Document Number: **SASoM/EQUIP/061.v2**

Title: **How to Acquire a Scan using the Celigo Cytometer**

Version: **v2**

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Effective from:	12/05/2019
Valid to:	11/05/2024

SOP History		
Number	Date	Reason for Change
v1	12/05/2014	Original
V2	12/05/2019	Five Year Review

1.0 Purpose –

The purpose of this SOP is to outline the principles of the routine use of the Celigo Cytometer in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to routine use and maintenance of the Celigo Cytometer within the SASoM.

3.0 Responsibilities –

It is the responsibility of all users of the Celigo Cytometer within the SASoM to comply with this SOP.

4.0 Procedure –

The Celigo Adherent cell cytometer is a semi-high-throughput static microscope with integrated camera and analysis software. This SOP should be read in conjunction with the instrument manuals

A: LOGGING IN TO THE CELIGO SCANNER:

Both the Celigo Scanner and the PC should be left switched **ON** all of the time. The main POWER switch is on the rear of the Celigo Scanner – the switch on the front of the Celigo Scanner simply turns the illumination OFF/ON.

Prior to using the Celigo Scanner you will need to request a user / Login ID. This should be done by emailing Peter Mullen (pm72@st-andrews.ac.uk). Once you have been given a user / login ID you will be able to open the software.

Double click the 'Celigo' icon on the desktop to launch the Celigo Scanner software after which you will be presented with a Login Screen.

Click inside the Login ID box and then type in your Login ID. Since your Login ID will be issued with no password protection, the Password field can be left blank. *Note that you have to physically click inside the login box before it will allow you to make an entry.*

Click Login after which the Task List for the Celigo scanner will appear.

- **Create a New Scan:** as detailed in this SOP.
- **View and Analyse Scans**: for selecting an individual scan to work with or perform analysis (separate SOP).
- **Batch Analysis**: for performing Batch Analysis on multiple scans (separate SOP).
- **Batch Export**: for exporting a batch of scans (separate SOP).
- **Manage Files** to look at files, archive files, delete files etc.

B: LOADING A PLATE INTO THE CELIGO SCANNER:

1. In the Start tab, click **CREATE NEW SCAN** after which the Enter Plate Detail screen then appears.

2. In the Enter Plate Details section make the following entries:

- **Plate Category**: Choose appropriate Plate Type and/or Format (eg flask, 24-well, 96-well etc).
- **Vendor Type**: Choose Plate Vendor / catalogue number (eg Corning 24-well Corning 3524 Plate)
- **Plate ID**: Enter Plate ID – this is essentially the folder name into which all of the data, analysis etc will be saved.
- **Folder**: Destination where the scans will be saved (the login user by default).
- **Name (optional):** Name the Experiment – this will appear on EVERY scan made under these settings. This should essentially be a description of the experiment).

- **Plate Description (optional):** Useful for providing detail of the experiment but again this will appear on EVERY scan made under these settings.
- **Scan Description (optional):** This refers to the SPECIFIC plate / scan you are performing (eg a time point) and should be specific to the scan being made at the time. *This is the single most important field to enter as it is the only way an individual plate can be tracked / identified.*

In the 'Select Experiment' section a previously saved Experimental Template can be loaded for use in a new scan. Only settings that are compatible with the chosen plate category are displayed for selection.

3. In the 'Start' section, click LOAD PLATE. Do **NOT** remove the lid from the plate.

4. Do one of the following:
- If you left the Experiment menu blank in Step 2, skip to Step 7.
- If you previously entered an Experiment menu selection in Step 2 with Focus Type in Focus Setup set to NONE, no registration occurs; skip to Step 7.
- If you previously entered an Experiment menu selection in Step 2 and you defined a Focus Type in Focus Setup in the experiment, a "Would you like to perform auto focus registration?" message appears. Continue to Step 5.

5. Click **YES** or **NO** as follows:
- **YES** – All of the image acquisition settings, including any offsets and focus registration settings that have been made in the Focus Setup dialog box will be applied to the upcoming scan.
- **NO** – All of the image acquisition settings except any offsets and focus registration settings made in the Focus Setup dialog box will be applied to the upcoming scan. Selecting NO will allow you to manually select Focus Setup settings to be applied instead of those in the Focus Setup dialog box.

6. Do one of the following:
   - If you entered an Experiment menu selection in Step 2 with the Focus Type in Focus Setup set to **NONE**, skip to step 7.
   - If you entered an Experiment menu selection in Step 2 and you defined the Focus Type in Focus Setup in the experiment, select one of the following in the resulting Plate Alignment message box:
   - Yes – A Plate Alignment will be performed using the settings in the experiment file:
   - No – A plate alignment will not be performed.
   - If you clicked YES, the system now performs a plate alignment.

7. A **LOAD PLATE** message box appears.

8. Carefully place a plate or flask onto the stage, using the following practices
   - For a plate – Manually push the plate into the inner left corner of the plate carrier. Ensure that the plate is seated FLAT in the stage, with well position

A1 in the upper-left hand corner of the plate as you face the front of the instrument.

