

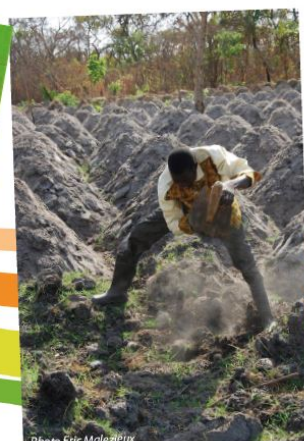
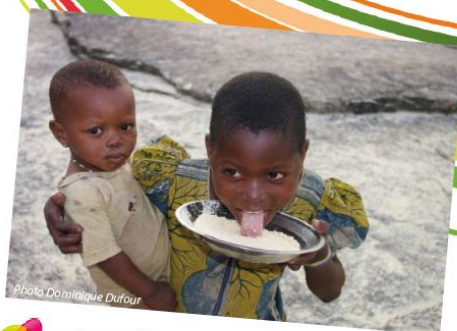
# SOP for Operating Mode and Parameters Configuration of Hyperspectral Camera Specim FX17

High-Throughput Phenotyping Protocols (HTPP), WP3

Montpellier, France, 13/09/2021

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<p align="center"><b>RTBfoods</b></p> <p><b>WP3: High Throughput Phenotyping Protocols</b></p>		
<p align="center"><b>SOP: Operating mode and parameters configuration of hyperspectral camera Specim FX17</b></p>		
<p align="center"><b>Date: 27/08/2021</b></p>	<p align="center"><b>Release: 1</b></p>	
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# ABSTRACT

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This document is a standard operating protocol (SOP) for the monitoring of hyperspectral camera SPECIM FX17. In this SOP, all required steps for the acquisition of hyperspectral images are described, from hyperspectral imaging (HSI) system and operating mode to start the camera and to adjust the configuration of parameters for image acquisition, to the control of instrumental noise and representativeness, repeatability of HSI measurements.

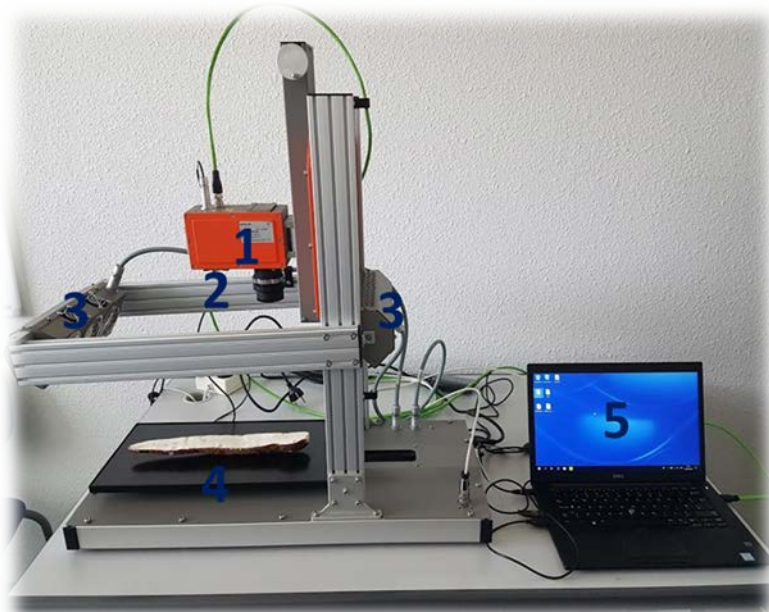
This SOP could be used by RTBfoods project partners as an instruction manual of HSI following a precise protocol for the acquisition of images, to check the instrumental noise of the camera and the estimation of repeatability and representativeness of HSI measurements.

**Key Words:** Hyperspectral imaging, instrumentation, configuration, instrumental noise, repeatability and representativeness.

# 1 INSTRUMENTATION

## 1.1 Hyperspectral imaging system

The hyperspectral imaging system is a laboratory-based pushbroom imaging equipment. The main components include: (1) a spectrograph with PGP optical structure (ImSpector, N17E, SPECIM, Finland), (2) a 12-bit CCD camera (V-light, Lowell Light Inc, USA), (3) 150-W tungsten halogen lamps (Fibre-Lite DC950 Illuminator, Dolan Jenner Industries Inc., Boxborough, MA, USA) and (5) a translation LabScanner with dimension (L × l) of 40 × 20 cm by a step motor. The harmonious work of the integral system is assured by using (5) control software LumoScanner (SPECIM, Finland).

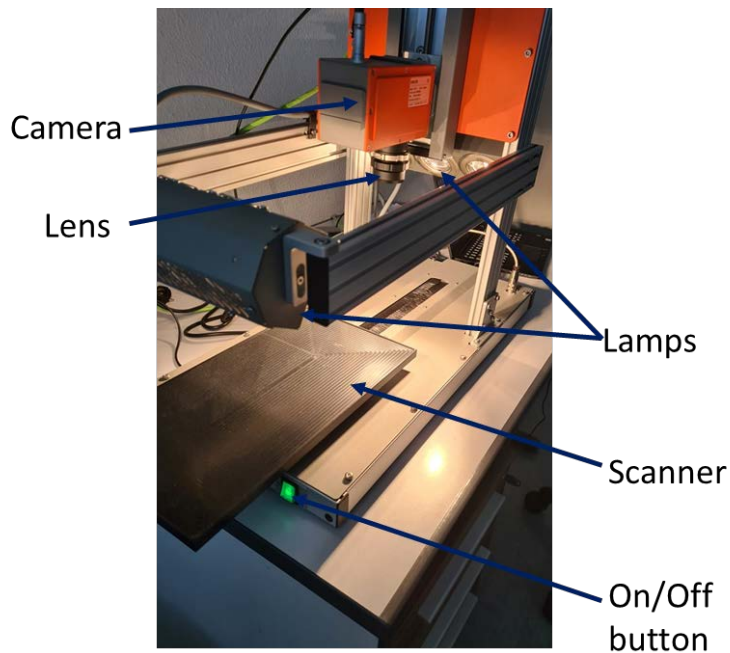


The assembly disperses the incoming line of light into the spectral and spatial matrices and then projects them onto the CCD. The optics, spectrograph and the camera, has high sensitivity from 900 to 1700 nm with a spectral resolution of 8 nm. After finishing the scans, a three-dimensional (x,y,z) spatial (x,y) and spectral (z) data space are constructed. Images are binned during acquisition in spatial direction to provide images with spatial dimension (xxy) pixels with 224 spectral (900-1700 nm). The spatial dimensions depend of the size of the sample.

