Progress report for project "Engineered surface platform for immobilization of microorganisms". June – August 2020 (Months 22 – 24).

Since the COVID-19-related restrictions have been softened it became possible for the project team members to continue their work at a more or less regular pace. This allowed to perform Fourier-transform infrared (FTIR) spectroscopy and photoelectron emission (PE) spectroscopy measurements on UV-exposed and non-exposed satellite samples and immobilization platforms from the second batch. Microbiological analysis of the attached cells was also possible. Additionally, it was decided to add silica-based glass as another type of unpatterned surface to see how surface charge and surface roughness would affect the attachment of S. cerevisiae cells. Thus, FTIR and PE measurements were performed on them as well. Summertime being what it is, there was no additional input from the students (aside from those employed within the project). Some of the project staff were also on scheduled leave during the summer months.

PE measurements for satellite samples from the second batch no longer exhibit the oscillating pattern of work function values in response to longer UV exposition times, but rather show an increase after 30 minutes and a slight increase after 60 minutes. A similar trend was noticed for all measured immobilization platforms from the second batch. Glass on the other hand exhibited a pattern of work function change similar to that of satellite samples in the first batch.

FTIR measurements of second-batch satellite samples (100-micron thick amorphous SiO2 layer thermally grown on a silicon wafer) show no difference in IR absorbance when compared to the satellite samples from the first batch. FTIR spectra for glass (non-exposed, 30-minute UV exposure and 60-minute UV exposure) also show no significant change to chemical structure. Representative IR absorbance spectra for glass are given in Figure 1.
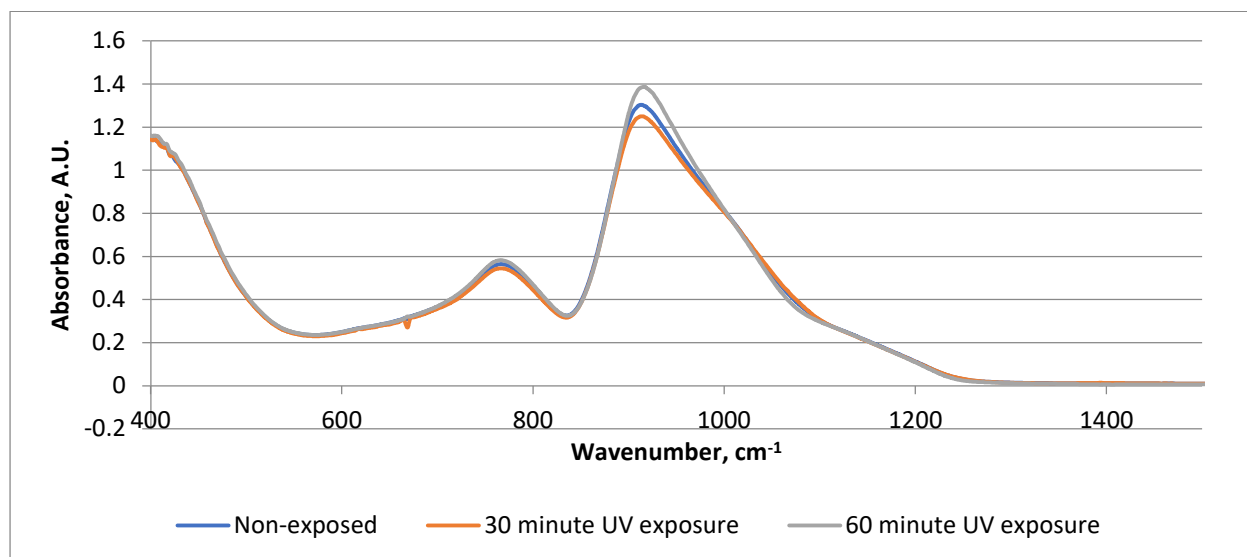

Figure 1. IR absorbance spectra for UV-exposed and non-exposed glass.

Cell deposition experiments were performed on UV-exposed and non-exposed glass and satellite samples from the second batch of immobilization platforms using the approach described in one of the previous project reports. Cell retention was negligible in all cases.

As was mentioned in the previous progress report, the project team planned to develop two pieces of software: one that packages the previously developed cell area estimation algorithm (Algorithm #2) into a user-friendly application that can be used on any computer running on Windows, and the other that allows the user to operate the motorized stage from a Windows-based PC which can be remotely controlled via TeamViewer, Chrome Remote Desktop or other remote access/remote control software. Both applications were developed using MATLAB App Designer.

The application for cell area estimation basically serves as a handy wrapper for letting the user to input all the data necessary for using Algorithm #2. The user needs to specify the difference mask and the binary mask images that are used to register the image and separate the areas into regions that correspond to the tops and the bottoms of the micropatterned surfaces, the registration/segmentation variable values, and the locations of image output/input folders. Figures 2 and 3 show the UI of the developed app.
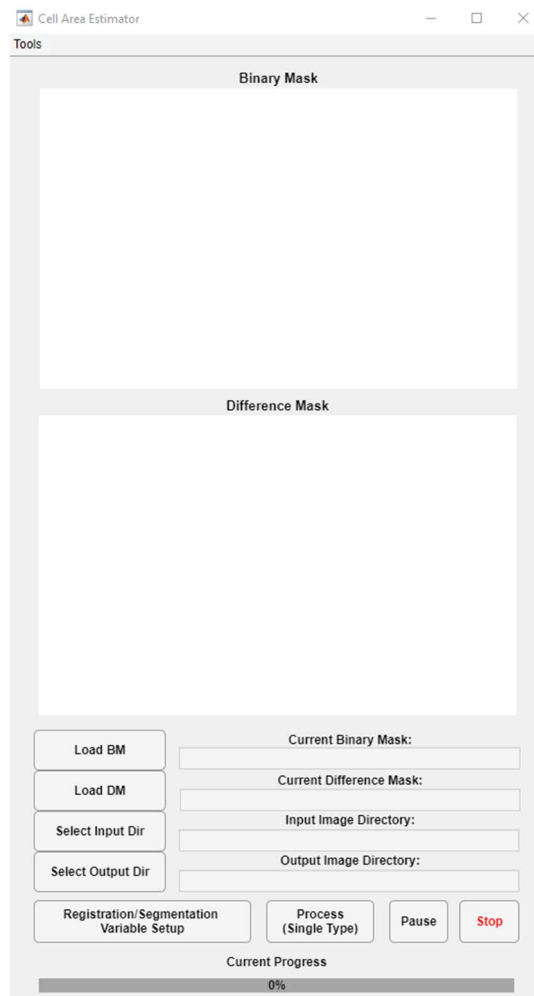

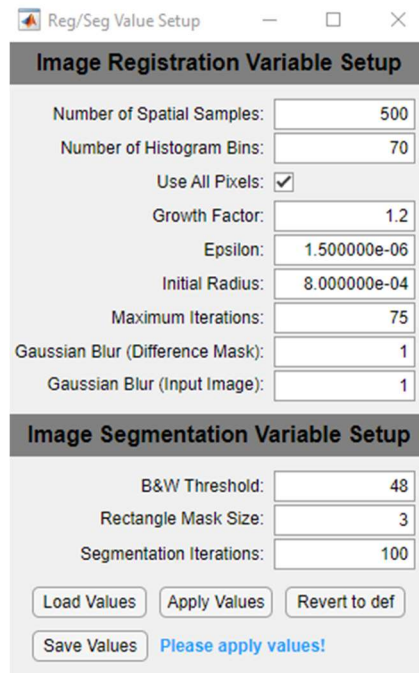**Figure 2.** Cell area estimator app main window.

Figure 3. Image registration/segmentation variable input window.

The application for controlling the movement of the motorized microscope stage was designed to communicate with the Arduino-based application that drives the motors of the Zeiss Jena NU-2 microscope stage and the digital camera mounted onto the microscope viewport. The application consists of three windows: the main window (seen in Figure 4) allows to the operator to manually move the stage in the XY plane, view and acquire an image of a sample, adjust the focus of the image, select the folder for saving the image, and change the name of an image to be acquired.
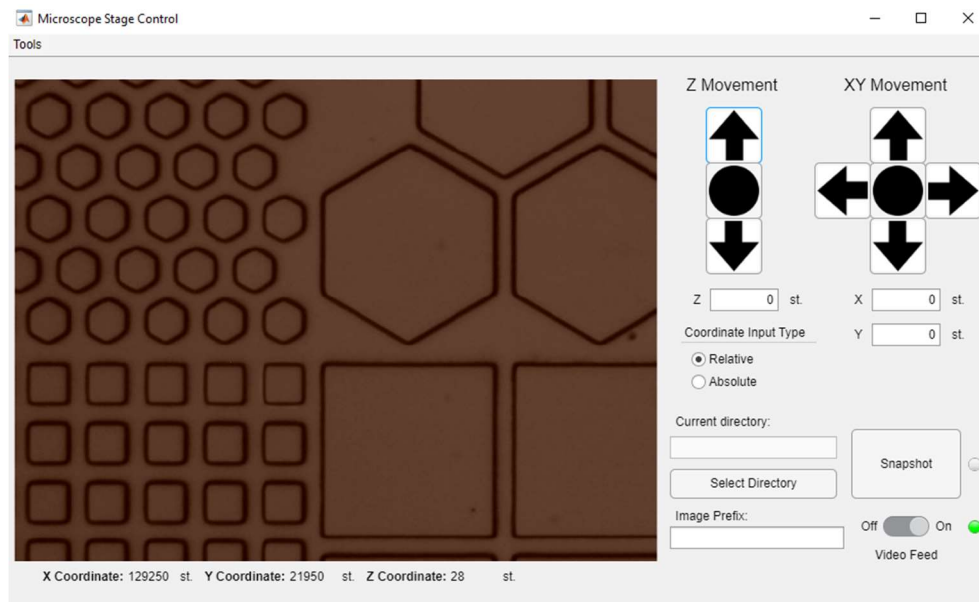

Figure 4. Main window of the microscope stage control app with the surface of a second-batch immobilization platform seen in the image section.

The second window (seen in Figure 5) allows the operator to produce a table of coordinates for performing serial imaging. The user needs to specify three points in the XY plane and their focus height value along the Z axis as well as the width and length of an imaging step in the XY plane and focus

correction step along the Z axis. Given these values, the app calculates a new focusing plane to keep the focus distance constant in case the sample surface is skewed and then generates a table populated with values of X, Y and Z coordinates of locations where imaging will be performed. This table is then imported into the serial imaging window (seen in Figure 6) which uses the coordinates to perform automated serial imaging of the sample surface.
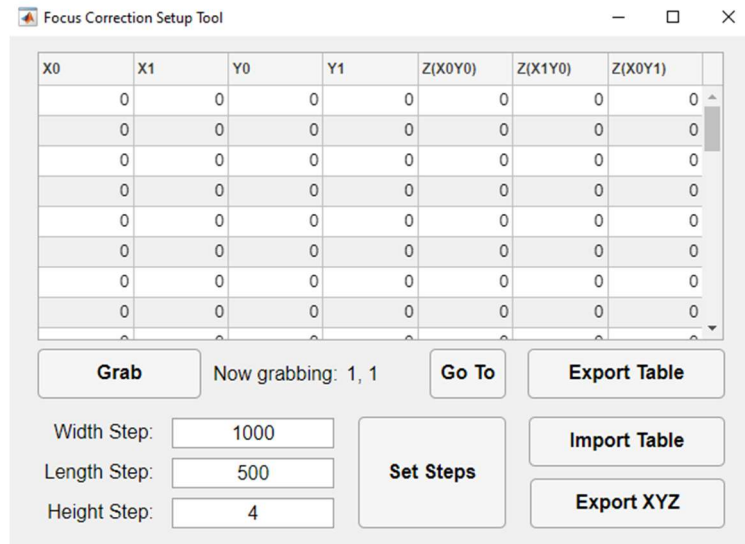

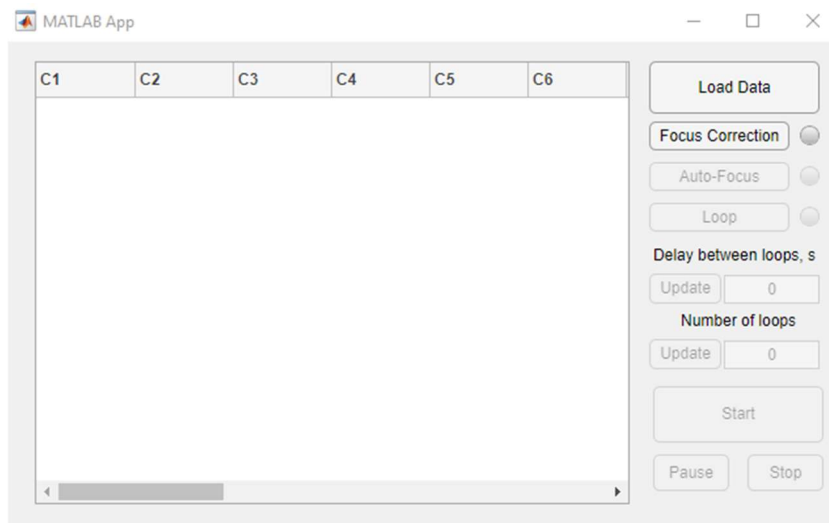**Figure 5.** Focus correction setup tool window.

Figure 6. Serial imaging tool window.

Test runs of cell metabolism studies were performed on several immobilization platforms from the second batch. Team members tried out fluorescent microcopy and confocal microscopy analysis approaches to ascertain the possibility of performing lipid composition analysis, live/dead stain analysis and mitochondrial analysis of the attached cells. It was found that for pyramid—like structure fluorescent microscopy is problematic due to the way light is reflected from the concave surfaces of microscale structure sides. Confocal microscopy gives much clearer images.