- For a T-25 flask, insert the FLASK HOLDER onto the stage. Load the flask into the Flask Holder by pulling back the spring mechanism. Ensure that the flask is seated FLAT, with the cap towards the left side in relation to an operator in front of the instrument.
- For a T-75 flask, load directly onto the stage with the cap towards the left side.

9. Click OK in the LOAD PLATE message box. The access door closes. If you had entered an Experiment menu selection, the settings in the selection will be used.

10. The **Scan Tab** now appears.

C: SCANNING A PLATE IN THE CELIGO SCANNER:

Scanning a plate is split into four sections, namely

- Select an Application.
- Selecting a channel
- Viewing wells
- Selecting Image Acquisition settings

Select an Application.

Applications are used to set the parameters needed for data acquisition for specific assays. Common components such as configuration settings are constant across all applications, components specific to each application are available only when that specific application is selected. The following procedure is based around the confluence application.

Selecting a channel

Some applications are associated with more than one channel (eg cell viability is associated with three channels – Live, dead and total cells). If using the 'Custom Channel' then each of the four channels (Brightfield / Green / Red / Blue) can be independently configured for 'Exposure Time' and 'Gain'.

Viewing wells

1. In the 'Navigate / Select Scan Areas' panel, click **NAVIGATION**.
2. Click the desired well to move to that location.
3. In the Camera Controls panel click **LIVE** or **SNAP** so that it is selected. Selection of LIVE will result in continuous illumination; selection of SNAP will make a single exposure and then turn off the illumination. The centre of the well appears in the scan tab. The small well view at the bottom right of the screen shows the Field of View (FOV) currently being displayed. To reset the centre FOV at any time, click NAVIGATION and then click a well.

4. *For scanning with fluorescence illumination it is recommended that you keep LIVE on for the shortest period possible in order to reduce photobleaching. The LIVE view can be turned OFF by clicking LIVE again so that it is deselected.*
5. To reduce photobleaching, swith OFF live view.

Selecting 'Image Acquisition Settings'

1. Select the Lighting type (Channel)
2. Select the Configuration settings
3. Select the Motion Control Settings (optional)
4. Select the Focus Settings (optional, default Focus Type is 'NONE').

1. **Selecting the Lighting Type**: When you make a Lighting type selection, you are selecting the exposure, and specifically whether you want to manually make image lighting adjustments or allow the software to make them. Select one of the two following options:

   1.1. **Custom Channel'** allows you to specify the light source to use for the channel (Brightfield, Green, Red or Blue) and to select from a wider choice of desired exposure and gain settings (not pre-determined settings), which will be applied to the entire plate.
   1.2. **Auto Exposure/Gain Channel'** – the software will automatically set the lighting by adjusting exposure and gain. Typically this setting is used only for scanning with illumination setting **Brightfield**. If using 'Auto Exposure' then only a single channel can be selected (Brightfield, Green, Red or Blue).

If you selected Auto Exposure/Gain Channel in the Type field, select one of the following Priority selections to apply the exposure and gain for obtaining the desired lighting level:

- Auto Exposure Only (no gain adjustment)
- Auto Exposure, Gain if necessary (exposure will be adjusted first with gain being used only if needed to increase light level)
- Auto Gain Only (Exposure will not be adjusted)
- Auto Gain, Integration if necessary (gain will be adjusted first with exposure being used only if needed to increase light level)

If you selected Auto Exposure / Gain Channel in the Type Field and therefore Frequency appears in the application select one of the following:

- Every Scan Area (applies the selected settings on an individual well-to-well basis but is more time consuming)
- Once for the Sample (apples the selected settings to the sample (plate) as a whole, using the first selected well as the reference point for the remainder of the selected wells.

2. **Selecting the Configuration Settings**: Configuration refers to a configuration name field and several lighting–related settings. Proceed as follows:

   2.1. Leave the Configuration Name as Custom, or Load in a previous file with pre-determined settings.

2.2. In Illumination, select Brightfield, Green, Red or Blue

2.3. If Exposure Time and Gain selections appear in the application you have selected, adjust Exposure time and then Gain using one of the following methods.

- Type an entry into the Exposure time and Gain fields
- Click the Up and Down Arrows to the right of the fields
- Move the Exposure time and Gain slidebars
- Click AutoCalc to the right of the fields to automatically calculate the Exposure Time and Gain (see Celigo Cytometer User Guide p45 for specific instructions). *Note: Adjust the exposure time first because a higher Gain Value increases the noise level. If a large amount of light is needed to view the well, you can improve (reduce) capture rime by increasing the Gain, which in turn reduces exposure times. Depending on the order of execution (exposure then gain or vice versa), different results may occur.*
- Select 2x2 binning as required – This function increases the pixel size while also increasing sensitivity. If enabled, a 2x2 area (4 adjacent pixels) is read as one, with the resulting additive intensity. This increased signal/noise ratio will therefore increase the sensitivity to less bright objects BUT will reduce the resolution by half. ***Consequently, 2x2 binning is not recommended for small objects (eg 2D cell cultures).***

3. **Selecting Motion Control Settings**: Motion Control settings are related to the settling time (scan delay) and the stage motion speed.