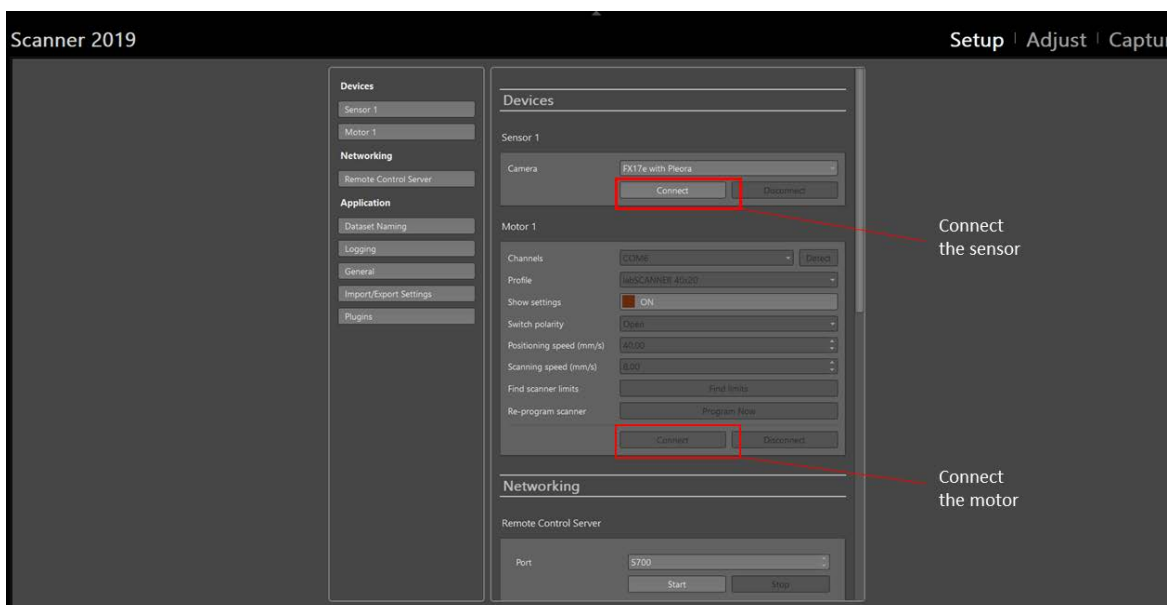


## 1.2 Operating mode to start instrument and configuration

### A. Switch on the halogen lamps 30 min before to start the measurements

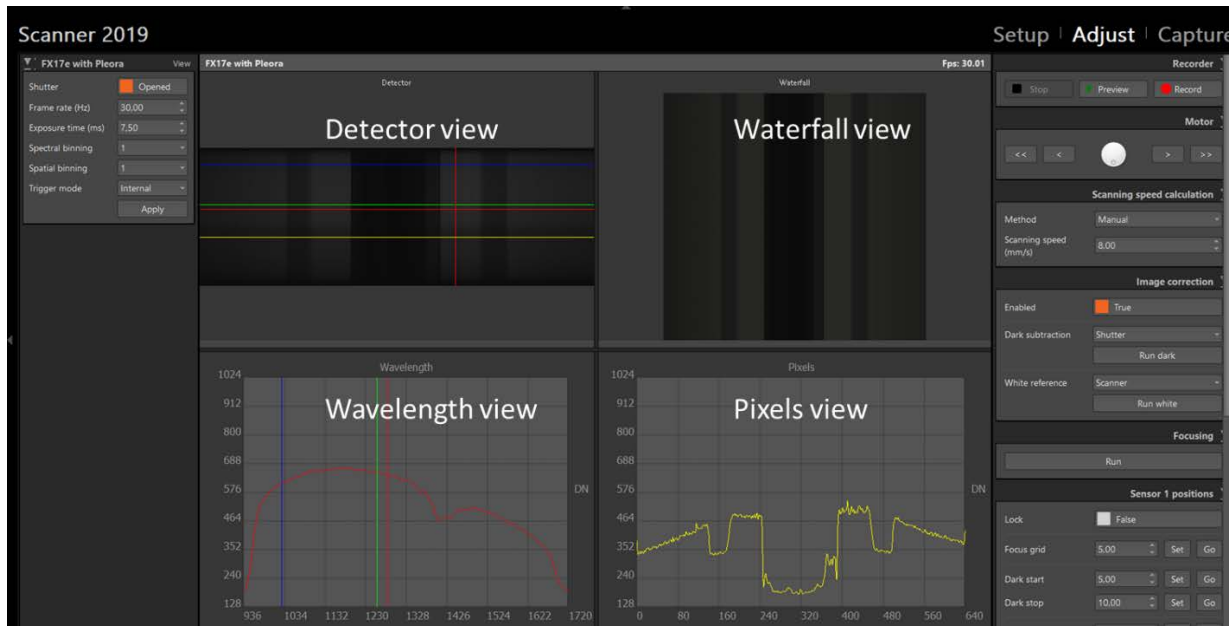


- B. Take off the lens cap and be careful to not touch the lens with your fingers, if you touch the lens, clean immediately with pepper towel and ethanol.
- C. Launch Lumo Scanner software and connect the sensor and the motor to the camera



➤ Lumo scanner contains three tabs, Setup, Adjust and Capture.

- In setup tab connect the sensor to the camera and the motor to the camera
- Set the path of the folder for data saving.
- In Adjust tab you will adjust the parameters of acquisition and there are 4 views



- Detector views shows a real time image from the detector short wavelength on the top and long wavelengths on the bottom horizontal axis covers spatial.
- Waterfall view shows a real time the camera with selected three visualisation bands.
- Wavelength view shows selected spectral profile the detector view and visualization bands.
- Pixels view show spatial profil from the detector view.

## D. Adjust Parameters of acquisition

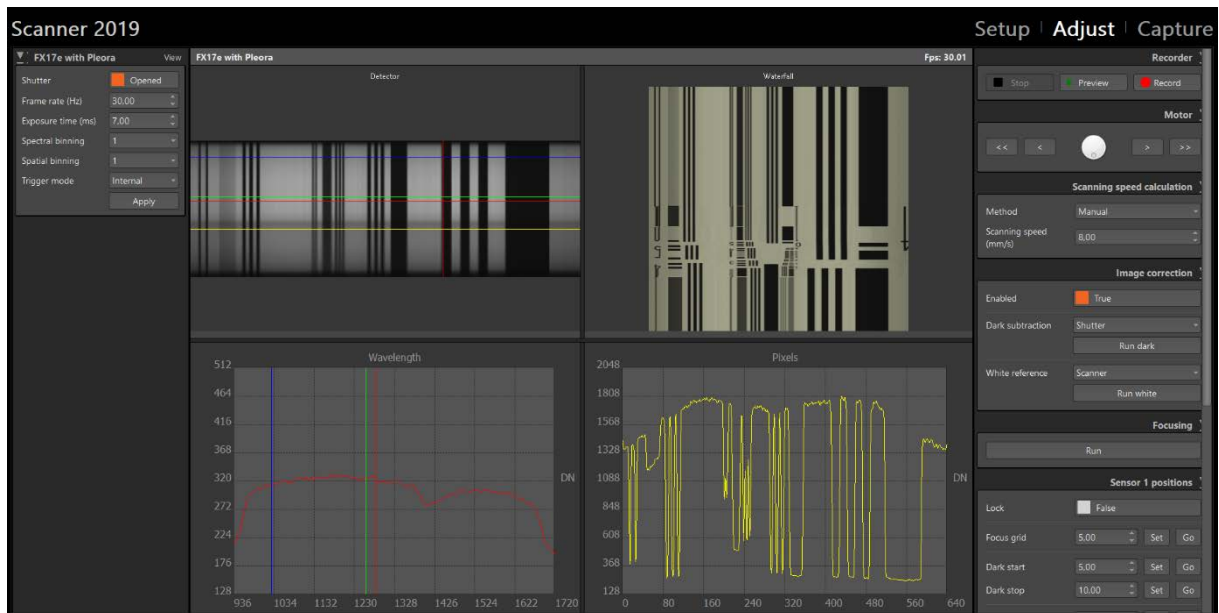
### D.1. Focusing the camera

- Put the focusing sheet on the scanner and run the scanner to a position where the camera is looking at the sheet by clicking on scanner moving button . At the detector view or waterfall view to make sure you stop the scanner when the camera is looking directly.



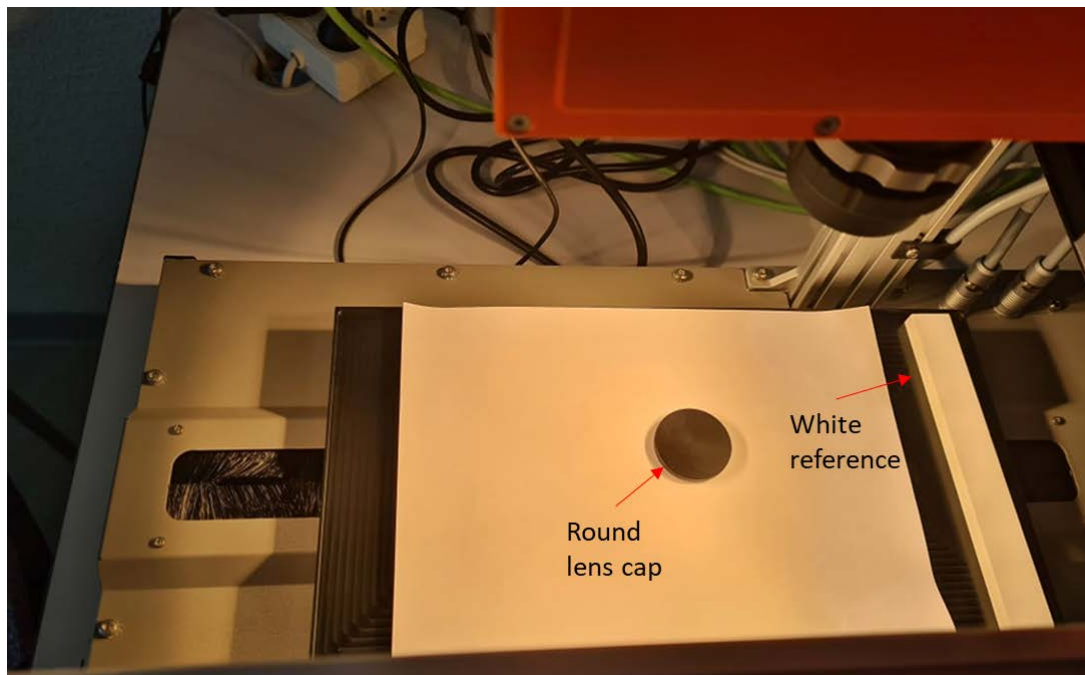


- When you see that the sensor is out of the focus you have unlock the focus of the camera and focus the sensor by rotating the for optics: image is improving when the pixels view starts to show a clear pattern. In addition, the difference between min and max in the pixels views increases. Ones the image is sharp, the focus is good and can be locked. When you change the field of view of the camera or the distance between the sample and the camera, you have to check if the focus is optimal.



## D.2 Adjust the scanning speed and frame rate

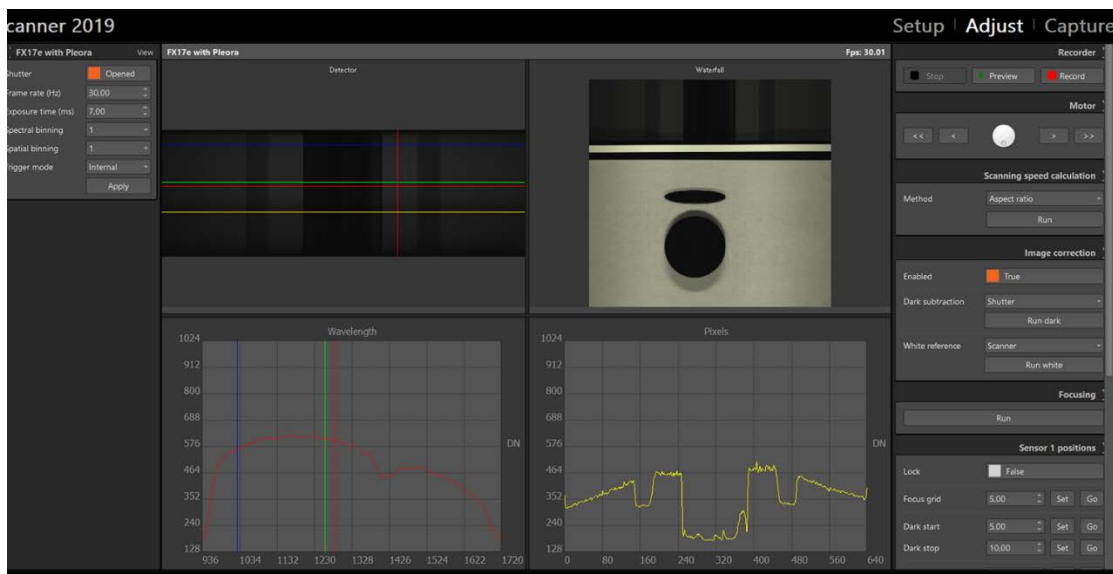
- Aspect ratio is the method to be used in order to adjust the scanning speed and camera frame rate in function of the current data.
- For the recording data, place a round object like a cap of lens on the white sheet on the scanner table.



- Find the correct positions for white reference start and stop and the target (lens cap) start and stop. For the white the reference (WR) move the scanner to the WR panel and select set for the WR start and stop. For the target (sample), we will find the correct target (lens

cap) positions, the good start position is just before the lens cap appears in the waterfall view and the good stop position and the target stop is just after the lens cap disappear to the waterfall view.

- Run preview: if you see from the preview that the lens cap looks too flat, this means that the aspect ratio needs to be adjusted. To do that, go to the scanning speed calculation and select method "aspect ratio" needs to be adjusted and click run, a new preview is run and new settings for scanner speed and camera frame rate are proposed.
- Choose the option we prefer scanner or camera and we can keep a frame rate and change scanner speed and select scanner.
- If the lens cover looks round, that means the scanner speed and frame rate are good.



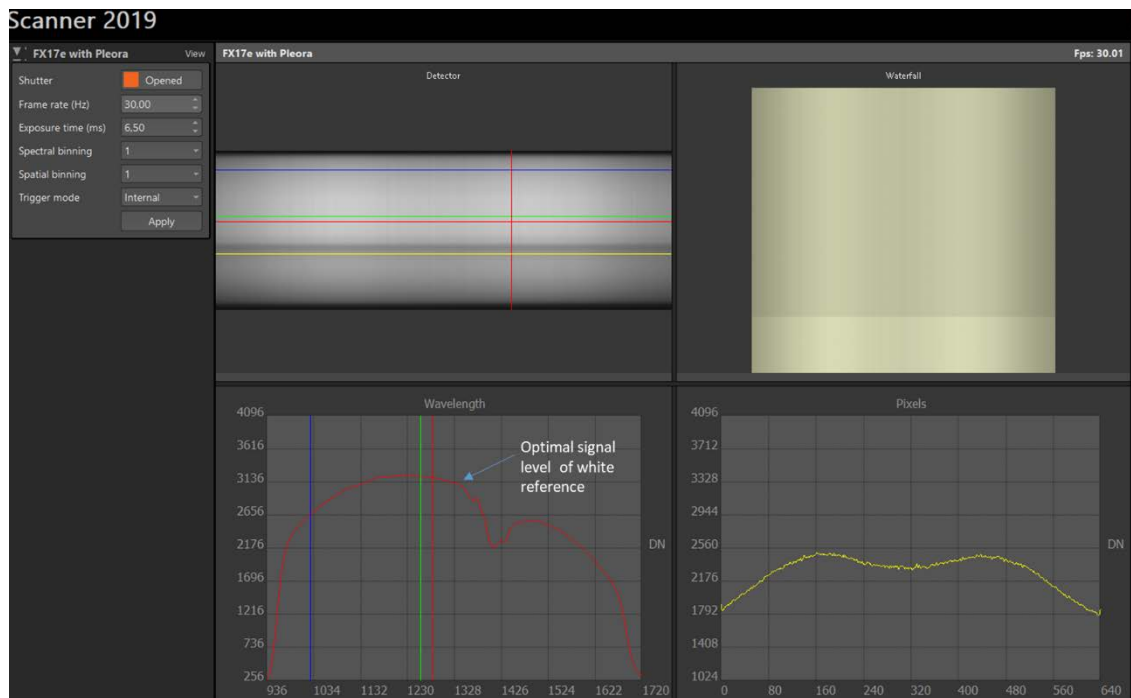
Note: when the motor is homing the scanner speed is different and the image might look flat: don't worry about this, the data is stored only from the scanning, not when the motor is homing.

### D.3. Adjust the optimal signal level for data acquisition

- Signal level must be 80-90% of the sensor dynamic range.
- Run the camera to the white reference and observe signal level :

If the signal level is less than 80 %=> increase exposure, time to increase signal level.

If the signal level is more than 90 %=> decrease exposure time to increase signal level.



Note: if you increase too much the exposure time the signal will saturate.

- If you change the spectral binning from 1 to 2, the amount of spectral bands in detector view is only half of the original => This happens because every two successive spectral bands are averaged together.
- If you change spatial binning from 1 to 2, the number of spatial pixels in the detector view is only half of original. Now you are ready for the image acquisition on your samples.

## E. Record data (image acquisition)

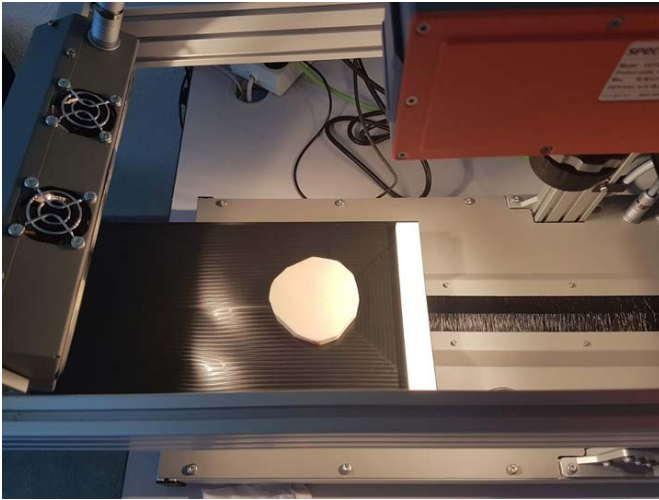
The system described above (SPECIM FX17) is used to acquire hyperspectral images. It employs push-broom imaging and acquires one line of the image at a time. Each line of the image has a fixed number of pixels of 640, and the number of lines depends on the length of the sample.

- Place, the sample and white reference sample (Teflon) into the black translation stage with a size of 40x20 cm.

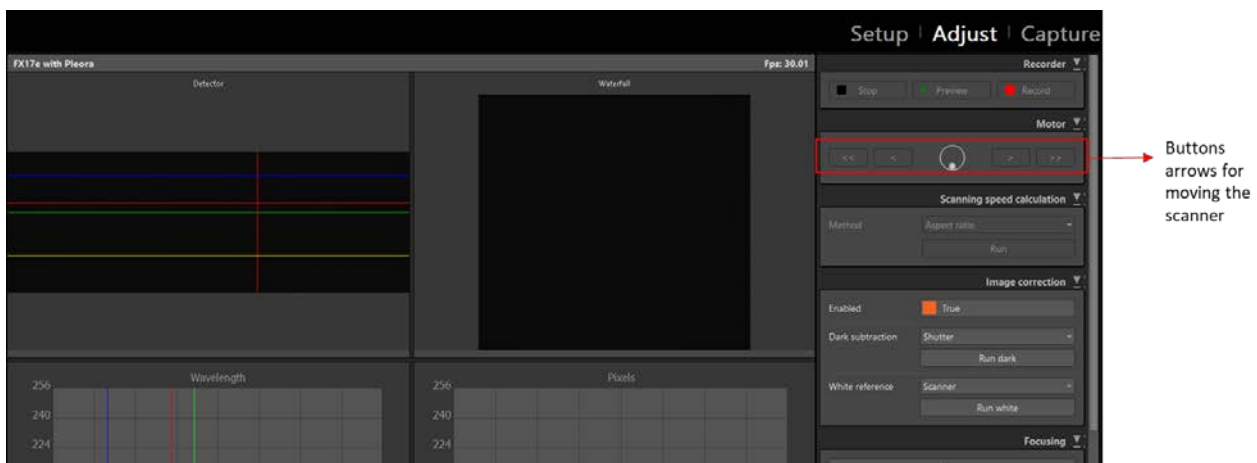
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Specim FX17**

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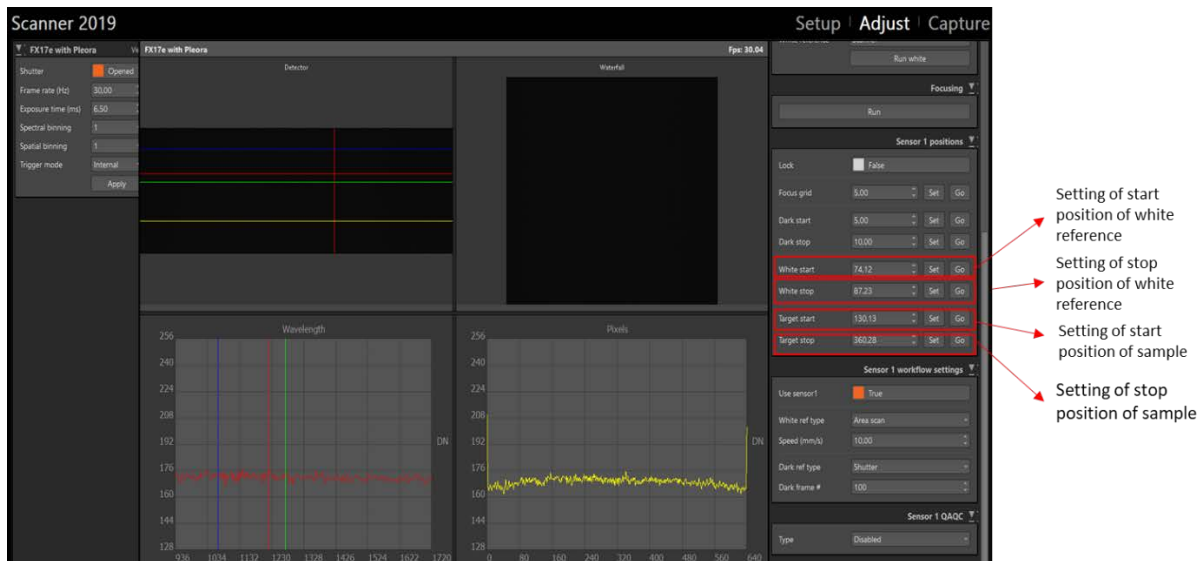


- In Adjust tab, fix the positions of the white and sample using the LumoScanner acquisition software. Use arrows buttons to move the scanner to find the correct positions for white reference start and stop and the target (sample) start and stop.



- For the white the reference (WR) move the scanner to the WR panel and select set for the WR start and stop. For the target (sample), the good start position is just before sample appears in the waterfall view and the good stop position and the target stop is just after the sample disappear to the waterfall view.





- In capture tab, add sample ID, metadata and record image by clicking in Record button.



Once the positions are fixed, the recording of the HSI image is started. The dark image (with 0% reflectance) is recorded first by turning off the lighting source with the lens of the camera completely closed, and then the white reference image (Teflon whiteboard with 99% reflectance) is recorded, in the end, the hyperspectral image of the target sample is recorded.

## 2 CONTROL OF INSTRUMENTAL NOISE

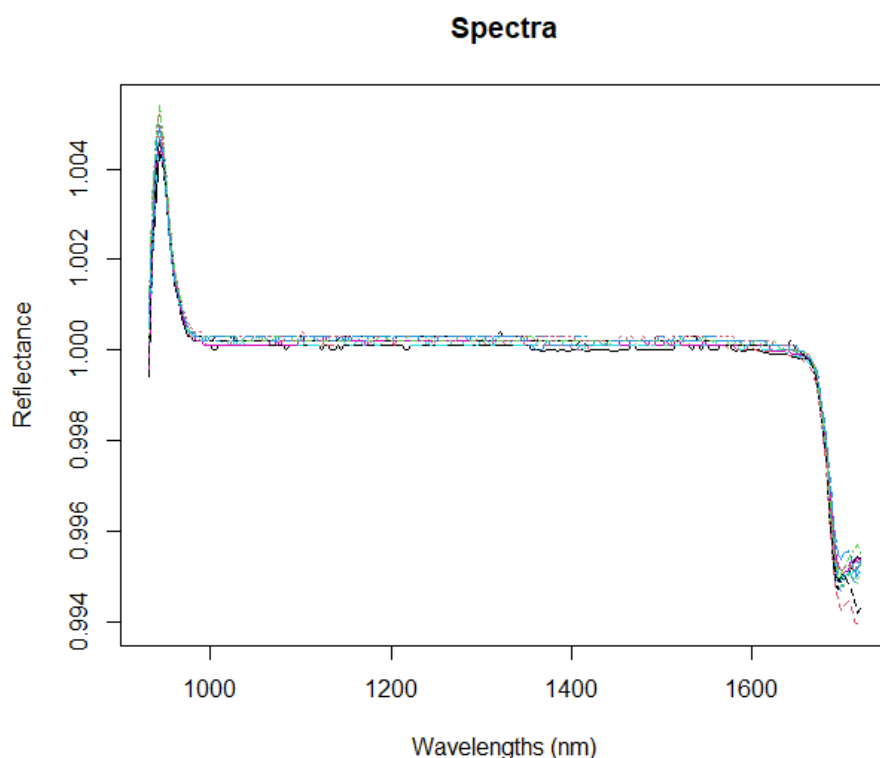
In order to estimate limits of instrumental noise of the camera Specim FX17,

- Take 10 hyperspectral images of white reference consecutively
- Calculate mean spectra of each image;



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Fig.1 below shows 10 average spectra calculated from the hyperspectral images taken on the white reference. The reflectance is around 1 between 1000 and 1632 nm for the white reference. The noise observed at the two extremes of spectra is due to the poor quantum efficiency of the CCD in these regions and therefore a poor signal-to-noise ratio.



*Figure 1 : 10 mean spectra replicates of white reference acquired consecutively on white reference.*

- Estimate the dispersion between each spectrum and the average spectrum by the root mean square error (RMS) calculation.

## 2.1 Method of calculation and formulae

The limits of instrumental noise were estimated by the calculation the root mean square error (RMS) between two spectra. This operation is applied on 10 spectra of the same white reference. The RMS was calculated using the equation below:

$$RMS = \sqrt{\frac{\sum_j^p (X_{aj} - X_{bj})^2}{p}}$$

Where:  $X_{aj}$ ,  $X_{bj}$  average of reflectance of wavelength  $j$  for the two spectra  $a$  spectrum and  $b$  mean spectrum of 10 spectra,  $p$  number of wavelengths ( $j$  variate from 1 to  $p$ ). Therefore, The average and standard deviation (SD) of all RMS were also calculated. The RMS of each spectrum must be within the interval mean RMS (RMSM)  $\pm 3SD$ . In this SOP, the RMS values were calculated using, the R script in Annex 1.

## 2.2 Results

The fig.2 shows the RMS values calculated from 10 spectra in spectral range 1000-1632 nm. The RMS obtained are from 37.07 to 133.95 uref. The RMSM=71.43 uref and the standard deviation is SD=27.51 uref. The results show that all RMS values are in the confident interval of RMSM (red line)  $\pm 3SD$  (dashed blue lines). Which means a high signal to noise ratio of the instrument for the spectral range 1000-1632 nm.

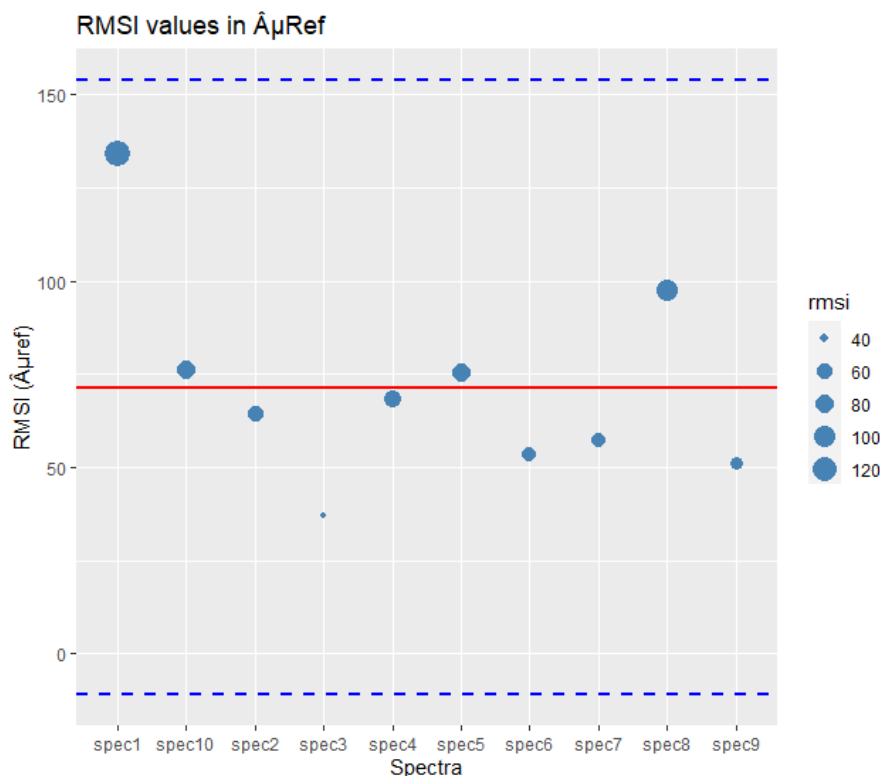


Figure 2 : RMS values of 10 replicates acquired on the white reference, the mean RMS represented in red line and  $\pm 3SD$  represented in dashed blue lines.

## 3 REPRESENTATIVENESS OF HYPERSPECTRAL IMAGING MEASUREMENTS

- For the determination of the representativeness, yam tuber is taken as an example
- Use two yam genotypes
- Choose 3 tuber from each genotype with regular shape, big, medium and small;
- Cut from each tuber, 3 slices (proximal, central and distal) (fig.3)
- Scan the two faces of each slice.

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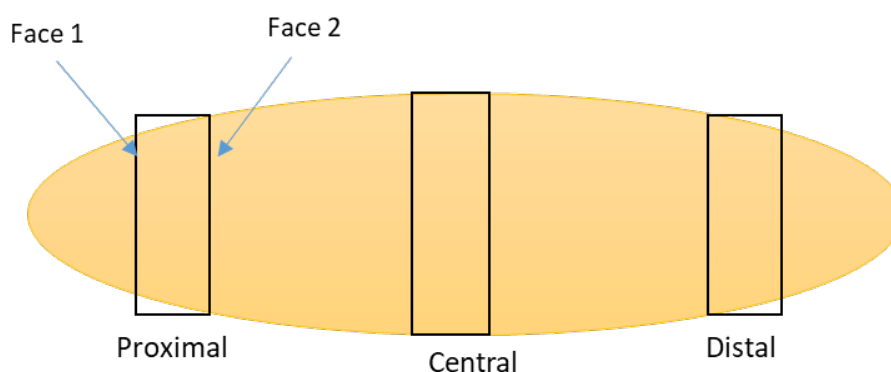


Figure 3 : schema of yam tuber divided into 3 slices of 3 zones proximal, central and distal.

- Do immediately DM measurements on each slice with two replicates
- Calculate RMS by size of tuber and by zone to estimate spectral variability between tubers of the same genotype and between zones inside tuber.

### 3.1 Variability between size of tubers

The fig.4 shows, the variability of RMS values between tubers of different size big, medium and small and then /the variability of RMS within tuber. These results highlight the spectral variability (RMS) between tubers of different size. We see also the spectral variability between samples of the same tuber. The variability in small tuber is higher than the variability in big and medium tubers.

Therefore, in order to capture all spectral variability in the sample, the HSI measurements must be taken on 3 yam tubers (big, medium, small) and on the three zones of the tuber (proximal, central and distal). In addition, two images per zone must be acquired (one for each face).

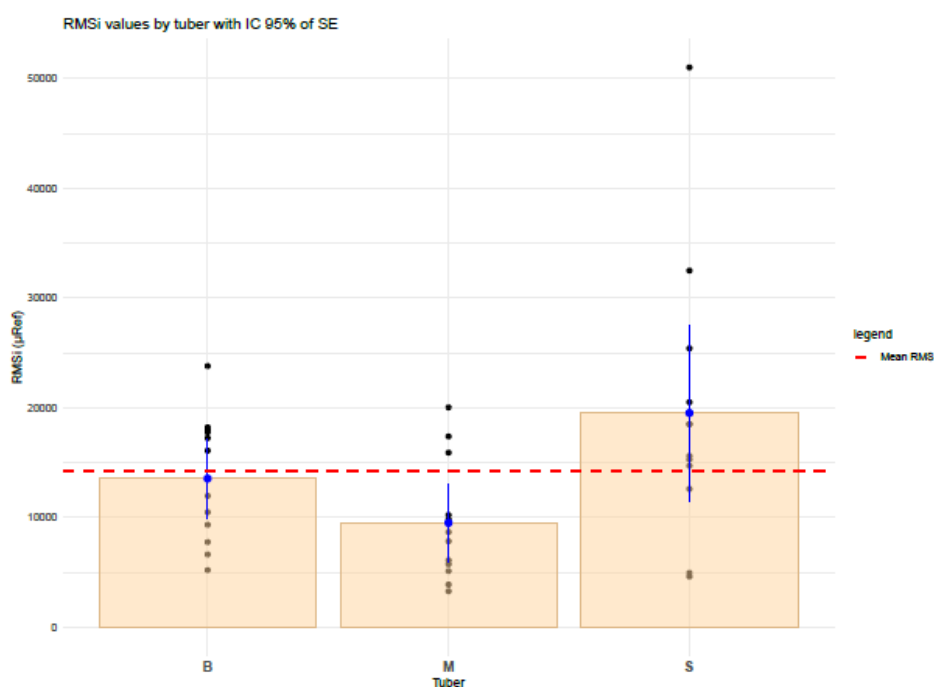


Figure 4 : RMS values by size of tuber and RMS values by zones within tuber.

## 4 CRITICAL POINTS OR NOTE ON THE PROCEDURE

- Switch on the lamps 30 min before the measurement.
- Do not touch the with your fingers
- Check the parameters of acquisition before each set of measurements and adjust them if needed.
- Check the focus when you change the distance between the camera and the samples
- The image acquisition should be done at the same distance for the sample as well the white reference.
- To avoid water evaporation, the image acquisition on fresh wet samples should be done very quickly.
- The lamps heat up and could accelerate the water evaporation.

## 5 APPENDICES

### 5.1 Annex 1: R script for the calculation of RMS

```

Libraries -----

library(tidyverse) # tidyverse library contents: ggplot2, dplyr, tidyr, purr,
readr, tibble forcats and stringr

# Create the function rms -----

## This function returns the individuals values of Root Mean Squares of n spect
ra
## the function returns a list of 2:
# the mean spectra ("meansp")
# the individual values of rms (in Åμ absorbance)
## the argument (x = data frame or matrix with numeric values only, here spectr
a)

rms <- function(x){
  meansp <- colMeans(x)
  difspec <- matrix(apply (x, 1, function(a)(a - meansp)), nrow=nrow(x), byrow
= T)
  difspecsqr <- matrix(apply(difspec,1,function(a)(a^2)), nrow=nrow(x), byrow =
T)
  sumsqr <- matrix(apply(difspecsqr,1,function(a)(sum(a))), nrow=nrow(x), byrow
= T)
  rmsi <- matrix(apply(sumsqr,1,function(a)(10^6*sqrt(a/length(meansp)))), nrow
=nrow(x),
                    byrow = T )
  return(list(rmsi=rmsi,meansp=meansp))
}

# demo1 -----

## import data: spectra with iundicatrice variable
demo1 <- read.csv2 ("D:/CIRAD/2021/test-noise-HSI/RMS_Fabrice240921/10spectra.
csv", header=T, dec=".", na.strings = c ("NA",""))

## data frame information
str(demo1)
dim(demo1)
class(demo1)
names (demo1[,2:6]) # to have the names of 5 first variables
names (demo1[,176:181]) # to have the names of 5 last variables
increment <- ((1632.8 -1000.1)+2)/length(demo1[, -1]) # wavelength acquisition s
tep

```

```

## create a vector of wavelength according to the instrument
wavelength <- seq(from = 1000.1,to = 1632.8, by=increment)

## visualization of the spectra (variable "id" is kept out)
matplot(wavelength, t(demo1[,-1]), type = "l", main="Spectra", xlab = "Wavelengths (nm)", ylab = "Reflectance" )

## calculation of RMSI using rms function (variable "id" is kept out)
result <- rms(x=demo1[,-1])

## extraction of the mean spectrum
meansp <- result$meansp

## graphic of average spectrum
plot(wavelength,meansp,type = "l", main="Average spectrum", xlab = "Wavelengths (nm)", ylab = "Reflectance")

## create a data frame with extraction of the rmsi from the list result and id of the samples from demo1
rmsi <- data.frame (id = demo1[,1] , rmsi = result$rmsi)
##Calculate the mean and sd of rmsi
sd<-sd(rmsi$rmsi)
mean<-mean(rmsi$rmsi)

## bar plot of rmsi values
ggplot(data=rmsi, aes(x=id, y=rmsi)) +
  geom_bar(stat="identity",width = 0.5, fill="steelblue")+
  coord_flip()+
  geom_text(aes(label = round(rmsi,0)), vjust=0.6, hjust=2, color="white", size =5)+
  labs(title="RMSI values in Âµref", x="RMSI (Âµref)" , y ="Spectra")
theme_minimal()

## scatter plot of rmsi with average value
ggplot(rmsi, aes(x=id, y=rmsi, size = rmsi)) +
  geom_point(color="steelblue") +
  labs(title="RMSI values in ÂµRef", x= "Spectra", y = "RMSI (Âµref)")+
  geom_hline(yintercept = mean(rmsi$rmsi),color = "red",size =1)+
  geom_hline(yintercept = mean+3*sd,color = "blue",linetype="dashed", size =1)+
  geom_hline(yintercept = mean-3*sd,color = "blue",linetype="dashed", size =1)

## boxplot des rmsi
boxplot(rmsi$rmsi, col = c("steelblue"),main = "Boxplot RMSi", ylab = "Quantiles")

```





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