Graphical user interface, application

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For every time-point, these steps must be repeated. Start up software. ① Press “Read Now” and “Existing Protocol…”. ② Select protocol that has been created in previous step. You will be asked to save experiment. This can be done at this step, but also at any later step manually by clicking the “Safe” button on the upper left.

Graphical user interface

Description automatically generated

① A pop-up window will ask you to wait for system to heat. Turn on the CO2 gas-controller and set to 5%.

Graphical user interface

Description automatically generated

Once the system has reached set temperature it will prompt you to insert a plate and read. ① Press “cancel”. This is because for each time-point the exposure of each fluorochrome must be adjusted individually.

Graphical user interface, application

Description automatically generated

After cancelling click on ① “Procedure”. ② In pop-up window select pre-set imaging step. Adjust focus and exposure on “DAPI” channel first. ③ Untick “Auto Exposure” box at all channels. Then click “microscope” icon next to “DAPI” channel. A new window will open.

A screenshot of a computer

Description automatically generated with medium confidence

① Select well you want to focus on. ②If signal is already visible, first click “Autofocus” then “Autoexposure”. If not, first increase exposure and repeat “Autofocus” and “Autoexposure” ③ If desired, exposure can be adjusted manually by selecting exposure drop down menu. It’s recommended to check your settings on multiple wells. ④ Once satisfied, “Save Settings”

Exposure has to be adjusted in the same manner for all channels. CAUTION: Do not focus on other channels, only adjust exposure. The focus plain should be the same for all channels.

A screenshot of a computer

Description automatically generated

Close “procedure” window. ① Select green “read now” button in top task bar. Safe experiment as indicated by the software.

Graphical user interface

Description automatically generated

The software will now automatically read all selected wells in selected settings.