How to use Dark Frame Subtraction in Anitoa ULVision™

When the light emission signal from a sample is very low, we would use long integration time to capture the image and detect the weak signal. Dark Subtraction is the method to eliminate the background noise of the sensor and enhance test reproducibility.

The dark subtraction feature in ULVision also has built-in temperature compensation. This will greatly enhance the data consistency across different operation conditions.

Dark Subtraction works the best when the integration time is long. This generally mean that the integration time is a few seconds or more in high gain mode, or 10 seconds or more in low gain mode.

Figure 1 is the GUI features for dark subtraction: there is a check box to enable dark subtraction, and a button to save the current frame as the dark frame.
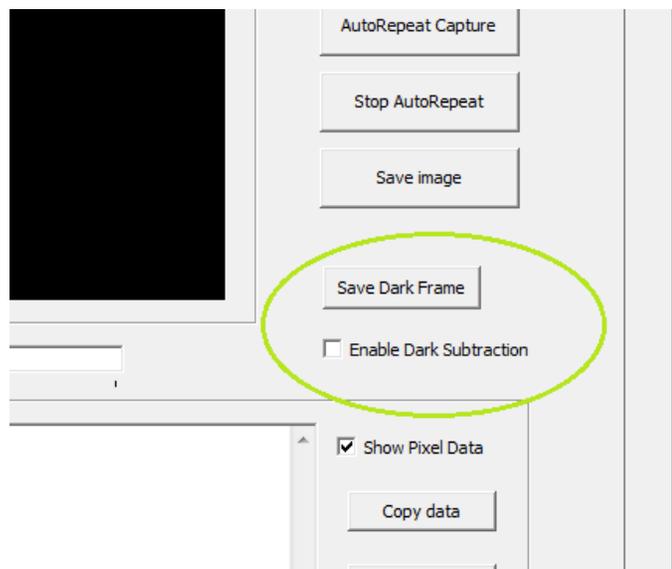

Figure 1. GUI items to manage Dark Subtraction

Below are steps to perform automatic dark subtraction:

Step 1. Collect the dark frame. First let's enable temperature capturing so we could perform temperature compensation when using dark subtraction (see Figure 2). With the automatic dark subtraction feature disabled, place the sensor in a dark environment and capture the image. Typically in application, the sensor is placed in a light-tight box with the samples. To collect dark frame, we typically do not add samples that may be emitting lights. For example, we can inject pure water into the sample chamber or channel, or just simply leave them empty. In the case of a fluorescence set up, we do not turn on the excitation light at this time.
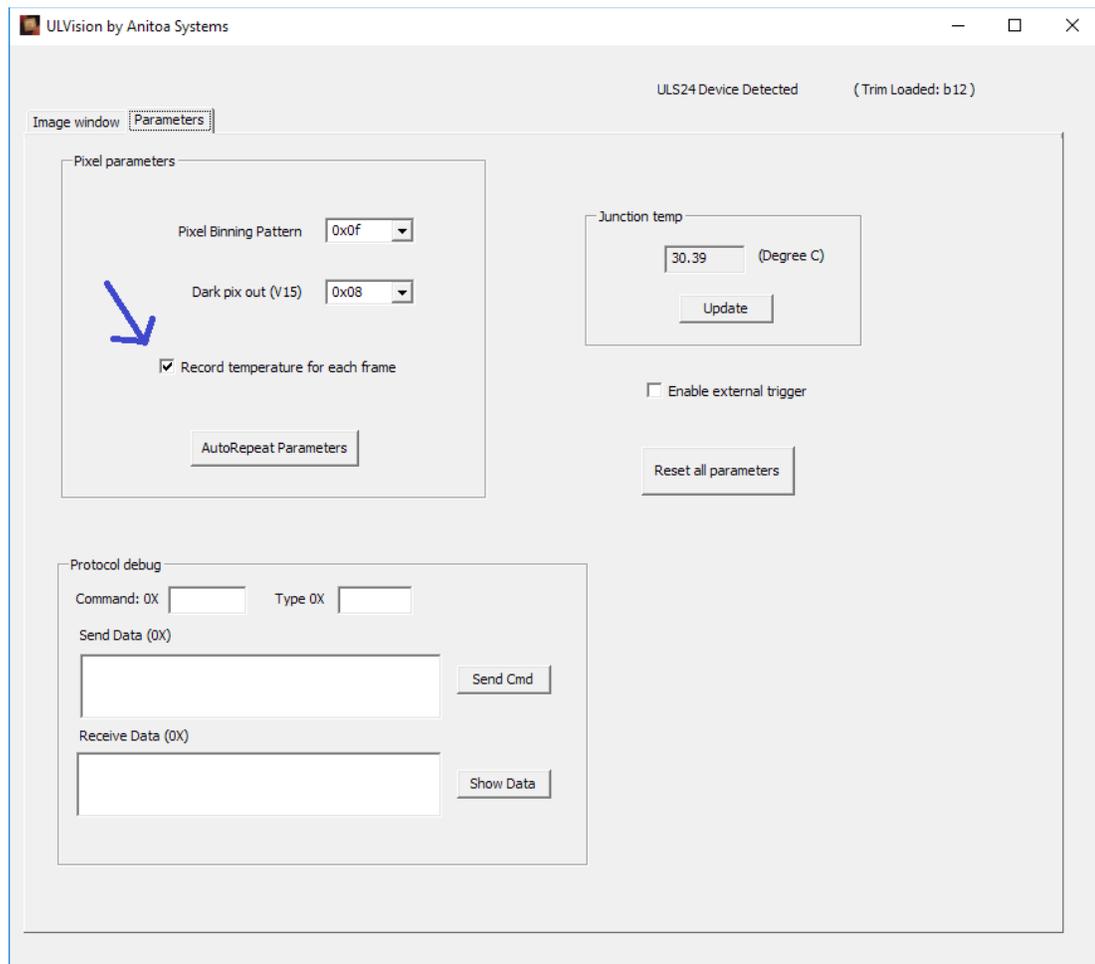

Figure 2. Select "Record temperature for each frame".

(Option: To benefit from the dark subtraction feature, the quality of the dark frame is very important. To improve the quality of the dark frame, we could use "AutoRepeat Capture" to capture multiple dark frames and take the average. When we use the AutoRepeat Capture mode in ULVision, the average is automatically calculated.)

Although it is not required that the dark frame be taken at the same integration time as the normal frame, it is still best if we use the same integration time (or longer) for dark frame. Needless to say, all other parameters, for example gain mode, sensor resolution and binning mode should be the same for dark frames and normal frames. (See Figure 3)
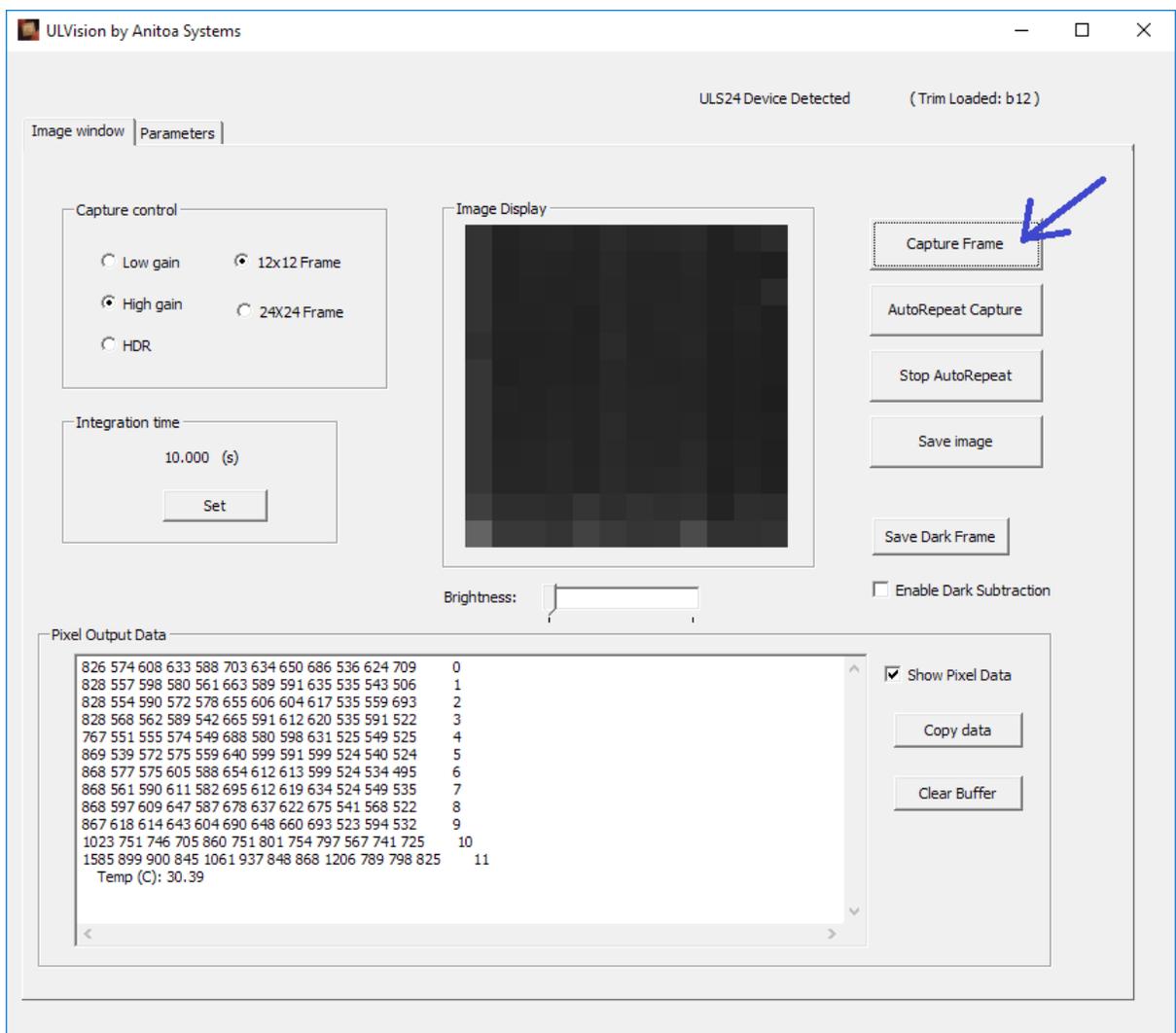

Figure 3. Capture the dark frame with the "Enable Dark Subtraction" feature turned off.

Step 2. Save dark frame. Once the dark frame is taken. We should save it and use it for dark subtraction. To do this, we first enable dark subtraction by check the "Enable dark subtraction" check box. We then click the button "Save Dark Frame". At this time, the dark frame is saved and dark subtraction is enabled.
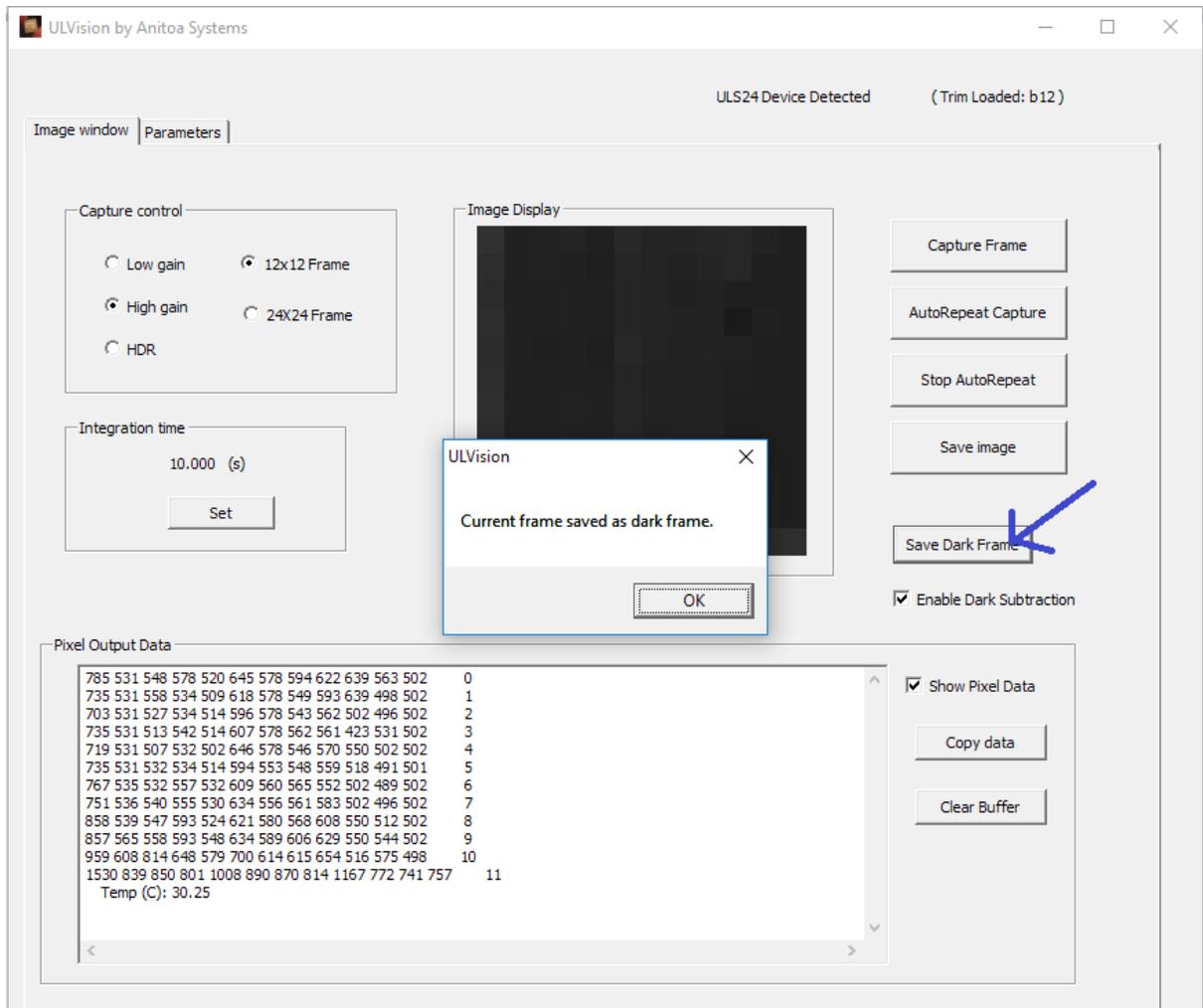

Figure 4. After taking dark frame, enable dark subtraction, then save the current frame as dark frame.

Step 3. Test dark subtraction. With the dark subtraction enabled and dark frame saved, we can now take normal frames and have the software automatically subtract the dark frame. To test this, we can keep the sensor still in a dark environment and take a normal frame. We should see a good quality dark image. If we observe the pixel data, they should all be around 100 or so. (Figure 5)
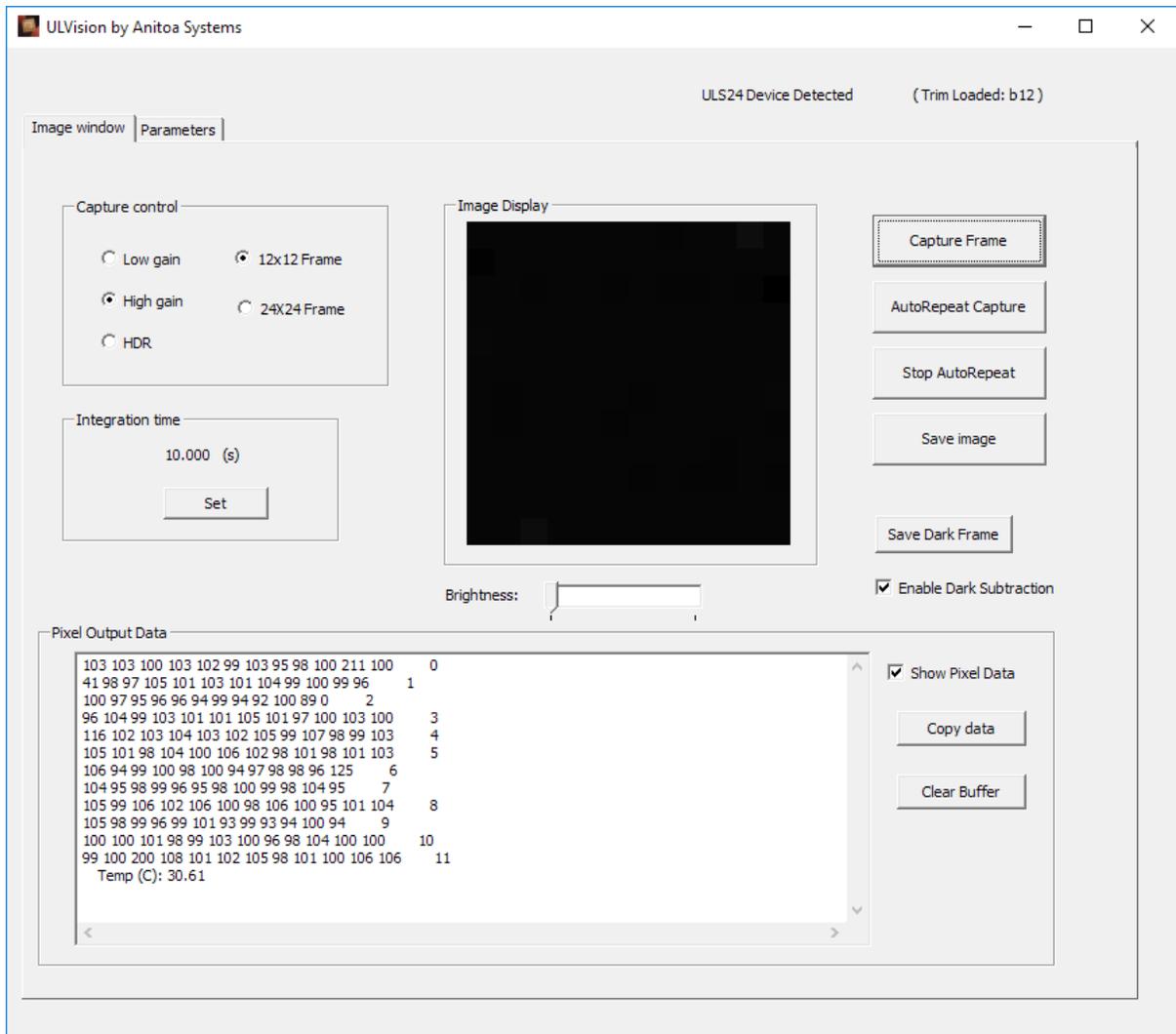

Figure 5. With Dark frame saved and dark subtraction enabled, test taking an image. Note the output is dark and even now (the numerical background level is 100).

Step 4. Take normal images. Now we can start take normal images of our sample.