

SOP for DigiEye Calibration

High-Throughput Phenotyping Protocols (HTPP), WP3

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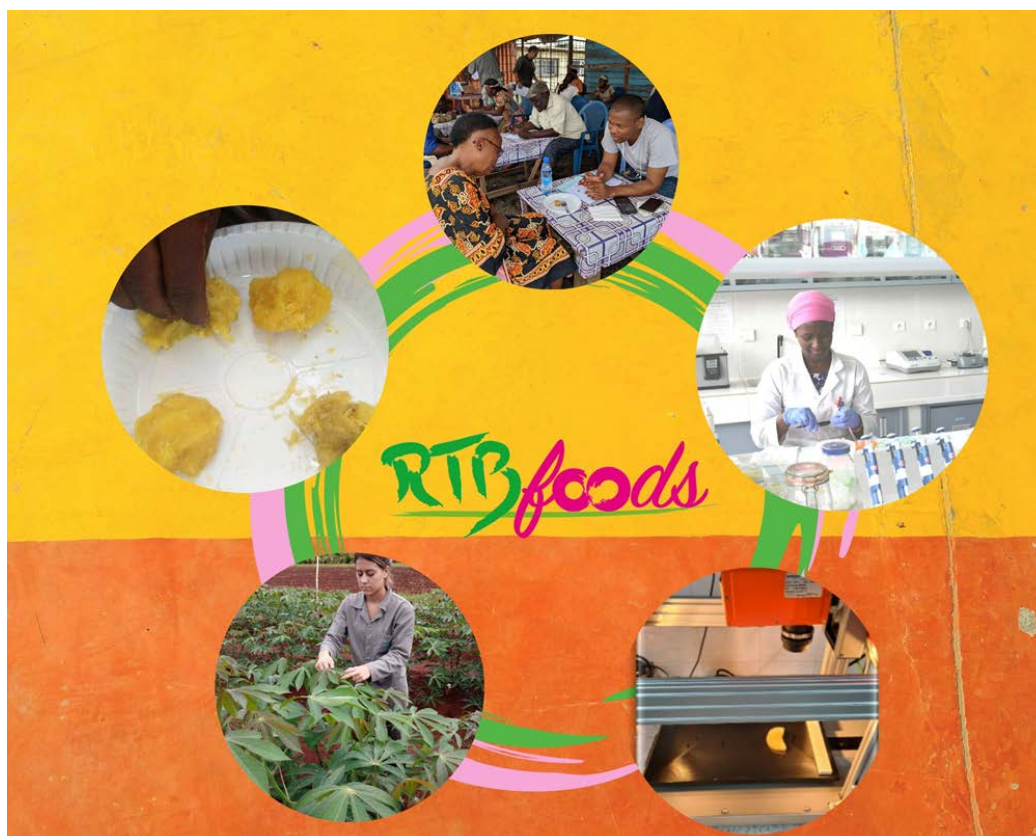
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ABSTRACT

A variety of imaging sensors are currently used in research and commercial practices to quantify complex crop traits for breeders. Imaging technology is a fast, non-destructive high-throughput phenotyping tool that has been used widely for accurate acquisition of crop traits on a large scale. The DigiEye is a computer-controlled digital camera system for measuring colour and capturing high-quality repeatable images. The SOP highlights the procedure of calibrating the DigiEye, for accuracy and consistence of results.

Key words: image analysis, DigiEye, set-up, calibration, high-throughput phenotyping tool
Type of document

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1 SCOPE AND APPLICATION

This SOP describes the steps taken to start up and calibrate the DigiEye imaging system in order to capture high resolution images of sweetpotato roots and potato tubers.

2 REFERENCES

DigiProduction 3.1 User Guide Incorporating DigiPix Lite, DigiGrading & Light Fastness, Stain Analysis & Index Mapping-Version 3.1 (rev a.1.6 September 2020) pp 7, pp 10, and pp 12-23.

3 DEFINITIONS

DigiProduction is a 64 bit modular computer controlled digital imaging system for measuring colour and capturing high quality repeatable images. DigiProduction (DigiEye), furthermore, can display not only colours but also surface details (e.g. texture, gloss, surface characteristics). Captured images can be coloured to specified standard shades to simulate final products. Standard data output formats allow data transfer into compatible commercial QC, recipe formulation, and colour communication systems.

4 EQUIPMENT

The current DigiProduction - DigiGrade system (**Figure 1** below) consists of the following components:

- 1) **An illumination cabinet** that uniformly illuminates objects for capturing repeatable images. The cabinet contains a combination of fluorescent D65 illuminant and additive LEDs to allow the production of a calibrated D65 simulator against ISO 23603:2005.
- 2) **A digital camera** allows previewing and capturing of the image of the sample and enables targeting of selected areas. DigiProduction can use a Nikon D90, Nikon D7000 or Nikon D7500.
- 3) **Desktop personal computer** running Windows operating system with a colour monitor.

DigiProduction software's main functions are: -

Camera Calibration: a procedure of standardising the camera response by relating its RGB signals to CIE specifications, **Monitor Calibration:** ensuring stable monitor performance over the time and **DigiGrade fastness assessments:** evaluating change of shade, staining, rubbing and light fastness for testing textile materials.

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Figure 1: DigiEye Imaging system; illumination cabinet, digital camera and personal computer.

5 DIGIEYE CALIBRATION PROCEDURE

Before starting to use the DigiProduction system, several steps must be taken to ensure that the system is launched properly and calibrated before any images can be captured. These steps taken are described below:

Step 1: Ensure the DigiEye USB dongle is plugged into the PC (**Figure 2** below)




Figure 2: DigiEye USB dongle plugged into PC.

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Step 2: Ensure that the DigiEye machine is plugged into a power source and then switch on the DigiEye by pressing the switch located at the back of the machine (**Figure 3** below)



Figure 3: DigiEye switch located at the back of the DigiEye machine.

Step 3: Open the DigiProduction software on the desktop. Select the correct camera. This is done by accessing “**Camera→Current**” at the top of the screen and selecting your camera model from the list provided. Click the **camera icon**  to open the **Camera TABS** and confirm that the aperture is 8 and shutter speed is 1/3 which are the recommended settings. If these settings were already set, proceed to step 4.


Step 4: Turn on the illumination cabinet (**Figure 4**). This is done by pressing the  button on the front of the DigiEye cube’s switch panel (**Figure 5**). Allow the digiEye machine to warm-up for 10 minutes (**Figure 6 and 7**).



Figure 4: Illumination cabinet before the warm-up process.

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Figure 5: Navigating to the Warm-up function on the digieye cube's switch panel.



Figure 6: Warm-up count down from 10 minutes warm up time.

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Figure 7: (L-R) Warm-up countdown till warm-up process is completed, Illumination cabinet after warm-up is complete.

Step 5: To calibrate the DigiEye imaging system, start by clicking the **camera calibration icon**. This opens up a calibration wizard (**Figure 8**).

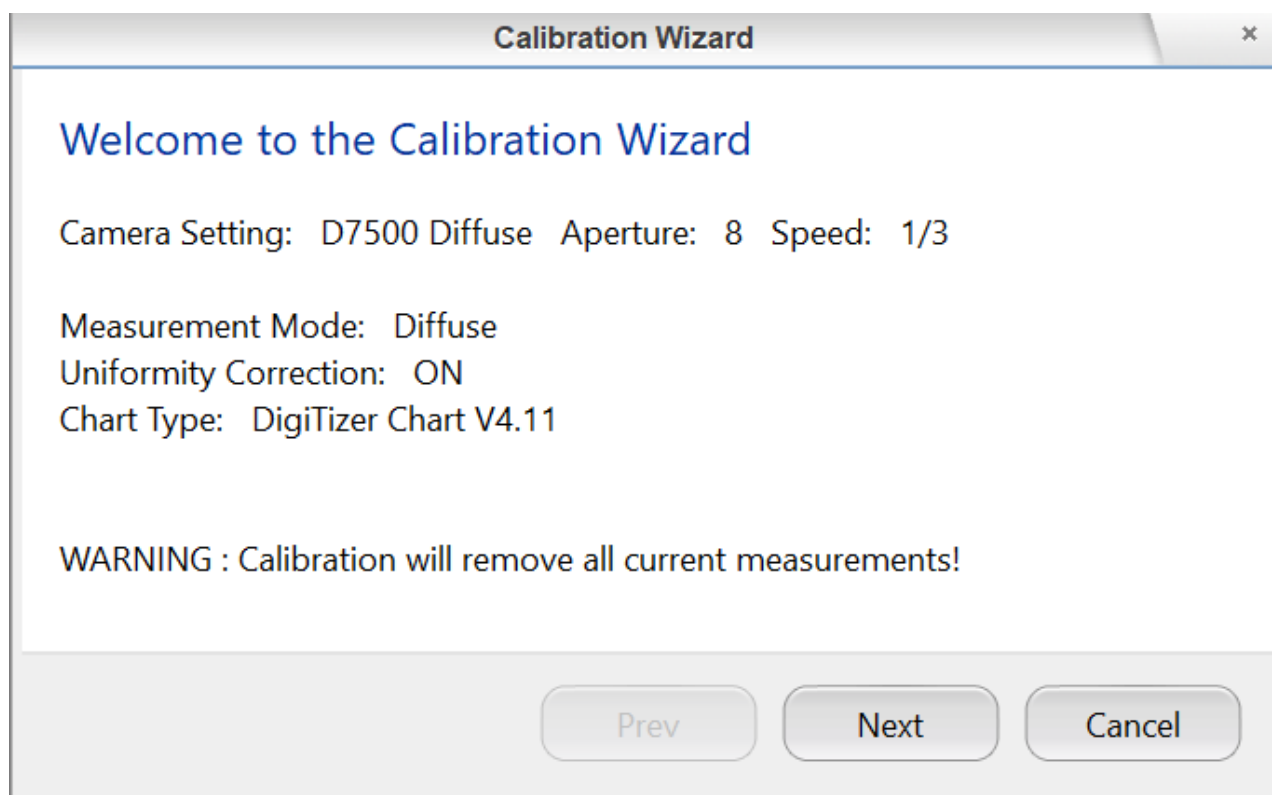


Figure 8: Calibration wizard.

Click Next to move to the next step of calibration.

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Step 6: The calibration wizard describes the first instructions for calibration (**Figure 9**) to be carried out in the stages as they are described.

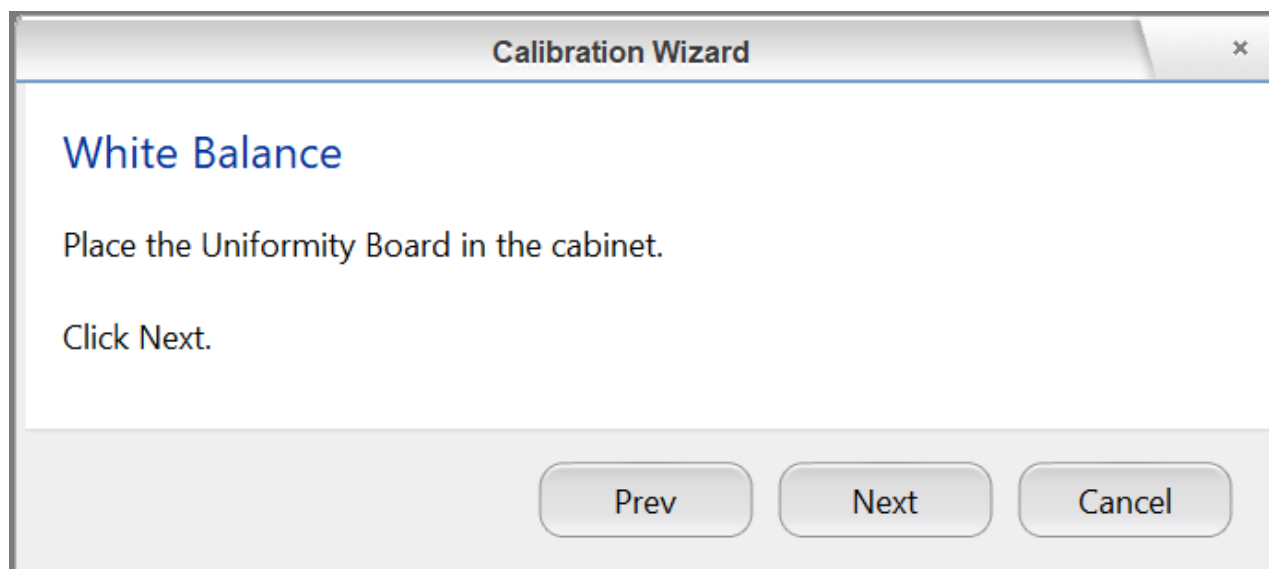


Figure 9: First calibration instructions on calibration wizard.

Step 7: Open the illumination cabinet and place the white uniformity board in the cabinet (**Figure 10**) to perform white balancing as instructed in **Figure 9**.



Figure 10: White uniformity board placed in the illumination cabinet.

Close the illumination cabinet and click Next. This action prompts the DigiEye camera to capture an image of the white board.

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Step 8: Follow the instructions that are stated by the calibration wizard (**Figure 11**).

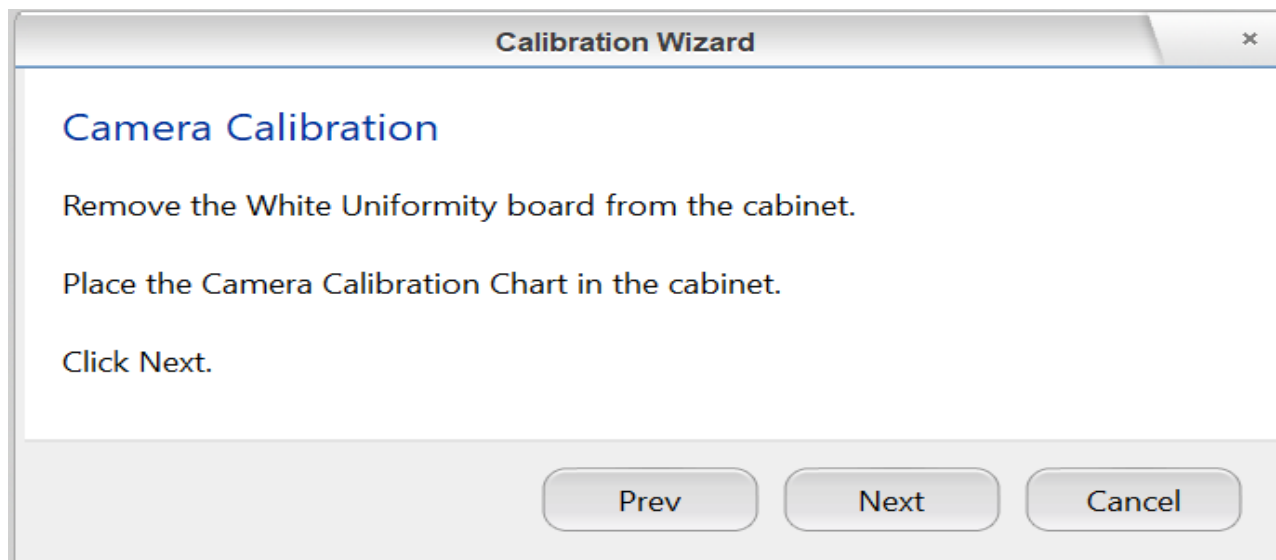


Figure 11: Calibration instructions that appear after an image of the white board has been captured.

Step 9: Open the illumination cabinet, remove the white board and store it away. Place the camera calibration chart (**Figure 12**) in the cabinet.

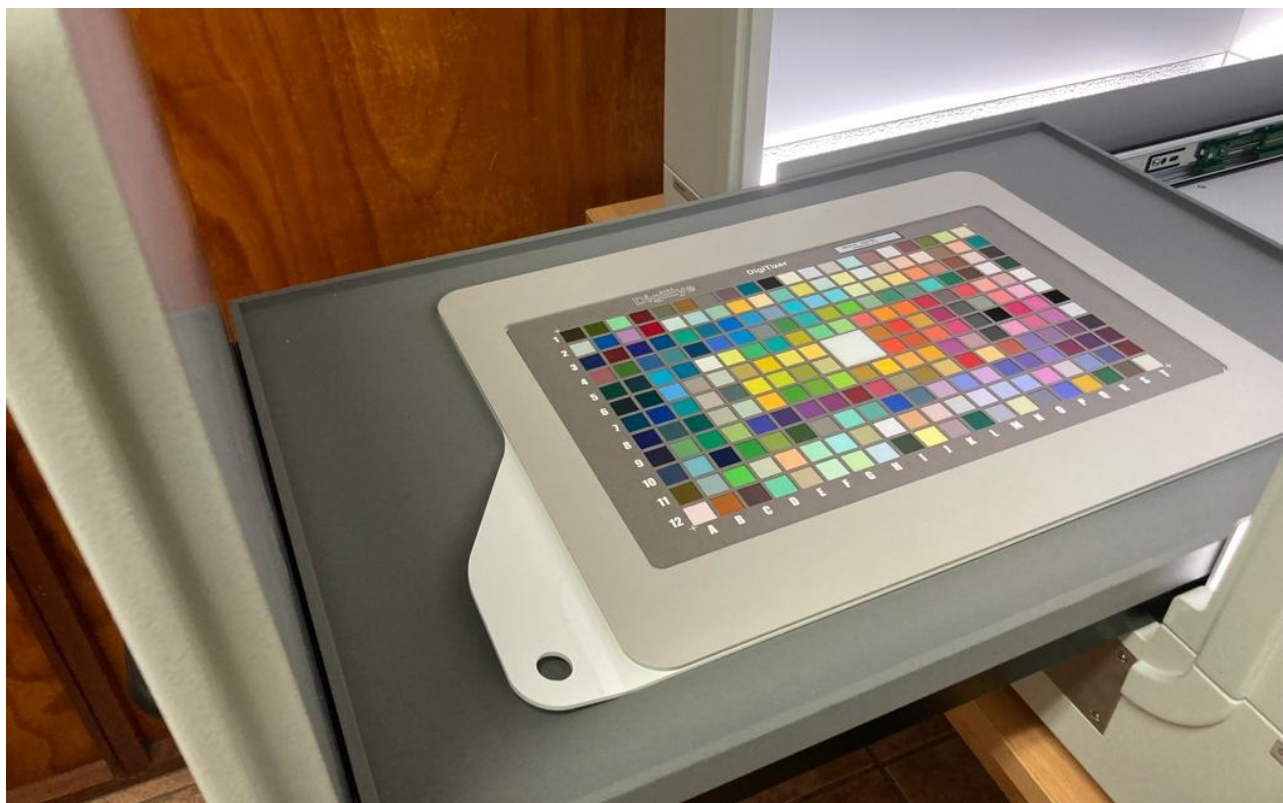


Figure 12: Camera calibration chart placed in the illumination cabinet.

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Step 10: Close the cabinet and click Next. This action prompts the DigiEye camera to capture an image of the camera calibration chart. Open the illumination cabinet and remove the calibration chart as prompted in the instructions (**Figure 13**)

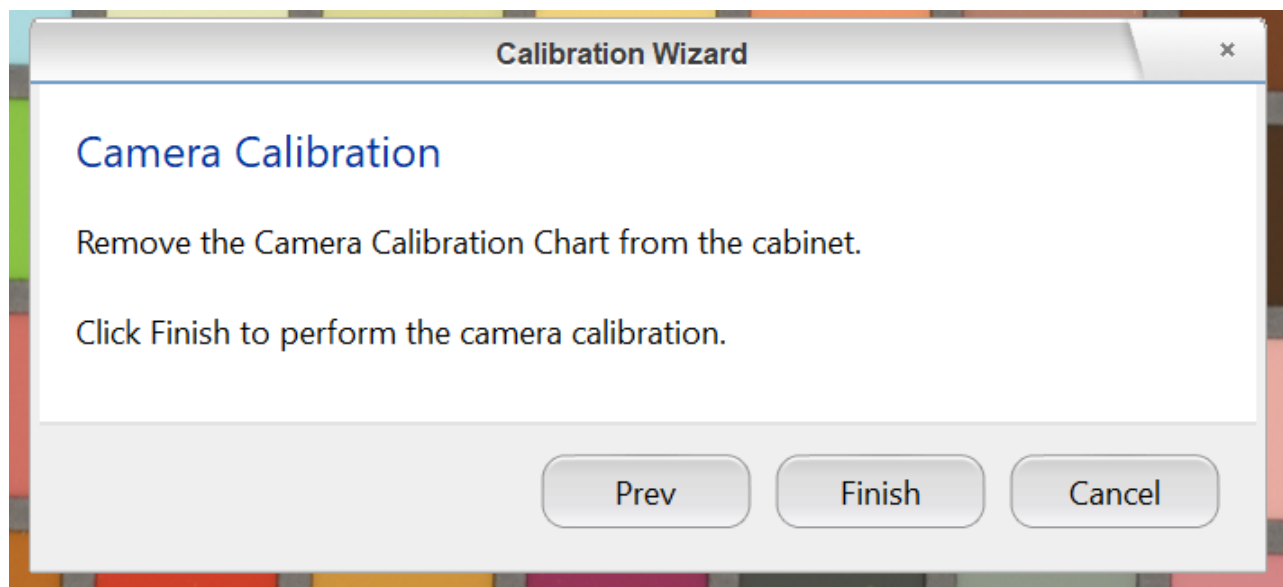


Figure 13: Instructions that appear after an image of the calibration board has been captured.

Step 11: Click the Finish button on the calibration wizard window to perform camera calibration. If the software can locate the target colour patches, the following message will appear:

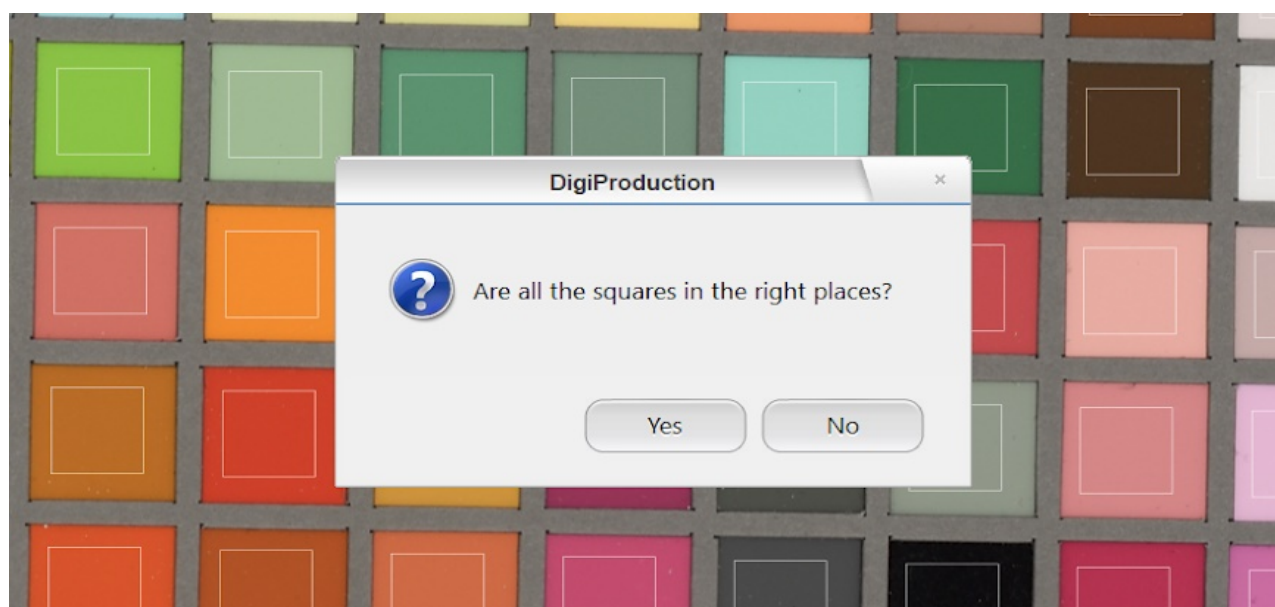


Figure 14: Verification of position of squares for calibration.

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Step 12: Verify that all the squares are in the right places and click Yes (**Figure 14**). The calibration results will appear in the results window (**Figure 15**)

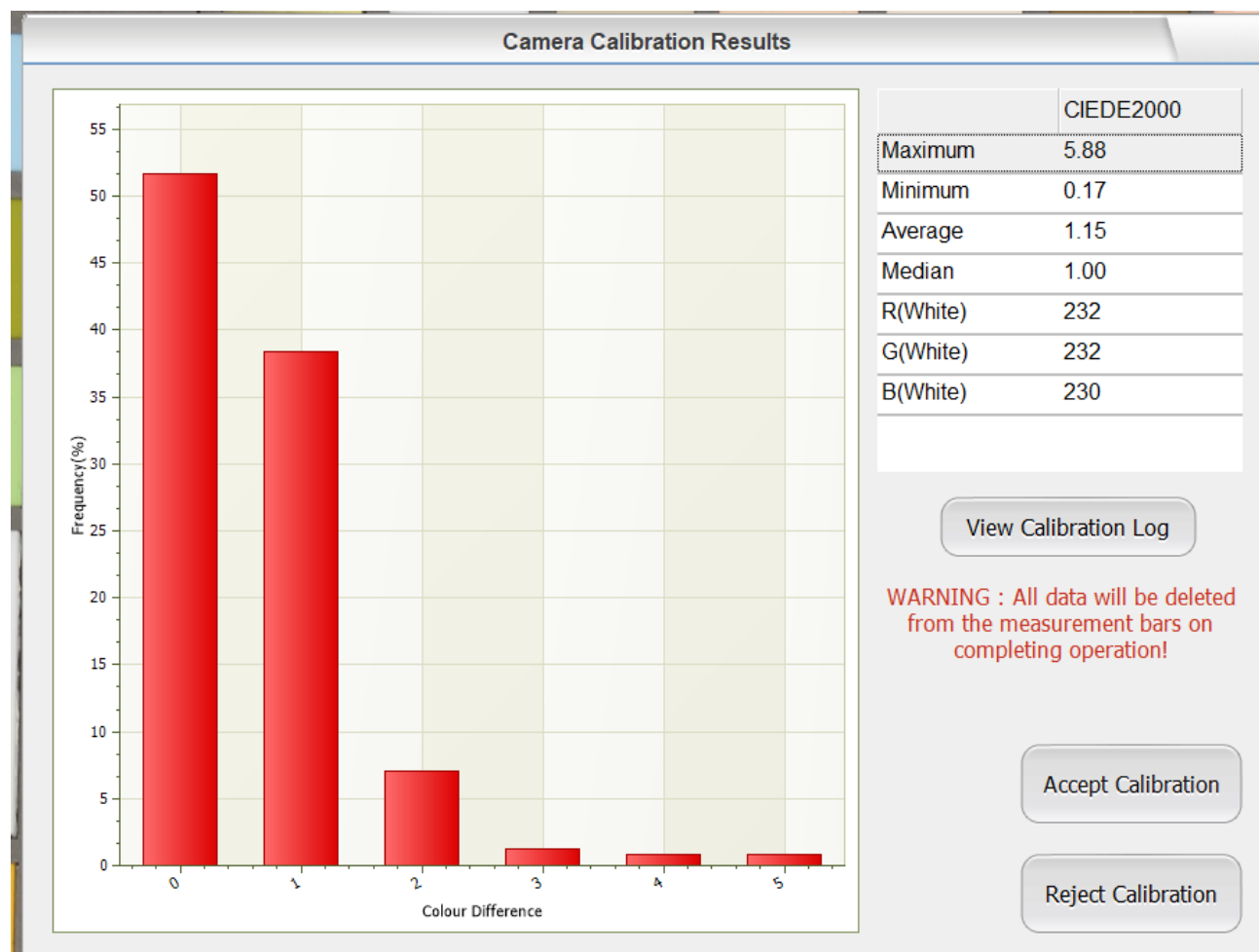


Figure 15: Calibration results window.

Step 13: If the calibration values are acceptable click the Accept Calibration button to complete the calibration. The values should ideally be no more than 229 or less than 205 to be taken as acceptable calibration values. Depending on the camera setup, it may not be possible to get exactly within the range of 205-229. If they fall outside of this range you should adjust the shutter speed, save the camera setting and repeat the calibration procedure to improve the results. Redo the calibration and when you are satisfied with the result values then click the Accept Calibration button.

Once the Accept Calibration button is clicked, the digieye system is calibrated and ready for use.

6 CRITICAL POINTS OR NOTE ON THE PROCEDURE

Some of the critical points to note while carrying out all the procedures described in this SOP include;

1. The DigiEye imaging system should always be calibrated before images of samples are taken as described in [section 5](#)

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2. If you change any camera setting you will need to perform the calibration using the new settings.
3. Each time the camera settings or illumination conditions in the cabinet are changed or after every 4 hours, a re-calibration of the camera is recommended.
4. Calibration Wizard Navigation Buttons (**Figure 8, 9, 11, 13**)
 - a. **Back Button:** The Back button will return to the previous calibration step and ignore or discard the current step
 - b. **Next Button:** The Next button will complete or confirm the current step and move to the next one.
 - c. **Finish Button:** The Finish button complete the calibration process
 - d. **Cancel Button:** This will stop the calibration process without completing a new calibration.



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