Standardized Protocol for Use of the Veggie Meter® in the Research Setting

The manufacturer's operating manual was modified for use of the Veggie Meter® for research purposes.

1. General

The Veggie Meter® uses reflection spectroscopy to measure the level of carotenoid pigments in an individual's skin. As a light source, the Veggie Meter® uses a white LED. To reduce inconsistencies in the research setting, the non-dominant ring finger should be selected for the measurement site. To obtain a measurement, the finger is inserted into the instrument's finger cradle to simultaneously bring the pad of the fingertip in close contact with the light source and light collecting contact lens. A spring-loaded clip gently applies pressure to the finger, such that blood flow is temporarily pushed away from the measured tissue site to avoid interfering with carotenoid detection.

A laptop computer interfaced to the instrument analyzes and quantifies the amount of white LED excitation light reflected from the finger and instantaneously derives a carotenoid score. The measurement takes approximately 10 seconds for a single measurement or 45 seconds for a triplicate measurement. Allow an additional 15 - 20 seconds for processing time and display information. In the multi-measurement mode, the finger is inserted and retracted three times and an average score is determined for the three measurement values.

Carotenoids are found in a wide variety of fruits and vegetables. They cannot be synthesized *de novo* in the human body and therefore are consumed through the diet. Thus, skin carotenoid scores are correlated with the dietary uptake of fruits and vegetables. In general, habitual consumption of fruits and vegetables results in an increase in skin carotenoid scores. The instrument provides a comparative output of an individual's skin carotenoid score in relation to

data from the general population. Additionally, changes in skin carotenoid scores may be tracked over time to determine responsivity to changes in dietary intake. Since carotenoid levels differ slightly between fingers, it is advisable to measure the same finger when tracking changes over time.

2. *Equipment*

- Veggie Meter®

- Interface Laptop and Corresponding Charger

- Connector Cords (2)

  o Black Cord

  o Grey Cord (device specific)

- Dark Calibration Reference Stick

- White Calibration Reference Stick

- Demonstrating tool and adaptor for different digit diameters (including toddlers and children)

- Access to hand-washing station, including anti-bacterial hand soap, warm water, and drying apparatus

  o If access to a hand-washing station is unavailable, alcohol-based prep pad or hand wipes may be used

- Optical cloth and glass cleaning fluid

- Data recording binder

3. *Set-up*

The Veggie Meter® instrument consists of a box shaped base (pedestal) that contains the electronics, and an oval shaped housing unit that sits on top of the base to provide the optical interface for measuring skin carotenoids.

Housing Unit

Spring-loaded Clip

Base/Pedestal

3.1 The Veggie Meter® and interfaced laptop have limited battery capacity (approximately 6 hours following a complete charge). Connect the laptop with a 110 Volt power outlet or make sure a power outlet is nearby if required.

3.2 Power up the laptop by pressing and releasing the power button, which is located on the top right corner above the keyboard.

Power button

To activate the user screen, tap on the mouse pad. When prompted, enter the password "project" to login.

* 3.3 **This step is device specific.**

If the device you are using comes equipped with one cable (newer version), connect the Veggie Meter® to the interfaced laptop using the black cable by inserting one end into either of the USB ports and the other end into the housing port.

If the device you are using comes equipped with two cables (older version), connect the Veggie Meter® to the interfaced laptop using both cables:

    Grey – connect the grey cable from the ***base (pedestal) port*** of the Veggie Meter® to the ***back port*** of the laptop

    Black – connect the black cable from the ***housing port*** of the Veggie Meter® to the ***front port*** of the laptop

Note: the USB ports are not interchangeable. The instrument will not function properly if the cords are reversed.

3.4   Power up the Veggie Meter® by pressing and releasing the power button on the

back of the instrument.

Note: There are two buttons located on the back of the instrument. The power

button is the bottom button, located closest to the black cable connection. Ignore

the top button.

* 3.5   The Veggie Meter® will take 5 minutes to warm-up. A colored display at the top

of the housing unit will initially display the number 5, and then will count down

the remaining minutes. **After 5 minutes, the instrument will display a logo. If

the Veggie Meter® is in an environment with consistent ambient

temperature, the 5-minute warm-up period is sufficient. If the Veggie Meter®

is introduced to a new environment, especially one with substantial changes

in temperature, lighting, or relative humidity, the device should run for 15-

minutes before use to allow for adequate acclimation.**

3.6   Once the logo is displayed, move the laptop curser to the "VeggieMeter Shortcut"

icon located on the desktop screen. Double click the icon to activate the program.

3.7   The Veggie Meter® program window should appear. There will be three panels

indicating the following:

> a. **Top Panel** – entry location for optional participant identification
>
> number and demographic information
>
> b. **Right Panel** – selection of desired measurement mode ("Single Scan or
>
> "Average of 3 Scans") and calibration measurements (DARK
>
> REFERNCES and WHITE REFERENCE)
>
> c. **Display Panel** – displays skin carotenoid score and histogram of general
>
> population. The display panel is blank prior to calibration.

Calibration

It is important to note that the expected ranges for calibration of the Veggie

Meter® are device specific. Please refer to the operating manual to locate the

expected range for dark and light calibration values for your device.

* 3.8   *Dark Calibration*

Gently slide the dark reference stick with the two side rails over the cradle,

located on the housing port, such that the rails are pointing down and the black

felt at the bottom of the stick is facing downwards, a few millimeters above the

lens. The reference stick should push against the two inside plastic pegs while

protruding from the housing port. When positioned properly, the instrument's

white LED light will illuminate the black felt. Keep felt clean by avoiding

touching the felt. Blow off lint or dust if needed.

Dark Reference
Stick

Once the dark calibration stick has been properly inserted, click on the DARK REFERNCE button, located in the right panel of the program window. The display panel will now show the reflection spectrum for the dark reference felt material. A jagged low-intensity narrow band should appear on the left and a low-intensity broad band should be represented on the right side of the light reflecting spectrum. The intensity (height) of the spectrum is indicated on the vertical axis and the number of pixels is recorded on the x-axis.

Refer to the reference image below. Exact values may differ somewhat from those presented in this reference image as the calibration values are device specific. If the general image of a low-intensity narrow band followed by a broad band does not correspond to the reference image, repeat the dark calibration. Note that any contamination of the contact lens (from fingerprints, etc.) or the felt material (lint, etc.) may lead to increased intensities. In other words, it is desirable to have clean optics, such that the reflection intensities are as low as possible, with a resulting appearance of a highly jagged low-intensity reflection trace.
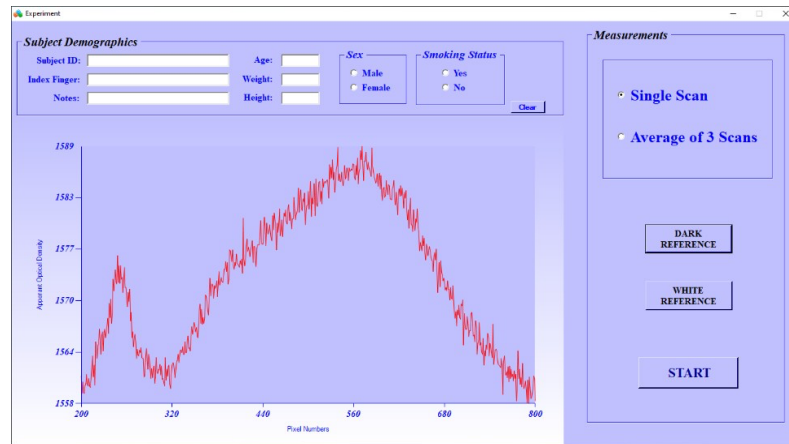
*3.9   *White Calibration*

Open the spring-loaded clip by pressing the back of the lever located on the

housing port with one hand. Using the other hand, insert the white reference stick

into the finger cradle, such that the indentation on the white plastic material fits

snugly over the contact lens. Make sure the white surface of the reference stick is

pointing downward.

White Reference

Stick

Once the white calibration stick has been properly inserted, click on the WHITE

REFERNCE button, located in the right panel of the program window. The

display panel will now show the reflection spectrum for the white reference. A

strong, high-intensity peak should appear on the left and a more intense broad

band should be represented on the right side of the light reflecting spectrum. The

intensity (height) of the strong peak is indicated on the vertical axis and the number of pixels is recorded on the x-axis. Note that the auto-scaled reflection intensities are much higher now compared to the dark reflection spectrum and as a consequence the trace is much smoother.

Refer to the reference image below. Exact values may slightly differ from those presented in this reference image as the calibration values are device specific. If the general image of a high-intensity narrow peak followed by a broad band does not correspond to the reference image, repeat the white calibration.
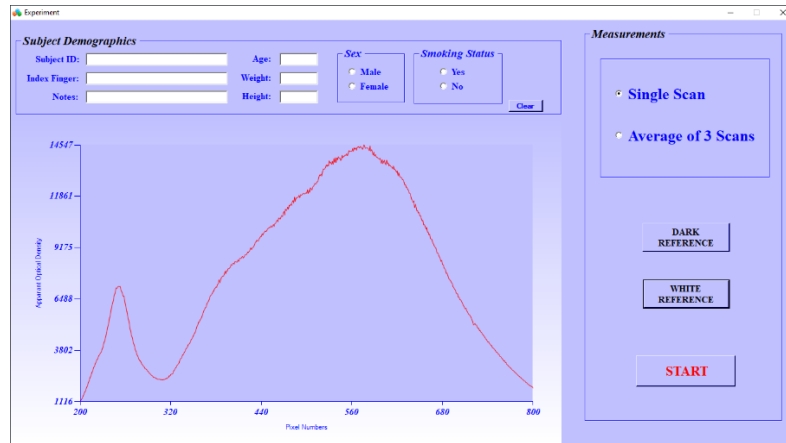
Calibration Note: Calibration should be performed every time the instrument is turned on. For improved accuracy in the research setting, it is recommended that re-calibration occur with both black and white reference sticks every **1 hour of operation or if the device is relocated or moved prior to the 1-hour time interval**. You DO NOT have to turn the instrument off and on and restart the software: When ~1 hour interval lapsed, insert the black reference stick and click the "DARK REFERENCE" button, then insert the white reference stick and click the "WHITE REFERNCE" button. The respective intensities will override the previously stored intensities.

4. *Skin Measurements*

4.1   Once the device is calibrated, the instrument is ready for skin measurements.

*4.2   Enter the subject identification information. **To ensure participant anonymity, it is recommended to use a coded identification number in lieu of a name. As skin carotenoid scores may be impacted by individual characteristics, it is encouraged to record the following information when possible: age, sex, BMI, smoking status, supplement use, and diagnosed chronic diseases. Although these data are not required for measuring skin carotenoids, individual level data will increase the diversity and generalizability of the universal data repository.** Select the desired measurement mode by selecting either "Single Scan" button or "Average of 3 Scans" button. **For research purposes, the "Average of 3 Scans" mode is recommended for higher accuracy.**

*4.3   Insert the **non-dominant, ring finger** and click on START.

In "Single Scan" mode, the display window will first flash SCANNING IN PROGRESS, display a progress bar, and after finishing the scanning, will show "Your Carotenoid Score" on a scale from 0 to 800. In the display panel on the right, the measured score is compared with a histogram of scores for the general population. The histogram region corresponding to the measured score is shown in red. Click on the "Close" button to return to the Measurements screen and start a new scan.

In the "Average of 3 Scans" mode, follow the prompts in the displays and obtain the final score after 3 consecutive measurements. **Remove the finger after each measurement to allow for reperfusion.** Click on the "Close" button to return to the measurement screen.

*4.4 **To ensure minimal lens interference, the surface of the contact lens should be cleaned using an optical cloth in combination with a glass cleaning fluid after every participant has completed the three scan (triplicate) mode.**

4.5 The measurement data will automatically save into a folder on disk C called ResultsM. A shortcut icon for the results is located on the desktop.

5. Shutting Down

5.1 Once data collected is completed, click on the upper right window (x) in the measurements screen to close the program window. In the following exit window, click the OK button to close. It is important to close the program prior to disconnecting the device, as communication between the Veggie Meter and laptop computer may be lost.

5.2   Turn off power to house of Veggie Meter® by pressing the lower button on the back of the base (pedestal).

5.3   Disconnect the black power cable from the Veggie Meter® and the USB port from the laptop. If the device contains two cords, after removing the black cord, disconnect the grey cable from the Veggie Meter® and the USB port from the laptop.

5.4   Pull up the main computer menu by sliding up on the mouse pad, click on "Power" and in the following window on the "Shut Down".

6. Notes

*6.1   In order to demonstrate the positioning, a spare cradle is supplied. The **non-dominant ring finger** should be positioned in the cradle, such that the pad of the finger fits snugly into the indentation of the cradle, and the tip of the finger touching the top edge of the cradle indentation. The user should fully feel the full rounded shape of the contact lens under the widest part of the measured finger.

*6.2   The Veggie Meter® is a highly sensitive optoelectronic device. Avoid mechanical shock and exposure to excessive heat or bright light. **For this reason, it is recommended to record environmental conditions, such as temperature and relative humidity when using the device outdoors or under variable environmental conditions where exposures to excessive heat or light may occur.**

6.3   Skin is a highly heterogenous tissue, with the degree of heterogeneity varying between individuals. It is normal that carotenoid scores vary from scan to scan for

the same individual. Any obtained score is usually within 10% of the average score that can be obtained via multiple measurements.

*6.4   Make sure the measured finger is clean. Any contamination needs to be removed prior to measurements. **Washing hands with soap and warm water is the most effective. If hand washing resources are unavailable, use a pre-moistened alcohol prep pad or hand wipes.**

*6.5   On occasion, the communication between the computer and instrument can be lost. This may occur if the shutting-down procedure is not followed. In those cases, corresponding messages will appear in the program window. To re-establish communication, abort or exit the program, and/or unplug and reconnect the instruments USB connections. This will require the standard operating procedure to be repeated to ensure the device is functioning properly. **It is recommended to back up the data on a USB drive and transfer to project-specific password-encrypted file to ensure no data is lost.**

6.6   Do not open the file ResultsM.csv or click on the Results shortcut icon while the instrument software is running. This may result in inaccurate syncing of data.

6.7   To avoid laptop battery discharge, remember to disconnect the black and grey cables between the Veggie Meter® and USB ports of the laptop as part of a normal shutdown procedure.