pm.

Out-of-hours work (e.g., early morning before 9:00 am, evening and night after 6:00 pm) and holiday work (e.g., Easter, Christmas, New Year, bank holidays) are not recommended.

If users would like to access SCH and conduct experiments out of normal working days and hours, they need to discuss and confirm with their supervisors and the concierge.

VI) Generator Test

Guy's Hospital conducts a monthly generator test (GT) during the weekend, which is a brief power-off for the SCH. For the GT, please pay attention to the floor manager's notification (normally via group email in the CSCRM).

Before the GT, the concierge will switch off the instruments at the SCH accordingly, which normally takes place at around 5:00 pm Friday. Users should plan their experiments accordingly to avoid any overlap with the weekend GT.

At the moment only the IncuCyte ZOOM has an external uninterrupted power supply (UPS) and is not affected by the GT power-off.

VII) Contacts

SCH website: www.kclstemcellhotel.org

Concierge email: **to be updated**

Further analysis / organisation assistance: erika.wiseman@kcl.ac.uk

Chapter 2 – Pricing & iLab

iLab (www.ilabsolutions.com, Agilent, USA) is the platform for the users of SCH to plan experiments and book instruments, including the use of the Analysis WorkStation PC. Users must register on iLab.

iLab is also for new users to request training on any instrument. Prior to formally using an instrument in the SCH, new users must be trained.

I) Pricing

Internal and external users will pay for their formal experiments at different rates, as below:

Instrument	External User Rate (£/hr)	Internal User Rate (£/hr)
Operetta CLS	50.00	35.00
Operetta Mk1	50.00	35.00
PhaseFocus Livecyte	50.00	35.00
IncuCyte ZOOM	2.50	1.50
JuLI Stage	2.50	1.50

Table 1: Experimental prices of each instrument for users at the SCH, subject to change

Before using any instrument at the SCH, new users need to

- Discuss with their supervisors which instrument they need for their research,
- Register on iLab and have a personal account (see **Part-II** of this chapter),
- Raise a training request of the instrument via iLab (see **Part-III** of this chapter),
- Once the request is proved by the admin, discuss with the concierge detailed information regarding the instrument, and arrange a date/time for a demo.

Users must book an instrument in advance on iLab and fill in their grant code (ask your Supervisors and Laboratory Managers). Additional assistance time will also be charged. The first initial test experiment and demo on any instrument at the SCH will typically not be charged.

II) iLab registration

All users need to register on iLab in advance.

To register for an account:

- 1) Navigate to the core page:

https://gstt.corefacilities.org/service_center/show_external/3223

- 2) In the upper-right-hand corner of the screen, select sign up
- 3) Complete the registration form on the sign-up page:

https://gstt.corefacilities.org/account/signup/396?sc_id=3223

choose 'Guy's hospital' as your centre.

- 4) Receive a "welcome" email from iLab (typically within one business day) with login credentials and basic instructions.

For further questions, iLab support team: iLab-support@agilent.com

Once a user has a valid account on iLab, please use the login page for the SCH:

<https://gstt.corefacilities.org/sc/3652/stem-cell-hotel>

III) Demo

Once registered on iLab, prior to formally booking and using an instrument of the SCH, new users need to arrange a time for a demo with the concierge. The process of arranging a demo is outlined below:

- New users need to first discuss with their supervisors and SCH which instrument they need for their experiments.
- New users need to log into iLab and raise a request for the demo of that instrument.
- The concierge will check and approve the request on iLab.
- The exact date and hour of the demo may be discussed with the concierge.
- New users need to arrive on time for the demo.
- After the demo, the concierge will verify the user's training on iLab, so that users may formally book and use the instrument at SCH.

If users encounter any problem regarding an iLab booking of an instrument, they need to contact the concierge.

IV) iLab booking

Some general policies of iLab booking:

- Users can only carry out experiments in the SCH after they have booked on iLab.
 - This also applies to the image analysis work on the Analysis WorkStation PC.
- Users can cancel or adjust bookings before using slots on iLab. However, if users are late for their bookings without specific reasons, the empty hours will also be charged.
- Users cannot cancel their bookings after their experiments.
- For specific instances that require a user to make a change to their iLab booking, please contact the concierge via email.

V) Experiment duration

Users need to follow the duration limits when they book on iLab, as below:

- For overnight live-cell imaging experiments on the Operetta (Mk1 or CLS), Users are asked to book instruments starting in afternoon/evening, e.g. around 5:00 pm.
- During the working days of a week, the Operetta (Mk1 or CLS) can only be booked for continuous live-cell imaging experiments for a maximum of 24 hours.
- For Operetta live-cell imaging experiments lasting more than 1 day, users are asked to image during the weekend, e.g., Friday afternoon/evening as the start. Please ensure that a monthly GT will not be running at this time.
- Live-cell imaging experiments during the weekend on the Operetta (Mk1 or CLS) can only be booked for a maximum of 3 days (from Friday to next Monday).
- For JuLI Stage, IncuCyte and Livecyte experiments, users cannot book instruments lasting longer than 1 week. Please contact us for special requests.

Chapter 4 – IT policy

Users need to follow the IT policy when using SCH instruments and services.

I) Use of PC

For the use of instrument PCs and the Analysis WorkStation PC, users need to:

- NOT to save their images or research data on the desktop.
 - This will occupy free space of the PC and therefore cause problems, such as Harmony software running slow.
- Creation of temporary folders on the PC's desktop for saving images is not permitted.
 - Users need to handle their images via the specific user-generated data folder.
- All PCs should be kept on, power on/off is only carried out by the concierge.

Any PC problem, such as

- the PC is running slow,
- the PC is running out of free storage space,
- the PC needs to be re-started, or,
- the PC has software updates,

please inform the concierge as soon as possible.

For the software on the PCs:

- The automatic Windows updates are not permitted on the PCs.
- Users are NOT allowed to install software onto the PCs of SCH by themselves. New software installation needs approval from the SCH.

For the hardware of PCs, users:

- Should not disturb cables of PCs and their connections in the SCH room, e.g., pulling and swapping the internet cables and sockets is not allowed.
- Should not move the locations of monitors or change their settings.

II) IT: Operetta systems

When using Operetta PCs (Mk1/CLS), users need to:

- Register their own accounts on Harmony4.8 software at the Mk1/CLS PC.
 - Please see **Chapter 5 Part-II** for more details.
- Check PC free space, prior to running imaging experiments on the Operetta.
 - For the Mk1/CLS PC, check > 200 GB free space in the local-c drive.
 - Imaging one full 96 well plate will typically generate 100 GB of images.

- If Mk1/CLS PC have < 200 GB free space, users need to inform the concierge.
- Only the concierge can conduct the image relocate.
- Do not change Mk1/CLS PC's settings (including the double-monitor).
- Do not change Harmony4.8 software settings (including panels, ODA, links, etc.).