3.1. In the Motion Control section, click **Advanced**.

3.2. **Settling Time** – select the number of minutes you want the system to wait before starting the scan

3.3. **Stage Motion** – select Fast (faster scanning but more movement of liquid within the wells) or Smooth (a slower S-curve motion to reduce liquid movement).

4. **Selecting Focus Settings**: Focusing can be done with either Manual Registration or Auto Registration. The User Guide recommends that Auto Registration be used but this may not be optimal. The choices are as follows:

4.1. **Manual registration** – You personally determine and set a focal position for image acquisition using the current well. The system will then use that focal position as the baseline to determine the different focal positions for the other wells.

4.1.1. Click LIVE in the camera controls section.

4.1.2. In the Plate View display, navigate to a well and view it.

4.1.3. In the focus selections (located below Motion Control), Adjust the focus using the Focus Up and Focus Down Arrows or the Find Focus button. Note: For brightfield, a useful practice is to set a focus position that slightly defocuses the cells so that they appear bright in the centre with dark edges, allowing for more accurate segmentation.

4.1.4. Click Focus Setup

4.1.5. In Focus Type, select one of the following:

- No autofocus will be applied during each capture and the current scan focal position will be used for the whole plate. *Note: You do not make a selection in the Target Focal Plane (Brightfield) menu when focusing with manual registration*.

- **Hardware Autofocus** – A software focus algorithm will be used during intitial capture in the setup well to determine the best scan lens position (focal position). Focusing will be carried out automatically on the plate bottom (not the cells) to determine focal plane and is therefore less reliable than Image Based Auto Focus. Hardware autofocus is however a faster scanning method.

- **Image Based Autofocus** – Focusing will be carried out automatically to determine the best focusing position to perform image capturing. This method is more consistent in focus but slower. *Note: you do not make a selection in the Target Focal Plane (Brightfield) menu when focusing with Manual Registration.*

4.1.6. Click **Register Manual** – the system focuses using the current scan position, executes the focus type method if selected (Hardware or Image based focus) and then displays the results in the live screen

4.1.7. Close the Focus setup dialog box by clicking **Focus Setup**.

4.2. **Auto registration** – The system will determine and set a focal position for image acquisition using the current well. The system will then use that focal position as the baseline to determine the different focal positions for the other wells. Perform the following steps to focus with automatic registration:

4.2.1. Click LIVE in the camera controls section.

4.2.2. In the Plate View display, navigate to a well and view it.

4.2.3. In the focus selections (located below Motion Control), verify that an object is visible in the well by using the Focus Up and Focus Down Arrows.

4.2.4. Click Focus Setup – the Focus dialog box appears. In Focus Type, select one of the following:

- Hardware Auto Focus – Focusing will be carried out automatically on the plate bottom (not the cells) o the setup well to determine focal plane and is therefore less reliable than Image Based Auto Focus (although faster).
- Image Based Auto Focus – Focusing will be carried out automatically to determine the best focusing position to perform image capturing. This method is more consistent in focus but slower.

4.2.5. For Brightfield only, select one of the Target Focal Plane options to set the criteria that the system will use during the scans to determine focus. This will be shown under **Find Focus Configuration**.

- Bright Focus (default for Brightfield): where the software calculates the scan lens position that results in a bright focus of the cells
- Dark Focus: where the software calculates the scan lens position that results in the darkest focus of cells
- Best Contrast (select for fluorescence illumination only): where the software calculates a scan lens position that results in the best contrast. Note: The Target Focal Plane menu is associated with the focus of Brightfield Images only. For fluorescent illumination the system will automatically use best contrast regardless of menu selection.

4.2.6. Click **Register Auto.** The software profiles the image, a focus is determined using the selected focus output, focus type method (hardware or image based focus) is executed, and the results are presented in the live screen.

4.2.7. Close the Focus Setup dialoge box by clicking **Focus Setup**.

4.2.8. Adjust the current registered focus position as required using (i) the Focus Up and Focus Down arrows, or (ii) Click Offset.

To start the Scan

1. Click Start Scan to begin scanning the plate. *Note: after removing the plate, you cannot adjust the image acquisition settings and so a new file must be created.*
2. *Data is automatically saved to the instrument ready for analysis.*

5.0 Personal protection -

Howie coat must be worn at all times.

6.0 Spillages -

It the Celigo is contaminated by spills it must be cleaned ***immediately after the spill has occurred***. A final wipe-down should be performed with 70% ETOH.

7.0 Training –

All users have to be trained before using the Instrument by a designated person.

8.0 Related documents –

8.1 Equipment Manual –
Celigo Cytometer User Guide
Celigo Cytometer Expression Analysis Application Guide
Celigo Cytometer Administration Guide0.

9.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature: Date:

Management Approval:

Name: Peter Mullen

Position: SOP Administrator

Signature: Date:

QA release by:

Name: Alex MacLellan

Position: QA Manager

Signature: Date:

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE