DropLLet Counter

Opening words

DropLLet Counter is a simple Matlab application for calculating the frequency of droplets on chip from a video captured by high-speed camera. The application was built under Matlab 2021b version and updated in Matlab 2022b version.

Motivation

Droplet-based microfluidic is a powerful and high throughput method suitable for generating uniform droplets. Droplets can be generated as a primary emulsion of aqueous phases in the immiscible continuous flow (typically oils or organic solvents, e.g., octanol). These can be even further used to generate double emulsions, where the droplets are dispersed in the aqueous phase. Droplets can be used as "isolated" containers for the reaction mixture. In combination with suitable assay and proper detection techniques, droplets can be used for screening enzyme activities or measuring various characteristics of the proteins, such as solubility or thermal stability.

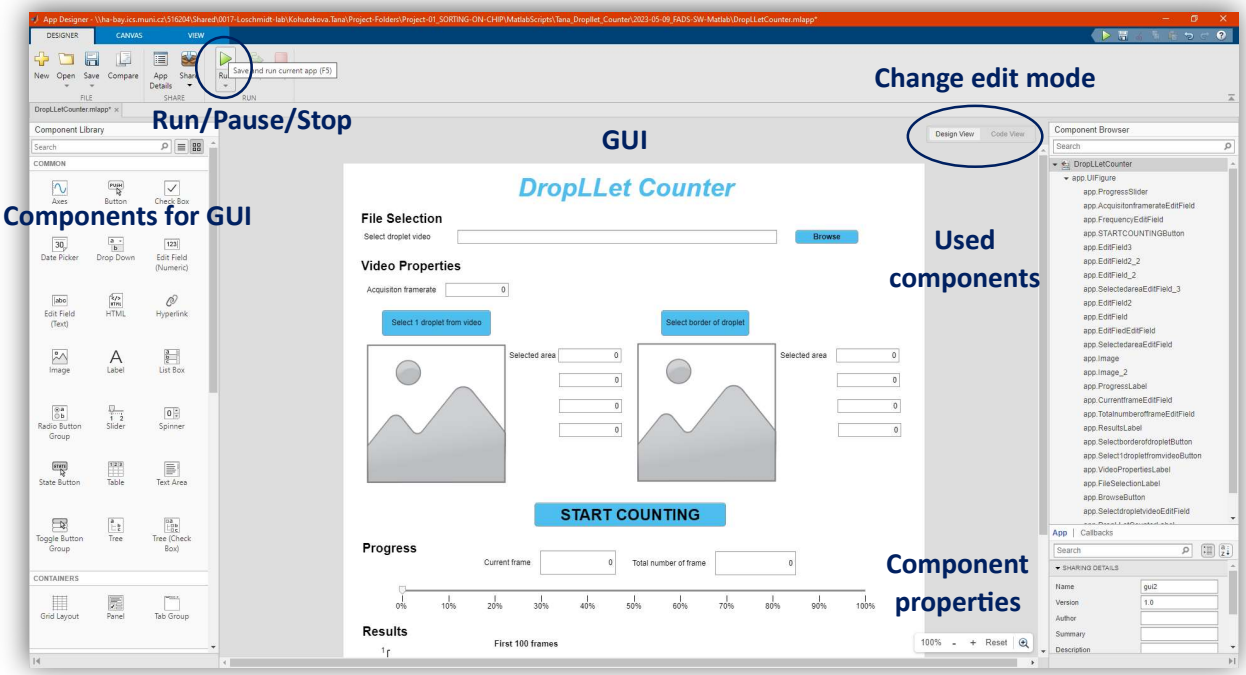
Several microfluidic chip designs are used to generate primary or double emulsions. The generation frequency usually varies between 1 – 25 kHz and mainly depends on the viscosity and flowrates of the used liquids. Thus, we need to monitor an exact generation frequency to ensure the final number of generated droplets applied in the following analysis will be high enough. Suppose the protein library of 10^8 variants is expressed in the *E. coli* cells. Regarding the Poisson distribution (λ=0.1) of the encapsulated cells, where only each 10^{th} droplet contains a bacterial cell, the number of the necessary droplets is at least 10^9 but usually needs to be higher. The proper knowledge of the frequency of generated droplets is then helpful for estimating the required time to generate all necessary droplets for the experiment.

Droplet Counter is a simple MATLAB application for calculating the frequency of droplets on a chip from a video captured by a high-speed camera. MATLAB 2021b or higher version is required for the running.

The program can process videos only in .avi or .mp4 format. It opens and analyses only one single file. The video framerate at which the video was captured is a basic prerequisite knowledge. Due to the variability of microfluidic chip designs, users can select a Region Of Interest containing only one droplet. As the program could also be used for the analysis of double emulsions, the user needs to select the droplet border as well. When the droplet counting is started, each frame is separately loaded, converted to a grayscale, and then the contrast is adjusted. Subsequently, the brightness of all pixels within the ROI is summed up. For each frame, the sum of bright pixels is stored – obtained data are considered signal. The logic of the analysis is as follows – as the droplet comes to the ROI, the image's brightness decreases since the border is darker, and as the droplet leaves the area, brightness rises. Due to the variability of captured and loaded video sequences, the analysis progress is displayed on the slider as a percentage progress and the number of currently processed images.

Start the app in App Designer

Open *DropLLetCounter.mlapp* via Matlab – App Designer window will open. This window allows user to modify the GUI (in Design View) or the functionality of components (in Code View). The app can be run via green Run button.



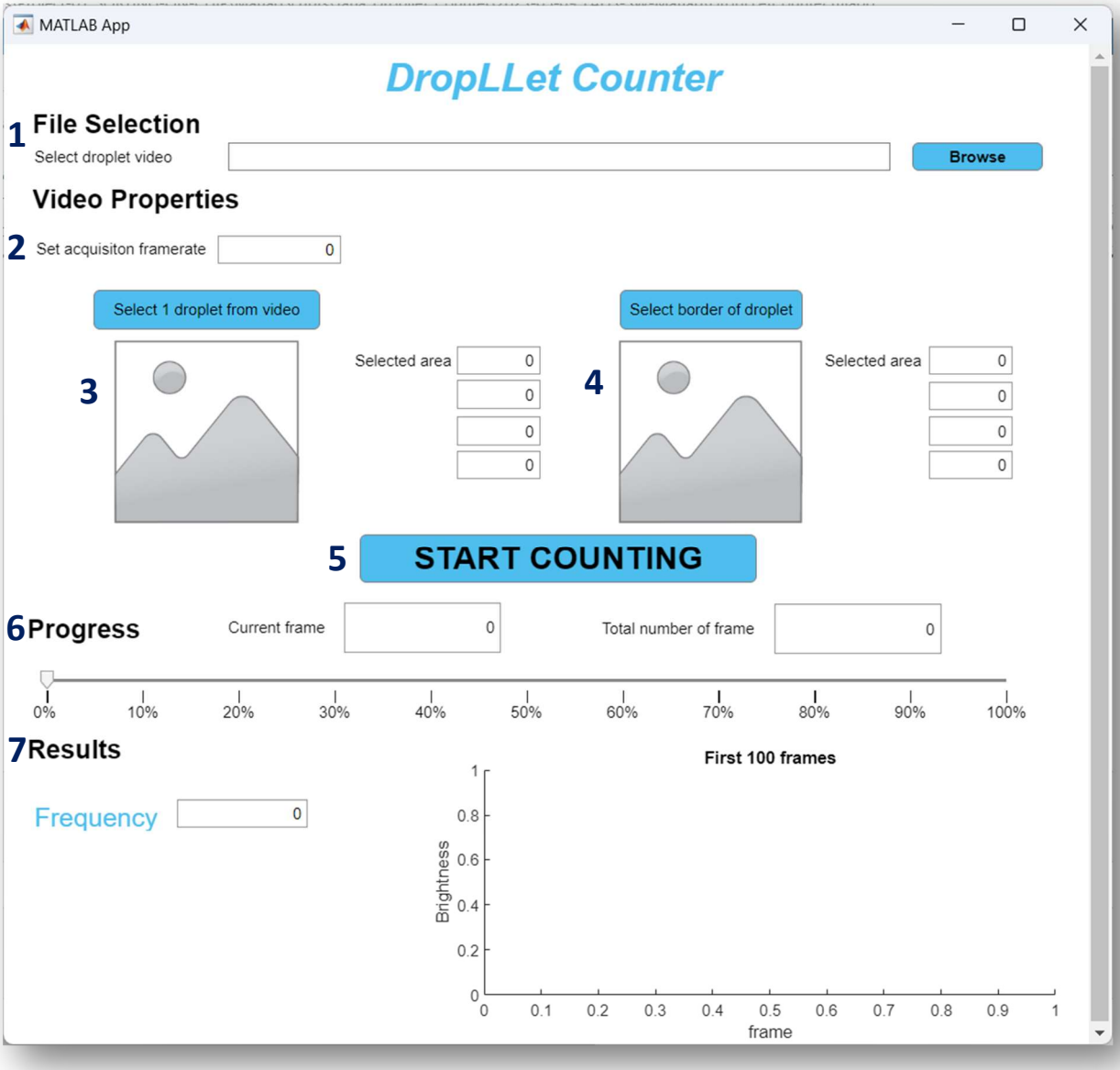
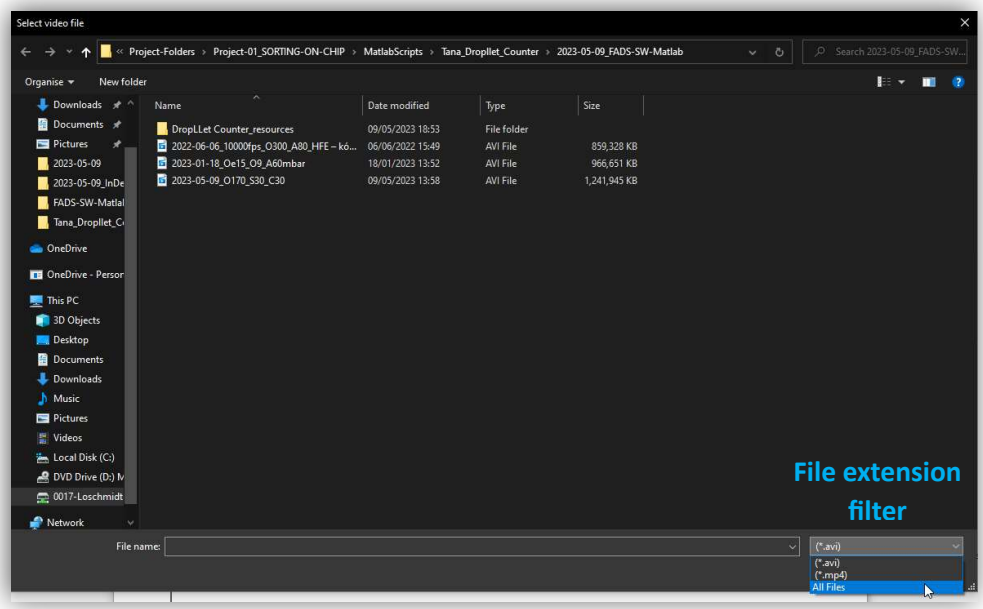


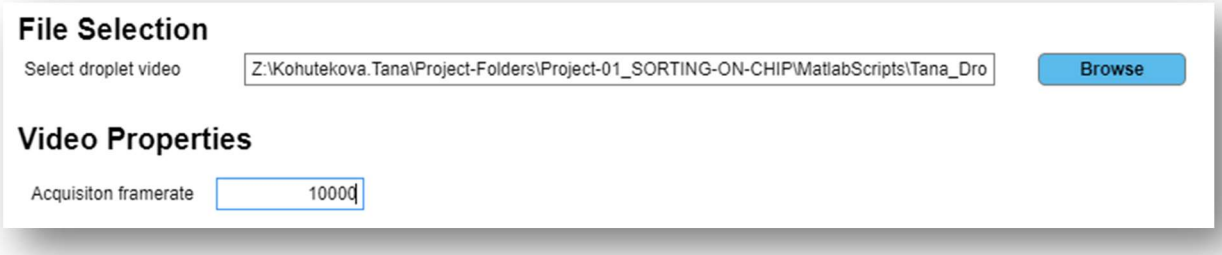
Figure 1: Graphical User Interface of the Droplet Counter. A video in the .avi, or .mp4 format needs to be chosen (1), the user needs to define the acquisition framerate (2), select ROI with one single droplet (3) and its border (4). Once the analysis is started (5) the progress can be seen during the analysis, of which resulted Frequency is shown in the Hertz (7).

1. **File selection**
   - Click Browse button – a new Windows Explorer window will open
   - Program can process videos only in .avi or .mp4 format, to show all files with format change the file extension filter in lower right corner
   - Choose only 1 video file
   - Click Open
   - The path to the file will be displayed in corresponding box newt to Browse button
   - It is also possible to write down the path to the box manually



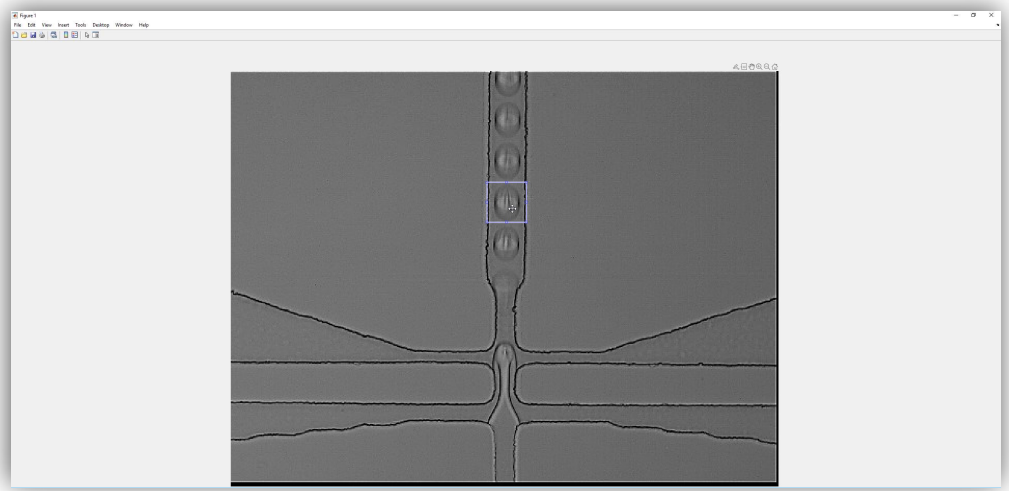
2. Video Properties

- Enter the framerate used for video capturing manually

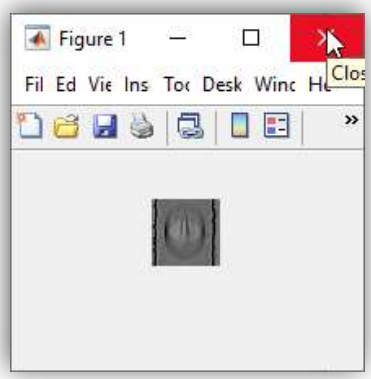


3. Select 1 droplet from the video

- Click the Select 1 droplet from the video button
- A new window with image from 1st frame will open
- Choose the region containing only one droplet
- Save selection by double clicking within the selected region

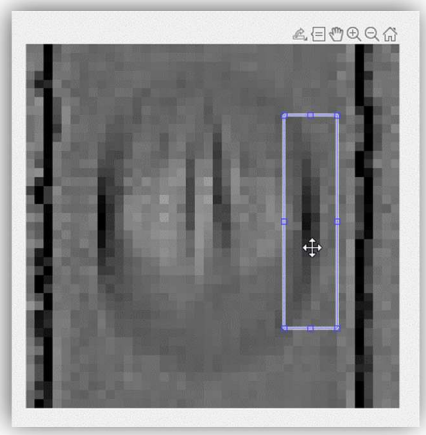


- Coordinates for the image selection will be saved into boxes next to the displayed image
- Another window displaying only the selected region will open – make sure to close it



4. Select border of droplet

- Click Select border of droplet button
- A new window with chosen droplet from 1st frame will open
- For better visibility maximise the window
- Try to choose the narrowest area containing border of droplet
- Select the border that is parallel to the flow of the droplets
- Save selection by double clicking within the selected region



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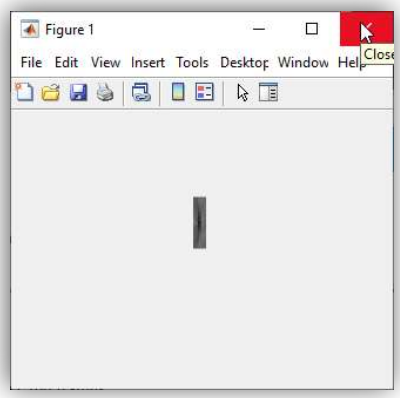
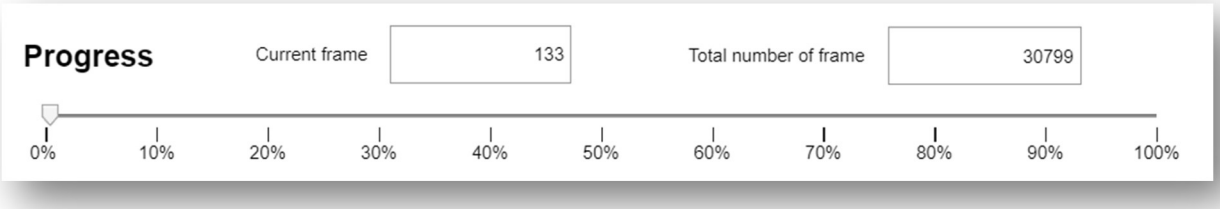
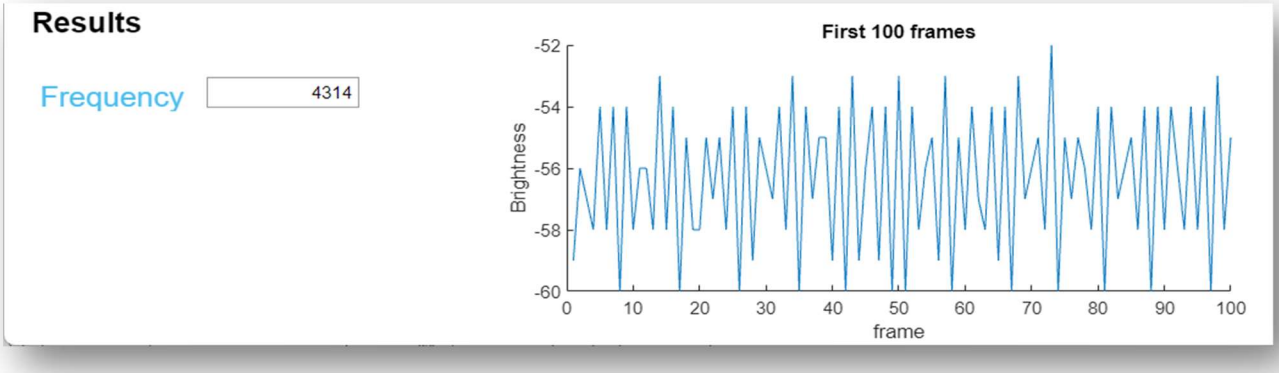


5. **Start counting**
   - Click Start Counting button
   - Make sure that the framerate is not set to default value

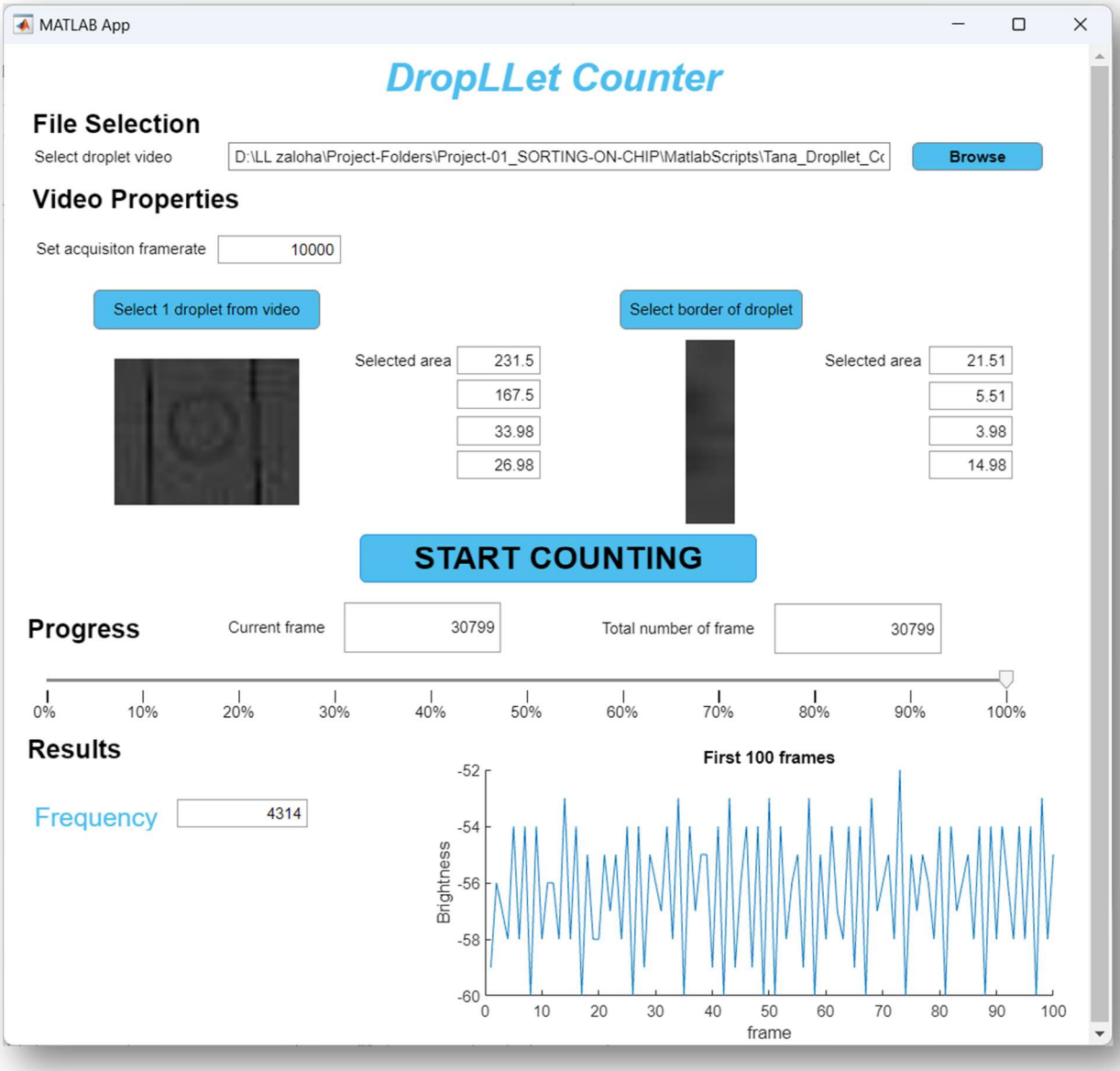
6. **Progress**
   - Each frame is separately loaded, converted to grayscale, then the contrast is adjusted
   - Subsequently, the brightness of all pixels within the ROI is summed
   - For each frame the sum of brightness is stored – obtained data are considered as signal
   - The idea: as the droplet comes to the ROI the brightness of image decreases, since the border is darker, as the droplet leaves the area, brightness rises
   - As the process takes some time, progress is displayed as the number of currently processed image and percentage progress on slider