In terms of Operetta data, users can:

- Archive their important measurements.
 - Harmony4.8 => Settings => Data Management => Archive
- Export their data after experiments
 - Harmony4.8 => Settings => Data Management => Export
 - If users need to export an Analysis Sequence (aka, Image Analysis Pipeline), choose 'Analysis Sequence' in the drag-down list.
 - If users need to export raw images, it is advised to select 'Measurement incl associate files', with 'Measurement' meaning original Operetta images in TIFF format, 'associated files' including metadata naming and mapping the images.
- Export their results
 - The last two options in the drag-down list, with 'well results' or 'well and single object results' (the latter include single-cell level results).
Notice that 'object results' can be very large in data size.
- Please be mindful of the quantity of data generated and avoid unnecessary exports or extremely long timepoints, z stacks, etc.
- Please ask the concierge to clean up and delete their data when they have finished a project, or they are leaving the lab (e.g., when students have finished their PhD).

III) IT: NAS network

The NAS (Network-Attached Storages) are for the SCH image storage and backups, with NAS-1 for storage, NAS-2 for backups, and NAS-3 for archiving.

- Users are not allowed to touch or move the NAS from their physical locations.
- Users are not allowed to change settings of NAS from PCs or the internet.
- Users are not allowed to touch or alternate cables and connections of the NAS.
- Do not place experimental reagents, water/solutions, ice, wet tissue/towel near the NAS.
- The NAS will be switched off during the GT (normally for one weekend per month, please see **Chapter 1 Part-VI**). At this time, users will be unable to access their Operetta images.

IV) IT: other instruments

For PC logins and initialisation,

- The PhaseFocus Livecyte PC does not have personal login. It has three major software packages on its desktop – 1) Assay Design, 2) Image Acquisition, and 3) Image Analysis, which enters another desktop.
- The JuLI Stage PC does not have personal login. The JuLI software may take a few minutes during initialisation.
- The IncuCyte software is installed on the CLS PC in the SCH room, and users need to connect and login to set up their experiments.
- The Analysis WorkStation PC located in the main lab does not have personal login. Users need to book in advance on iLabs to use this PC.
 - Harmony4.8 software is installed which requires a user's personal login. Users must pay attention to the ODA (Operetta Database) setting (the Mk1 ODA is for Mk1 image analysis, whereas the CLS ODA is for the CLS image analysis).

For image storage,

- The PhaseFocus Livecyte has its own NAS (12 TB in size, bottom layer in the black box).
 - Users need to save their images onto the Z-drive (which is the NAS drive) by creating their own folders using the Livecyte PC.
 - Users are not allowed to save their images directly onto the Livecyte PC.
 - Users are not allowed to leave any personal data or images on the desktop of the Livecyte PC.
- The JuLI Stage PC has its own drive.
 - Users need to check that there is sufficient free space in storage before their JuLI Stage imaging.
 - If there is not enough space for their experiment, users need to contact the concierge.
 - Users need to check its Data Path before their JuLI Stage imaging.
 - Users are allowed to export images onto their own drive connected to the JuLI Stage PC.
- The IncuCyte has its own storage, which is placed on the top of TC room incubator and named IncuCyte Store.

V) IT: Columbus

Columbus is the PerkinElmer platform for online image storage and analysis. Please also see **Chapter 5 Part-VI** for more details.

King's hosts Columbus and users can log in using their personal Mk1/CLS Harmony accounts.

At the moment, users may access and use Columbus when they are in the King's Campus.

VI) Data storage policy

Users must follow the **90-day data storage policy**:

- Once data has been acquired with the Operetta, they will be stored for 90 days in a dedicated area of Columbus server. During this time, Users will have access to the data and will be entitled to analysis and process using the Columbus tools.
- Users are solely responsible for backing up their images and data within 90 days as these will be automatically deleted 90 days after acquisition.

VII) IT: remote access

Users are not allowed to remotely access the PCs of SCH, unless under specific conditions.

Users' remote access of the PCs of SCH needs to be approved by the SCH team.

For troubleshooting purposes, King's IT or instrument providers may access the PCs remotely.

VIII) IT: contacts

For any other questions concerning IT, users shall contact the concierge who will raise a ticket to King's IT (8888@kcl.ac.uk) or phone [020 7848 8888](tel:02078488888).

King's IT helpdesk: <https://www.kcl.ac.uk/it/services-software>

Chapter 5 – Specific guidelines for each instrument

There are some specific guidelines for each instrument of SCH that users must follow.

I) PerkinElmer EVOportal

For problems related to the Operetta, Harmony, Columbus, and the Analysis WorkStation PC, users are asked to first contact the concierge.

If the problems could not be resolved by the concierge, the concierge may raise a request on PerkinElmer's platform for trouble-shooting, EVOportal. Once a request has been raised, experts from PerkinElmer will give advice and solutions online promptly.

EVOportal website: <https://evportal.perkinelmer.com/>

II) Account setup

The Operetta Mk1 and Operetta CLS both use Harmony4.8 software. New users must set up their own accounts for Harmony4.8. For assistance, please contact the concierge.

- The account should be the user's name in a standard format (NameSurname), with a simple password that the user must remember.
- For every experiment, users need to login into Harmony4.8 using their accounts.
- For re-setting up an account, users need to contact the concierge.

III) Plates & plate formats

For experiments on Operetta Mk1 and Operetta CLS, plate format is critically important.

- Before beginning experiments, users should know their plate format and detailed dimensions (e.g., plate length, width, height; plate bottom height and thickness; well diameters).
 - Users can obtain this information by searching online or email/contact the plate manufacturer.
- Harmony4.8 in Operetta Mk1 or Operetta CLS PC has a database of existing plate formats saved by previous and current users.
 - If the plate format does not exist in the Harmony database, users need to establish a new format and name it properly, and it is best practice to include the plate's catalogue number and manufacturer.
- For glass-slide samples, there is a slide holder in the drawer for each Operetta.
 - For the Operetta Mk1, the slide holder has one position with clamps;

- For the Operetta CLS, the slide holder has four positions with magnetic fixation.
- Both the Operetta Mk1 and the Operetta CLS cannot image V-bottom microwell plates, petri dishes, or any other unusual plates of specific formats.
- U-bottom microwell plates can only be imaged at the central fields of the well bottom, using a low magnification (5× or 10×).

IV) Operetta Mk1

IV.1) Introduction to Operetta Mk1



Figure 2: Operetta Mk1

The **Operetta Mark1 High-Content Imaging System** (Mk1) delivers all the required features for the generation of unbiased, statistically significant data of high-content imaging and high-content analysis for SCH users, including:

- Fast widefield imaging for routine applications.
- True spinning disk confocal imaging option to eliminate background, and protect live-cell samples, by minimizing photobleaching and toxicity.
- Spinning disk pinhole size optimized for good confocality across a wide range of objectives.
- Equipped with 8-position dichroic wheel for maximum excitation flexibility.
- Unsupervised automated image acquisition and autofocus for reliable high-content imaging from slides to multiple plates.
- Digital Phase contrast for imaging and segmentation of cells that have not been fluorescently labelled.
- High numerical aperture objectives which give exceptional images for higher quality data and more reliable results.
- Low and high magnification plus a large field of view allows you to look at the whole well and choose an area of interest for detailed analysis.

Mk1 runs **Harmony4.8** (Harmony® High-Content Imaging and Analysis Software) which allows:

- Easily setup of assays, automation of experiments, analysis of data and storage, retrieval and presentation of results.
- Plate wizard for simpler setup of new plate types - automatically detects the important parameters plate bottom height and plate bottom thickness.
- Analysis templates for a range of common applications.
- User-configurable image analysis building blocks for the easy creation of customized high content analysis applications.

- STAR analysis, which provides a more detailed analysis of cellular morphology for accurate differentiation of cellular phenotypes.
- Texture analysis for applications where intensity and morphology are not suitable or do not deliver robust readout and reproducible results.
- Transferring and viewing data from any computer that has an installation of the Harmony software.
- Cell tracking to see temporal changes of biological systems and study cell dynamics in 2D.
- Advanced building blocks for easier analysis of complex applications such as FRET.
- Improved memory management for quicker processing – up to 3 times faster than Harmony 3.5.
- XYZ View which shows an xy-, xz- and yz-section through an image stack of a thick sample e.g. microtissues.

The **Columbus™** image data storage and analysis system is used to store images allowing online processing and analysis.

Applications of Mk1 include imaging wells, stem cells, primary cells, 3D cultures, assay development, siRNA experiments, compound screens.

IV.2) Operetta Mk1 lamp box

Operetta Mk1 has a separated lamp box, linked to the Operetta Mk1 machine via a cable. Users should not bend or twist the cable.

Once switched on at the start of the day, the lamp box, should be kept on for the whole day. Frequently switching on/off the lamp box is forbidden, because that will damage the lamp.

Once switched on, the lamp box will need a few minutes to warm up and be stable.

IV.3) Mk1 PC and IT policy

The Operetta Mk1 PC normally should be kept on. Users are not allowed to change or disturb its monitor settings and cables, including the Internet cable, the monitor cables, the cable linking the PC and Mk1.

Re-starting the Operetta Mk1 PC is allowed when the PC is running slowly. For example, after being kept on for a few weeks, the Operetta Mk1 PC may begin to run slowly. In this case, please contact the concierge to re-start the PC.

An imaging experiment of a 96well plate at 40× magnification may occupy as much as 100 GB in Operetta Mk1 PC's local-c drive. It is advised for the local-c drive to have at least 200 GB free space. Before starting an experiment, the user should check how much free storage is available

in the local-c drive. If the Operetta Mk1 PC is running low on memory space (e.g., less than 200 GB in the local-c drive), please immediately report to the concierge.

Please also refer to **Chapter 4 Part-I** and **Part-II**.

IV.4) Prior to beginning an Operetta Mk1 experiment

IV.4.1) Science before the Operetta Mk1 experiment

Before setting up experiments, users need to study the science first, e.g., read manuals, papers, and discuss with their supervisors. Some important aspects are listed below:

- Biology.
- Assay plan, including positive/negative controls and conditions.
- When culturing cells, try to avoid the edge and corner wells in multiwell plates (e.g., 96well format). The media evaporation in those wells would be faster than central wells, therefore causing potential condition differences.
- Which culture format to image, including plate format, slide, etc.
- Which antibodies to use in the immunostaining protocol, including primary and secondary antibodies.
Try to avoid using expired or contaminated antibodies.
- Which fluorophore to use.
Please try to avoid fluorescence cross-excitation and overlap of fluorescence (especially the emission spectra).

IV.4.2) Operetta Mk1 system switch-on order

Prior to an experiment, users need to start the Operetta Mk1 system. When starting the whole Mk1 system (in the case of the whole system is off at the very beginning), the switch-on order is:

- Lamp Box (Xenon Lamp) => Machine (Operetta Mk1) => PC => Software (Harmony4.8).
 - The lamp box needs to be first to switch on.
 - The Operetta Mk1 machine can be switched on (blue button) once the lamp is on and stable.
 - After switching on the Operetta Mk1 machine, the blue-lighting band on the machine will blink, which means the machine is preparing.
Wait for the band to be stable which indicates the machine is ready for image acquisition.
 - Once the whole system is on, it will be kept on for the whole day.

IV.4.3) Operetta Mk1 lenses & brightfield imaging

2× is the lowest magnification lens installed in the Operetta Mk1 machine.

The low-magnification lenses (2× and 10×) do not have a correction collar, only 20× and 40× have.

To change a lens and adjust the lens collar on the Operetta Mk1, please contact the concierge.

For imaging on brightfield channel, please use the little arm inside the Operetta Mk1, to improve the image quality.

IV.5) During the Operetta Mk1 experiment

IV.5.1) Operetta Mk1 status during an experiment

When running experiment smoothly, users should not disrupt the Operetta Mk1 machine or Harmony4.8 software in the Operetta Mk1 PC.

- The blue-lighting band on the Operetta Mk1 machine should be constantly on;
- If it shows up Red, this indicates there is a problem with the machine or imaging process;
- If it shows up Yellow, it needs users to pay attention or wait.
- For any Error Messages that show up, please contact the concierge.

IV.5.2) Operetta Mk1 lid

During imaging, the Operetta Mk1 machine's lid should be kept closed and users should not open it.

Sometimes the Operetta Mk1 machine's lid does need to be held, to prevent falling, e.g., when a user is examining a plate inside the machine.

To open and close the lid, firstly press the Eject/Load button in the Harmony4.8 software. Then the lid can manually be opened/closed.

If users do not activate the software step first, and instead, open the lid manually, an error message will be generated.

IV.6) End of the Operetta Mk1 experiment

IV.6.1) Operetta Mk1 plate handling at the end of the experiment

Both the opening and re-closing of the Operetta Mk1 machine should be imitated through the Harmony4.8 software of the Operetta Mk1 PC, via the Eject/Load button.

After image acquisition, users should remove their plate from the Operetta Mk1.

After taking out the plate, users should re-close the machine lid. It is NOT permitted to leave the machine open because any dust that enters could cause problems.

After experiments, users are responsible for removing or disposing of their plates properly.

IV.6.2) Operetta Mk1 tidy-up after experiments

After experiments, users should not leave any experimental plates and materials, aluminium wraps, or lab tissue on the Operetta Mk1 bench.

After experiments, users are responsible for keeping the Operetta Mk1 bench and seats clean and should take all their belongings with them.

IV.6.3) Operetta Mk1 switch-off order

The switch-off order for the Operetta Mk1 system normally follows (the reverse-order of the Mk1 switch-on):

- Software (Harmony4.8) => Machine (Mk1) => Lamp Box
 - The software Harmony4.8 should be logged out/closed at the end of an experiment by the user.
 - The Operetta Mk1 PC should be kept on.
 - The Operetta Mk1 will be switched off at the end of the day, either by the last user or by the concierge.
 - When pressing the off button (the same blue button as the 'on') on the Operetta Mk1 machine, the blue-lighting band on the Operetta Mk1 machine will start blinking. It needs to be completely off before proceeding.
 - The lamp box should be switched off afterwards.

IV.7) Operetta Mk1 data management

Users are not allowed to bring their own drive to export their data. For important measurements, it is suggested that users make an Archive for themselves.

Users are not permitted to save their data just on the desktop of the Operetta Mk1 PC, because this will seriously occupy the storage space of the PC. This will negatively influence the PC and software performance, e.g., Harmony4.8 running slowly or crashing.

If users have temporarily saved their images on the desktop of the Operetta Mk1 PC, they should delete them from the desktop after their experiments or transfer onto their own drive.

For image analysis purposes, users do not need to occupy the Operetta Mk1 PC but should book (via iLab) and use the Analysis WorkStation PC. This is located in the main lab, with Harmony4.8

installed and connected to the Mk1 ODA. Alternatively, users may perform analysis online using Columbus.

The Image Relocate (relocation of images from the local Operetta Mk1 PC to the NAS-1) should be only carried out by the concierge.

V) Operetta CLS

V.1) Introduction to Operetta CLS



Figure 3: Operetta CLS

PerkinElmer Operetta CLS™ High-Content Analysis System (CLS) can reveal fine sub-cellular details at high speed, sensitivity, resolution, and flexibility. CLS hardware includes:

- Transmitted light capabilities (brightfield, digital, phase contrast).
- Widefield and confocal fluorescence.
- Powerful, stable 8× LED light source for optimal excitation of fluorophores and confidence in results.
- Automated water-immersion objectives with a very high numerical aperture for high resolution and fast read times with minimal photodamage.
- Confocal spinning disk technology which allows a fast imaging process, efficient background rejection, live-cell experiments and 3D imaging.
- Large format 16-bit sCMOS camera (4.7 Megapixel, 2160×2160, 6.5 microns pixel size) which ensures low noise, wide dynamic range and high resolution (50nm) for sensitive and quantitative measurements at short exposure times.
- User accessible bar-coded emission filters which provide flexibility, sensitive imaging, and error-free operation.

CLS comes with the **Harmony® 4.8 High-Content Imaging and Analysis Software** for the set-up of assays, automation of experiments. image acquisition, data analysis, and storage and retrieval of results (all in a simple, powerful and easy way). **Harmony4.8** software allows:

- Image acquisition
- Computer controlled excitation power
- High-speed autofocus in every field.
- Full exposure control and channel editor.
- Simultaneous acquisition and analysis, and visualization of data during acquisition.
- Variable plate formats: 6, 24, 48, 96, 384, 1536-well.
- Multi-field imaging acquisition.
- Z-sectioning for identification of the z-plane of interest.
- Automated flat field correction.
- Time kinetics capabilities.

CLS image analysis allows:

- Fully automated cell segmentation with automatic or manual parameter tuning.
- Ready-made turnkey applications.
- Easy and intuitive creation and modification of analysis sequences.
- PhenoLOGIC™ machine learning plug-in to teach the software to create tailored algorithms.
- Texture analysis capabilities.

CLS data management allows:

- Scalable database and search via sortable tree.
- Automatic storage of metadata and additional experiment information.
- Allows for image storage on a remote server.
- Export results automatically or in batches into Columbus™ image data storage and analysis system. (allows access, re-analysis, storage, and share of data).
- Export results for further analysis (i.e.: High Content Profiler™ platform by TIBCO Spotfire).
- Accessible from office installations of the Harmony software for data inspection, analysis, and result export.

Examples of CLS applications include:

- Fixed-cell assays.
- Live-cell assays.
- Imaging of complex cellular models.
- Advanced assays such as FRET.

- Phenotypic fingerprinting.

V.2) Difference between the Operetta CLS and the Operetta Mk1

The Operetta CLS is a more advanced version of the Operetta Mk1, with C stands for the Confocal, L for LED, and S for SMOS Camera. Some major differences are:

- Operetta CLS applies integrated LED to illuminate and excite samples, while Operetta Mk1 has a separate xenon lamp box.
- When imaging, the Operetta CLS is faster than the Operetta Mk1.
- The Operetta CLS has an updated camera compared to the Operetta Mk1.
- Besides the air lenses, the Operetta CLS has the water immersion objectives (shorter name: water lens) which allow higher resolution and brighter images.
The Operetta Mk1 only has the air lenses installed.

V.3) Operetta CLS PC and IT policy

The Operetta CLS PC should normally be kept on. Users are not allowed to change or disturb the settings of CLS monitors and cables.

Re-starting the Operetta CLS PC is allowed when the PC is running slowly. For example, after being kept on continuously for a few weeks the Operetta CLS PC might run slowly and, in this case, the PC is allowed to be re-started. Please inform the concierge if users would like to re-start the Operetta CLS PC.

An imaging experiment of 96well plate at 40× may occupy 100 GB in the Operetta CLS PC's local-c drive. It is advised that the local-c drive has at least 200 GB of free space. Before starting an experiment, users should check how much free storage is available in the local-c drive. If the Operetta CLS PC is running low on memory space, e.g., less than 200 GB in the local-c drive, please immediately report to the concierge.

The Image Relocation (relocation of images from the local Operetta CLS PC into the NAS-1) should be only carried out by the concierge.

Please also refer to **Chapter 4 Part-I** and **Part-II**.

V.4) PerkinElmer water immersion objectives

The water immersion objectives are patented by PerkinElmer. The Operetta CLS is installed with three water lenses of 20×, 40×, and 63× magnifications, respectively. The water lenses are immobilised in the machine and therefore cannot be moved or taken out.

During imaging, the water lens creates a small water droplet filling in between the plate bottom and the lens glass piece, which allows more light transmission to the sample.

Procedure and guidelines for using the water lens:

- Check.
 - Before applying the water lens, users should first check the Milli-Q water bottle and the wastewater bottle (both placed inside the Operetta CLS machine).
 - If the Milli-Q water level is low and/or the wastewater bottle is full, please contact concierge promptly.
 - The concierge will re-fill the Milli-Q water and clean the wastewater bottle.
 - For water lenses, it is strictly Milli-Q water that is used (with a resistance value higher than $18.0 \text{ M}\Omega \text{ cm}^{-1}$).
- Flush.
 - Before applying the water lens, flush the Milli-Q water through the water lens once or twice.
 - This is operated through the Harmony4.8 software:
Harmony4.8 => Settings => Operetta CLS => Flush the water lens
- Swap.
 - When the imaging is changed from water to air lens, the user needs to first eject the plate and wipe off the water droplet on the plate bottom, before re-loading the plate and imaging using the air lens.
- Clean.
 - If the cleaning of air/water lenses is needed, please inform the concierge.
 - The concierge will clean the lenses using a special lens tissue.

For more information about the water lens, please visit:

<https://www.perkinelmer.com/uk/category/water-immersion-objectives>

V.5) Prior to Operetta CLS experiment

V.5.1) Science before the Operetta CLS experiment

This part is similar to the Operetta Mk1's. Please also refer to **Part-VI.3.1** of this Chapter.

Before setting up experiments, users need to study the science first, e.g., read manuals, papers, and discuss with their supervisors. Some important aspects are listed below:

- Biology.
- Assay plan, including positive/negative controls and conditions.
- When culturing cells, try to avoid the edge and corner wells in multiwell plates (e.g., 96 well format
 - 1) s). Cells in these positions would experience different media evaporation. And,

2) the Operetta CLS water lens is unable to image edge/corner wells due to its mechanical structure.

- Which culture format to image, including plate format, slide, etc.
- Which antibodies to use in the immunostaining protocol, including primary and secondary antibodies.

Try to avoid using expired or contaminated antibodies.

- Which fluorophore to use.

Please try to avoid fluorescence cross-excitation and overlapping.

V.5.2) Operetta CLS filters

The Operetta CLS has a filter wheel of 8 positions, of which 4 filters have been installed.

Users should not touch or alter the filter settings.

If users would like to install new filters to cover certain spectra for their fluorophores, they need to contact the concierge.

V.5.3) Operetta CLS lenses

Different from the Operetta Mk1's 2×, the lowest magnification lens installed in the Operetta CLS is 5×.

The low-magnification lenses (5× and 10×) and the water immersion objectives (20×Water, 40×Water, and 63×Water) do not have correction collars, only 20×Air and 40×Air have.

For water lenses, please refer to **Part-V.3** in this chapter.

For air lenses, there are only 3 positions in the Operetta CLS machine and there are 4 air lenses - 5×, 10×, 20×, and 40×. The spare air lens is placed in the lens case in the cupboard under the Operetta CLS.

Installing a new air lens should be first conducted through the Harmony4.8 software. To manually change lenses and adjust collar correction, please contact the concierge.

V.6) Start the Operetta CLS experiment

When starting the whole system (in the case of the whole Operetta CLS system was off at the very beginning), the switch-on order would be:

- Machine (Operetta CLS) => Software (Harmony4.8)

Different from the Operetta Mk1, Operetta CLS uses an LED to illuminate/excite samples, which is already integrated into the machine body.

However, when users switch on the Operetta CLS machine (by pressing the green button, on the left-hand side), the instrument needs to initialise for 1-2 minutes and become stable.

Once the Operetta machine is ready, the lighting band on the front will be shining green and stable, at which point users can progress further.

Once the whole system is on, it is kept on for a whole day.

V.7) During the Operetta CLS experiment

Once an Operetta CLS experiment is set up and run, users must not disrupt the Operetta CLS or Harmony4.8.

If the experiment is running smoothly, the lighting band on the front of the Operetta CLS machine keeps shining stably, with a lighting array showing its imaging progress.

If the lighting band shows up Yellow, it indicates a caution that users must check or wait.

If the lighting band becomes Red, it means there is a problem and the imaging must stop. Normally it would generate an error message on Harmony4.8. For any error messages that show, please contact the concierge.

V.8) End of the Operetta CLS experiment

V.8.1) Plate ejection and the platform reload

Users should eject their plate from the Operetta CLS machine by pressing the 'Eject' button on the Harmony4.8 software.

After the plate has been ejected out of the Operetta CLS, users should re-load the little plate platform back into the Operetta CLS and close the plate entrance gate. This is conducted by pressing the 'Load' button on the Harmony4.8 software.

It is NOT allowed to leave the machine open, because any dust that enters could cause problems.

V.8.2) Operetta CLS tidy-up after experiments

After experiments, users are responsible for removing or disposing of their plates. Users should not leave any experimental plates, aluminium wraps, or lab tissue on the bench of Operetta CLS

After experiments, Users are responsible for keeping the Operetta CLS bench and seats clean and taking their belongings with them.

V.8.3) Operetta CLS switch-off

The Operetta CLS machine will be switched off at the end of the day, either by the last user or by the concierge. Its power button is the same as the 'on'.

The Harmony4.8 software should be logged out/closed at the end of the day, either by the last user or by the concierge.

V.9) Operetta CLS data management

Users are not allowed to use their own drive to export their data. For important measurement, it is suggested that users make an archive for themselves.

Users are not allowed to save their data on the desktop of the Operetta CLS PC, because this will seriously occupy the limited space of the PC. This will negatively affect the PC and software performance, e.g., Harmony4.8 running slowly or crashing.

If users temporarily saved their images during the Operetta CLS experiment, they should clean up their data on the desktop of the Operetta CLS PC.

VI) Columbus

Columbus is the online image storage and analysis platform developed by PerkinElmer. Its website is: <http://columbus.biomed.kcl.ac.uk/login?next=/>

The image analysis building blocks in Columbus online are the same as in the Harmony4.8 software installed on the PCs of SCH. A major difference would be Columbus does not have the function of Global Image as in Harmony4.8.

Users are advised to use the platform when at the King's Campus. It is advised that users first export their measurements from Harmony4.8 at the SCH to their own drive, then upload to Columbus via their computer. However, because such exports usually would take a long time, it is suggested that users do this via the Analysis WorkStation PC (please book first on iLabs), instead of occupying the Mk1/CLS PCs in the SCH room. Additionally, users should delete any of their temporarily saved folders on the SCH PC desktops.

VI.1) Non-Operetta images

If users would like to upload and analyse non-Operetta images on the Columbus platform, the Columbus Insight 2.8 (installed in the Analysis WorkStation PC) should be used, with a demo possible from the concierge. All of the demos for Columbus and Columbus Insight 2.8 will be held at the Analysis Station PC for which users should book in advance on iLabs.

VI.2) Off-Campus Columbus access

If users would like to access King's Columbus when they are off-campus (i.e., remote access), they would need to follow steps:

- Request remote VPN through King's remedyforce self-service,
- Install and configure Forticlient,
- Access Columbus off-campus.

VII) PhaseFocus Livecyte

VII.1) Introduction to Livecyte



Figure 4: PhaseFocus Livecyte

The **Phasefocus Livecyte**[®] provides non-toxic phenotypic screening for live cells, enabling long-term time-lapse assays without the need for fluorescent labels. This is an important consideration for the screening of sensitive primary cells, and stem cells. Based on an imaging method known as ptychography, this label-free microscopy approach is particularly suitable for reporting cellular changes such as mitosis, apoptosis, and cell differentiation. The Phasefocus Virtual Lens provides an extremely versatile platform for time-lapse label-free imaging, with post-acquisition capabilities for the analysis of cell state and cell cycle. Operation is fully automated, but with options to tailor data acquisition protocols to suit user's needs.

Key features of Livecyte include:

- Label-free and non-toxic: Use of low laser power, thereby allowing users to perform long-term time-lapse assays on sensitive primary and stem cells that previously were only practicable with immortalised cell lines due to the need for labelling.

- Flexible fields of view without loss in resolution: Users can perform fully automated high-resolution measurements on statistically significant numbers of cells, and over multiple fields of view, to produce flow cytometry-type measurements without removing cells from their culture medium.
- Consistent outputs: No photo-bleaching effects during measurements or inconsistencies due to label variability result in better reproducibility of measurements.
- Simple experiment workflow: Users save time and reduce variability in results caused by complex experimental protocols associated with labelling, fixing and harvesting of cells.
- Quantitative phase images: Direct measurements of morphological changes in cells such as volume, thickness or dry mass, which enables previously impossible assays such as contraction of primary smooth muscle cells.
- Robust cell tracking: Liveocyte can automatically follow multiple cells individually, through multiple cell divisions, and compare multiple generations of cells to their ancestors.
- Multiplexed data from analysis provide outputs equivalent to multiple assays without the need to repeat experiments, saving time and money.
- Perfect focus: Post-acquisition autofocussing means that users will never suffer from focal drift issues during a time-lapse experiment again.
- All cells in a time-lapse video may be analysed independently and simultaneously.
- The cells in the field of view of a time-lapse series may be colour-coded reflecting different states/stages.

Main applications of Liveocyte include:

- Cell count, confluence, and proliferation, at multiple time-points, which allows simple yet robust cell population monitoring.
- Toxicity, cell viability and apoptosis rate, which provides the kinetics of the cytotoxic response rather than just a snapshot like many colourimetric endpoint assays.
- Mitotic index vs time, with measurements at multiple time-points which allow identification of changes in proliferation as a population evolves.
- Morphology such as sphericity, nucleus-cytoplasm ratio, which highlight changes in cell behaviour such as the formation of neuronal processes.
- Cell motility, including scratch-wound assays, for robust measurement cell migration parameters.
- Cell thickness, volume and dry mass.

VII.2) Prior to the Livecyte experiment

VII.2.1) CO₂ for Livecyte experiments

Currently, the CO₂ supply for Livecyte experiments is from a gas cylinder. It is already mixed with 5% CO₂. For safety reasons the cylinder is placed in the corner of the SCH room and locked tightly onto the wall.

Before Livecyte experiments, users need to make sure the CO₂ cylinder has enough gas, by checking its meters. Otherwise, users need to contact the lab manager or concierge to replace the cylinder.

VII.2.2) Incubation

For preparing Livecyte experiments, Users need to warm up the chamber (by turning on the Temperature Controller) to 37°C for incubation of at least 4 hours or even overnight.

VII.2.3) Plate care

Users need to take care of their cell culture plates, to prevent any scratches, dust, hairs, or condensation on the bottom surfaces. The plate surface conditions will directly influence image quality and the analysis result in Livecyte.

Besides well plates, there is also a slide holder that users could use for Livecyte imaging.

VII.3) Switch-on the Livecyte System

When starting the whole system (in the case of the whole system is off at the very beginning), the switch-on order would be:

- Electrical sockets => Temperature Controller => CO₂ cylinder / CO₂ Mixer => Microscope platform controller => Olympus system => Black Box (Server, Pi-box, NAS) => PC
 - When turning on electrical switches (in total five of them) on the wall, there should be a 5-second delay in between.
 - Normally the switches are already kept on.
 - After switching on the Temperature Controller, it needs time to rise to 37 °C. It may take 5-10 minutes (rise from the SCH room temperature 21 °C).
 - For a formal live-cell imaging experiment, the temperature needs to be on for at least 4 hours in advance. Please refer to **Part-VII.1.2** above for the Incubation.
 - When switching on the CO₂ cylinder, please pay attention to the values of two meters:

- The right-hand side meter indicates how much CO₂ remains in the cylinder.
- The left-hand side meter indicates CO₂ flow rate coming out of the cylinder.
- When switching on the CO₂ Mixer, please pay attention to the bubbling (and the bubbling rate) in the little plastic bottle inside the Livecyte Chamber.
- The Olympus system has two parts, switch on from the right one to the left one.
 - When switching on the right part, please pay attention to the orange-coloured LED, wait until it becomes stable.
 - The left part is a touch screen.
- The Black Box is under the bench of Livecyte microscope
 - There is a red main button on the right-hand side (when facing the Black Box), open the right door of the Black Box and switch on the button first, and wait until all green LEDs are lit up.
 - Once the red button is on, open the left door of the Black Box, and switch on the three parts inside from top to bottom.
 - The top part is the server, which connects all parts of Livecyte internally and also the internet.
 - The middle part is the Pi-Box. When switching it on, please wait for the fan noise and wait until it disappears.
 - The bottom part is the 12TB NAS, where all the data is stored.
 - When sequentially switching on these three parts from the top to the bottom, please pay attention to their green-LEDs (these should be lit up and be constantly on) and wait for 30 sec – 1 min in between.
 - When switching on the Livecyte PC, it does NOT have a login.

VII.4) Livecyte PC

The Livecyte PC has three software packages on its desktop:

- Assay Design
- Image Acquisition (also has assay layout design function)
- Image Analysis

VII.5) Start the Livecyte experiment

To start and set up an imaging experiment on Livecyte:

- 1) Click the Image Acquisition software on the desktop;
- 2) You will be prompted to create a database file.

Please create, save, and name this file, into the Z-drive (which is the NAS).

Please do NOT save any data, file, or image to the local-c drive or desktop, because the Livecyte PC itself has limited space.

If users save results into the local-c drive and run a live-cell imaging experiment of several days, it may crash the PC.

Once have created your own database file in the Livecyte NAS, users would just need to follow the experimental setup procedure according to the Livecyte user's manual. Please notice that:

- After editing the Assay Layout, it would be better to SAVE it into your folder in the Z-drive, so that next time you can reload it or modify based on it.
- After choosing your favoured settings e.g. image fields and magnification etc., it is worth saving them again into your folder in the Z-drive, so that next time you can reload from it and directly apply.
- When setting up the focus, this focus is different from the conventional Phase Contrast microscope's focus.
 - This QPI (Quantitative Phase Imaging) focus would need cells in your field being "disappeared" (Zangle & Teitell, 2014; Kasprowicza, Suman, & O'Toole, 2017).
- It has an "Autofocus" function. You could manually set up your focus or apply "Autofocus" by the software, or a combination of both.
 - E.g., for the first few wells you manually setup focuses, which would be a kind of training for the software, and based on that, you apply the "Autofocus" for the rest of the wells in the plate.
- When setting up the Time-lapse, you can always right-click the Timetable panel and "Edit".
 - If there is any "Conflict", the software will warn you.

VII.6) During the Livecyte experiment

Do NOT close the software window, please leave the software as it is on the desktop. It will show the realtime progress of imaging, including on-going wells and timeframes.

During the imaging, users can check the fields and wells which have already finished via simply clicking their titles on the Livecyte software window (left-hand side panel).

Users should not disrupt the microscopic and hardware parts of Livecyte during the imaging.

VII.7) End of the Livecyte experiment & switching-off the system

When an experiment is finished, all images have been automatically saved into your named folder in the Livecyte NAS.

To completely switch off the whole Livecyte system, users need to follow the reversed order of its switch-on:

- Livecyte PC => Black Box (NAS, Pi-box, Server) => Olympus system => Microscope platform controller => CO₂ Mixer / CO₂ cylinder => Temperature Controller => Electrical sockets
 - Once the experiment is finished, users can shut down the image acquisition window on the PC. If the image analysis software had been used, users need to first log out and close the analysis window on the PC.
Only after properly closing all running Livecyte software packages, users can switch off the Livecyte PC.
 - After switching off the PC, users need to turn to the Black Box under the Livecyte bench
 - Firstly, switch off the bottom layer of the Black Box – the NAS, by briefly pressing the off button and then wait for all LEDs to be off.
 - Secondly, switch off the middle layer of the Black Box – the Pi-box, by briefly pressing its off button and then wait for the fan to stop.
 - Thirdly, to switch off the top layer of the Black Box – the Server, by briefly pressing its off button.
 - Users need to wait for about 30 sec in between.
 - Finally, turn around to the other side of the Black Box and switch off the red main button and wait for all green LEDs to be off.
 - Switch off the Olympus units, starting from the touching screen (i.e., the left-hand side unit). When the monitor is off, wait for a few seconds and then switch off the right unit.
 - Switch off the microscope platform controller.
 - Switch off the CO₂ Mixer, and then turn off both vales of the CO₂ Cylinder (therefore there will be no CO₂ leakage from the Cylinder).
 - Switch off the temperature controller.
 - Switch off the electrical sockets on the wall one by one.

In case there would be next users, the CO₂ and Temperature parts of the Livecyte system could be switched off but leaving other parts on.

VII.8) Livecyte data management & image analysis

All images are automatically saved into your folder in the Z-drive. Image analysis software on the desktop will show up as a second desktop, once you click on it.

For a demo of basic image analysis, e.g., cell segmentation, users could also contact the concierge.

For image analysis questions, the PhaseFocus team can provide answers and a remote demo. Please contact Dr James Russell (james.russell@phasefocus.com) for more information.

VIII) IncuCyte ZOOM

VIII.1) Introduction to IncuCyte



Figure 5: IncuCyte ZOOM

Essen Bioscience IncuCyte® ZOOM is a device used for in vivo assays. This device is simply a microscope inside an incubator, solving potential problems that may result from disturbing cells during the observation process rendering long-term kinetic imaging practical and easy. Users can use this instrument to see what happened and when, without ever removing your cells from the incubator. Key features include:

- Simple and flexible sample preparation, which also ensure minimal cell perturbation in your choice of vessels.
- Automated setup: Users can set up automated acquisition and analysis parameters with confidence, having no need to pre-define the assay endpoint.
- Automatic acquisition of images for hours, days or weeks.
- Powerful image visualization and analysis tools which enable real-time decision making. This includes efficient and reproducible image analysis and powerful visualization of images and timecourses.

- Support for multiple users, accommodating up to 6 microplates at a time. Users can schedule experiments at different image acquisition frequencies and magnifications in parallel.
- Remote, networked access with unlimited, free licenses.
- A unique optical design which ensures that cells remain stationary while optics move.
- Multiple imaging modes: HD phase plus red and green fluorescence. Please notice it does not have a DAPI (blue) channel.
- It applies autofocus without z-stack.
- CMOS camera and efficient optics enable sensitive, long-term live-cell imaging.
- 16 total core processors for enhanced speed of image processing and data analysis.

Application of IncuCyte include:

- Live imaging.
- Stem cells.
- Primary cells.
- 3D cultures.
- Assay development.
- siRNA experiments.
- Compound screens.

VIII.2) IncuCyte parts & structure

The IncuCyte microscope and vessel are integrated into the bottom incubator in the Tissue Culture Room-1, and its touch screen control and drive parts (IncuCyte Store) are placed on top of the incubator. The images acquired by IncuCyte ZOOM are stored in the IncuStore hard drives. Its software is installed in the Operetta CLS PC.

The objective not currently in use at any one time should remain in the protective plastic encasing in the incubator besides the Incucyte; this will keep the objective at the right temperature (37°C), saving time for when that objective needs to be swapped in (rather than having to wait a while for it to warm up from room temperature before scanning).

VIII.3) After IncuCyte experiments

Please remove your vessels when your scheduled scan is over, or just instruct the person scheduled after you in that slot on what to do with your vessel (e.g. in case that your scan is scheduled to end in the middle of the night).

VIII.4) IncuCyte software & image analysis

The IncuCyte software has a database of all available culture formats, mostly well plates. Users are advised to use the available formats for their cell culture vessels.

It also offers basic analysis for cell culture, including confluency plot. Therefore, for more complicated analysis, it will rely on third-party software, e.g., ImageJ, CellProfiler, Icy.

IX) JuLI Stage

IX.1) Introduction to JuLI Stage



Figure 6: JuLI Stage

NanoEntek JuLI™ Stage Real-Time Cell History Recorder is a Real-Time CHR (Cell History Recorder) designed for live-cell imaging and analysis. This live imaging device allows high-quality imaging with different focuses on Z-planes, for a total of 4 different channels (red, green, blue, and brightfield, but NOT far-red) and on selected areas of each well in different vessels. It is designed to support Users to approach kinetic images and data from the start to the end so that they can save their time and can focus on more advanced and valuable work for their research.

Main features of JuLI Stage include:

- A fully automated x-y-z stage and multi-channel fluorescent colours to acquire cell images and videos from various cell culture plates (up to 384 wells) and dishes in an incubator.
- Image-based analysis for a bright field, which enables users to obtain quantified cell confluence results with low variations as well as the growth curve.
- Incubator-compatible.
- Interchangeable objective lens (4×, 10×, 20×), which are placed in the lens boxes (in the cabinet under the Operetta CLS Bench).
- Manual and auto-focusing.
- Compatibility with various planes and vessels.
- Data management with all-in-one PC.

Main functions of JuLI Stage include:

- Image capturing.
- Time-lapse recording.
- Automated video making.
- Image stitching.
- Image and video editing.
- Multi-channel fluorescent colours (GFP, RFP, DAPI).

- Multi-well monitoring (up to 384 wells).
- Multi-position monitoring.

Applications and assays of JuLI Stage include:

- Wound healing.
- Fluorescence expression.
- Apoptosis and cytotoxicity.
- Cell proliferation.
- 3D-spheroid culture.
- Stem cell monitoring.
- Cell growth monitoring.
- Differentiation of neurons.
- Angiogenesis.

JuLI Stage has only one plate holder, so users can only run one plate at a time. Its microscope part is integrated into the bottom incubator, and its software is installed in the JuLI Stage PC, both are in the SCH room. The JuLI Stage microscope and JuLI Stage PC are linked together via cables, users shall not touch or modify the cables.

IX.2) JuLI Stage PC and IT policy

The JuLI Stage PC is placed next to the SCH sink and close to the SCH incubators. It is always on and does not require a user's login.

It has two major JuLI software packages – JuLI Stage and JuLI EDDIT. To conduct live-cell imaging, JuLI Stage is the one; to edit images and movies, users should use JuLI EDDIT.

Users are not allowed to install their own software or third-party software into the JuLI Stage PC.

JuLI Stage PC runs the Windows 10 System and users are forbidden to perform Windows updates or any other software updates on the PC.

Users are allowed to bring in their own drive (e.g., a USB stick) to connect with the JuLI Stage PC, for image export purposes.

IX.3) Prior to JuLI Stage experiments

Prior to a JuLI Stage experiment, users are allowed to place their cell culture plates temporarily in the SCH incubators.

Users are not permitted to run a JuLI Stage experiment continuously for more than 1 week.

IX.3.1) JuLI Stage software initialisation

The software for JuLI experiments is called 'Realtime Cell History Recorder', on the desktop of the JuLI Stage PC. Users need to double-click its icon to initialise it.

The initialisation of the JuLI software takes about 1 min.

IX.3.2) JuLI Stage lens change

For changing lens, please inform the concierge. After changing a lens, users need to go to the "Setting" part of the software and confirm the installed lens.

IX.3.3) JuLI Stage PC storage check & Data Path

Before starting an experiment, besides confirming the lens, users also need to check whether there is enough storage space for imaging in the JuLI Stage PC.

A typical JuLI Stage experiment would generate 10-30 GB.

Before starting an experiment, users can manually set up its 'Data Path' where all the images will be saved. However, users need to remember this path so that later can easily find the images when the experiment finishes.

IX.3.4) JuLI Stage plate calibration

If users are using a plate with precise dimensions, the system needs to conduct the "Calibration" so that the imaging will be in right coordinates and positions. Please ask the concierge for further assistance.

IX.4) JuLI Stage experiments setup

JuLI Stage has functions including positioning, mapping, autofocus, and z-stack.

When setting up an experiment, users should not forget to SAVE the list of positions (with their optimised focuses) and their imaging protocols.

For setting up time-lapse imaging, the interval should be always longer than a single cycle duration. The JuLI Stage software will generate a reminder.

IX.5) After JuLI Stage experiments

There is no power off button for the JuLI Stage, when the image acquisition finishes, users can just close the JuLI Stage software and leave the JuLI Stage PC on.

After the JuLI Stage imaging, users must take their plate out of the JuLI Stage microscope and incubator, and either take the plate with them or dispose it properly into the yellow waste bin in the SCH

Users must clean up the JuLI Stage PC bench, and take their notebooks, samples, reagents, etc. with them. The tissues and aluminium foil should be disposed of properly, e.g., into the yellow waste bin of the SCH.

Users are not allowed to leave their images and personal data on the desktop of the JuLI Stage PC after their experiments.

IX.6) JuLI Stage image export & analysis

The images acquired by the JuLI Stage are stored in the JuLI Stage PC drive. Users can export their images via JuLI Stage software using the “Export” function. Users are also allowed to bring in their drive to export their images at the JuLI Stage PC.

JuLI Stage software also offers some basic analysis, e.g., 1) confluency plot, for 2D cell culture, and 2) spheroid diameter/area plot, for spheroid culture. However more sophisticated analysis may be performed using third-party software, e.g., ImageJ, CellProfiler, Icy.

IX.7) JuLI EDDIT

The JuLI EDDIT software is for image editing and movie making. Its icon is also on the desktop of JuLI Stage PC. It can add scale bars and time stamps, crop images, and make video clips.

X) Analysis WorkStation PC

The Analysis WorkStation PC (short name: Analysis PC) is only for image analysis purposes. It is located in the main lab next to the HipSci lab bench, not in the SCH room.

X.1) Harmony software

The Analysis PC has Harmony4.8 software installed for analysing Operetta images (for both Operetta Mk1 and Operetta CLS). The PC is linked to the NAS (image storage) and the ODAs (databases) of Operetta Mk1 and Operetta CLS. If users experience difficulty loading their images into Harmony4.8, this is likely a networking problem and needs to be reported to the concierge with relevant error messages.

X.2) Columbus software

Columbus Insight 2.8 is also installed in the Analysis PC. It is for converting and transferring non-Operetta images onto the Columbus platform for image analysis. Please refer also to Part-**VI** of this chapter.

X.3) Miscellaneous software

Third-party software ImageJ, CellProfiler, Spotfire are also installed in the PC that users can use. Microsoft Office, including Word, Excel, PowerPoint etc. are installed in the PC for assisting users' analyses. For example, users can export image analysis results by Excel and also conduct basic statistics using Excel functions.

X.4) IT policy for the Analysis PC

Installation of other software into the PC by users themselves is not permitted unless approved by the SCH and IT.

Users are also not permitted to save their images and image analysis results on the Analysis WorkStation PC. These would occupy a lot of its free storage space and therefore cause issues, such as the PC to run slowly.

During the analysis task, if users temporarily have saved their images or results in the Analysis PC, e.g., on its desktop, then users need to remove them after their analysis.

USB sticks are allowed to be used on the Analysis PC for users' image and results transfer and for saving purposes.

XI) Lab space & accessories

XI.1) Incubators in the SCH

There are two incubators in the SCH room, with the bottom one hosting the JuLI Stage microscope. Users can temporarily use the top incubator, e.g., for preparing the live-cell imaging of Operetta Mk1, Operetta CLS, PhaseFocus LiveCyte, and JuLI Stage.

Users should remove or dispose of their cells properly after their experiments at SCH, instead of keeping them in the incubators.

XI.2) Accessories

Besides incubators, shared equipment includes:

- 1 centrifuge
- 1 water bath
- 1 fridge (4 °C)
- 1 freezer (-20 °C)

Users need to ask permission before using them.

XI.3) SCH fridge

The PerkinElmer plates are permanently stored in the 4 °C fridge, and they are for the demo and calibration purposes of the Operettas. The PerkinElmer demo plates are HeLa cells immunostained in 384-well PerkinElmer Cell Carrier Ultra plates. If users would like to try the PerkinElmer demo plates on the Operettas, they need to contact the concierge.

Users can temporarily put their immunostained plates in the SCH fridge when preparing their imaging experiments. However, after experiments, users need to remove them.

When temporarily storing plates in the SCH fridge, users need to wrap their plates well using aluminium foil or clean lab tissue and label them clearly with plate name, date, and the owner.

Chapter 6 – Contacts

Users must follow guidelines detailed in this handbook. For any questions, please contact the SCH team:

- Director - Dr Davide Danovi: davide.danovi@kcl.ac.uk
- Senior Scientist - Erika Wiseman: erika.wiseman@kcl.ac.uk
- Concierge – To be updated

For questions regarding the image analysis on Operetta systems and Columbus platform, please contact the concierge.

For questions regarding the image analysis on Livecyte (PhaseFocus), please contact:



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