CellenONE (SCI)

CellenONE is the automated cell detection software that is use to detect and dispense cells in the nozzle. With cells at a good concentration it takes about 20 minutes to spot 200 cells (10 cells/min).

Procedure for cell spotting:

1. Turn off the light in the clean room for any cell dispense steps. Place the black out panels on the spotter casing. This helps avoid image artifacts due to reflections on the nozzle.
2. To use the CellenONE software, navigate to the desktop folder that contains the latest version (4.5) and open the program.
3. Use a PDC90 type 4 (or cell qualified) for all cell spotting.
4. Uptake your cells at a concentration of 220,000 cells/mL and make sure you have a stable droplet.
5. Use a 72x72 fld so the locations will be recorded correctly. Use a 1 drop fld for 1 cell per well.

Running the software:

Change the run directory to delete_me folder on desktop. We don't want to record all data during set-up, just logs of the background and the cells dispensed.

Set Run and Target from main menu.

- Run: Set_CellenONE-run_2ref_pt_right-v4.
- Target: CellenONE_v4_3.

Set up nozzle. Autofocus, make sure the drop is stable, change LED pulse to 10.

At the camera station, use the 'Do Task' panel to use the 'Cell_Monitor'. This will open the CellenONE window which shows the nozzle with cells inside.

Change run directory to store the images and the log file which gives row and column spotted, circularity, elongation, and size of the cell dispensed. File>Select Run Directories and make a folder labeled with date and chip ID: YYYYMMDD_A#####?. Do not use sub directories. Use this directory for all cell spotting for this library. Use a different directory for other libraries in a chip.

Click 'Test Droplet' to dispense cells. When there is no cell visible in the nozzle image, turn on 'Save Images' in the 'Advance Settings' tab in the cell monitor.and click 'Get Background' to save a background image in the main tab. Turn off save images.

Click 'Test Droplet' to dispense cells and read their circularity, elongation, and area from the cell monitor. Based on the values you see with representative cells, adjust these settings in the 'Advance Settings' tab. Typcial GM18507 settings: min area 20, max area 250, circularity 1.35, elongation 2.5.

Click 'Start Mapping' and ensure there is clear separation between the green and blue labeled cells. Mapping density threshold should be between 0.25 and 0.3.

> ***If the mapping is poor:***
>
> - Before changing anything, try repeating it.
> - Try changing the focal plane in Nozzle offset, ideally cells are slightly out of focus. Autofocus works fairly well.
> - Change the LED pulse from the default 5 to 10 for CellenONE spotting if you have not yet done so.
> - Dilute the cells if they seem too concentrated.

Once you have good mapping, enable "Print Run Data File" and enter the ID of the library entered in the box in the main tab of the cell monitor. This will label the log and images with the ID.

Go to the run tab of the main Scienion software, and click run. Select the chip (there should only be one option if the target was set correctly) and hit run.

Monitor the spotter to ensure droplet stability is maintained.

Make sure to spot the corners if the chip needs to be imaged after. Do this as a separate run with the delete_me directory so the images/log are not stored in the run file.

Spotting cell suspension fluid for NCC controls

Select desired wells in fld.

In 'Advance Settings', enable 'Negative Control'. Start run as usual.

Notes:

- Circularity, elongation, and area settings can be updated during the run if acceptable cells that do not fall within your original settings are seen.
- If you need to stop the run, the X and Y displayed on the Scienion software run monitor are the real locations -1. Add 1 to get the correct location. The row and column on the images are the real location.

Data entry

In the single cell database under create libraries, add the IDs of the chips you are spotting into (note that if multiple libraries are planned for the same chip, they should be designated with the chip id plus a letter (e. A90564A, A90564B, ...) and record relevant sample details such as viability and passage number. Create a JIRA ticket for queuing the analysis of the library, but self-assign it until the data for the library is received. Ticket titles should contain the sample ID and chip ID, and the title should have a note on what the experiment may be, if necessary.

Next...

- **Chip imaging and calling (SCI)**