7. **Results**
   - Obtained signal is then negated so each peak in signal now represents a droplet in ROI
   - Peaks in signal are detected and the total amount of detected peaks divided by duration of the video (calculated as total number of framerates / acquisition framerate)
   - Signal obtained by processing first 100 frames and the final calculated frequency are displayed

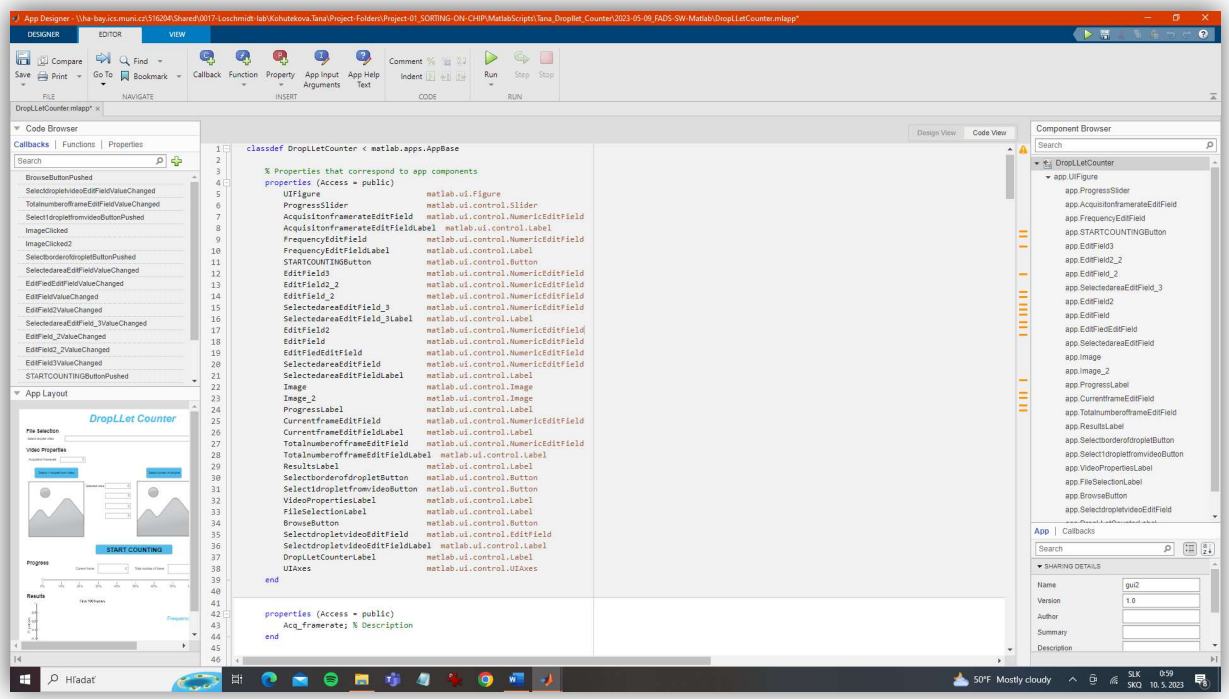


8. Final GUI after counting



9. Code View

- It is possible to change or add another functionality into program in Code View in App Designer



- To change the appearance of components, adjust component properties in Design View in lower right corner (for example ticks in slider, background colour of buttons)
- To change the function, add callback by clicking the left mouse button and moving to the callback section
- Click Go to function callback (Go to YourButtonAndAction callback) – the program will open the Code View on the corresponding row