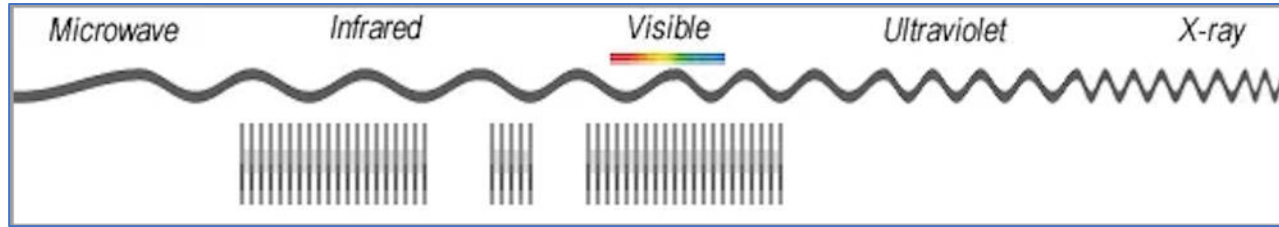


# New tools in plant disease assessments

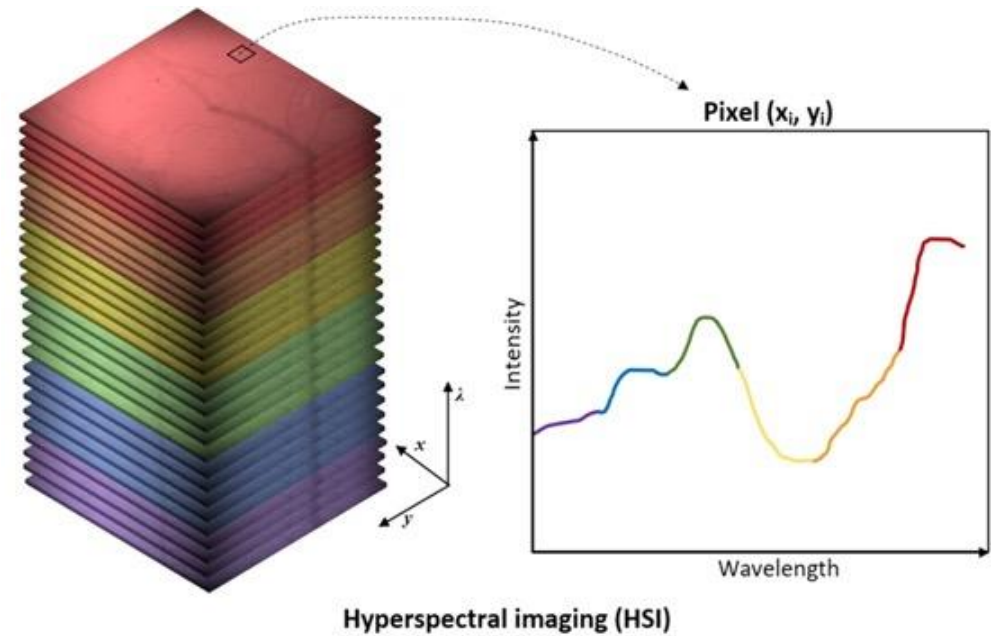
Mathews L. Paret, [paret@ufl.edu](mailto:paret@ufl.edu)



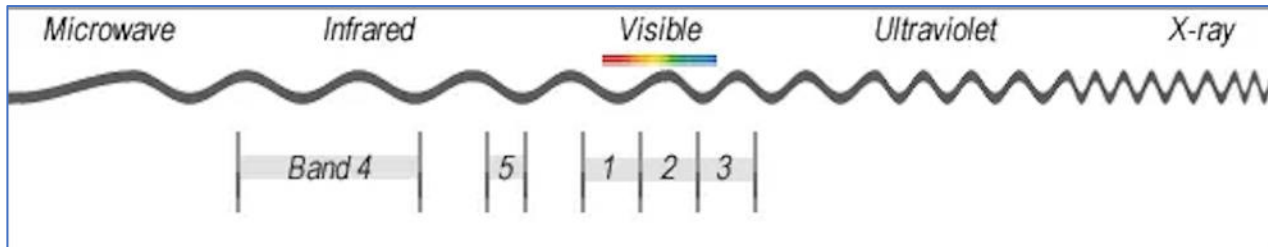
# Hyperspectral sensor



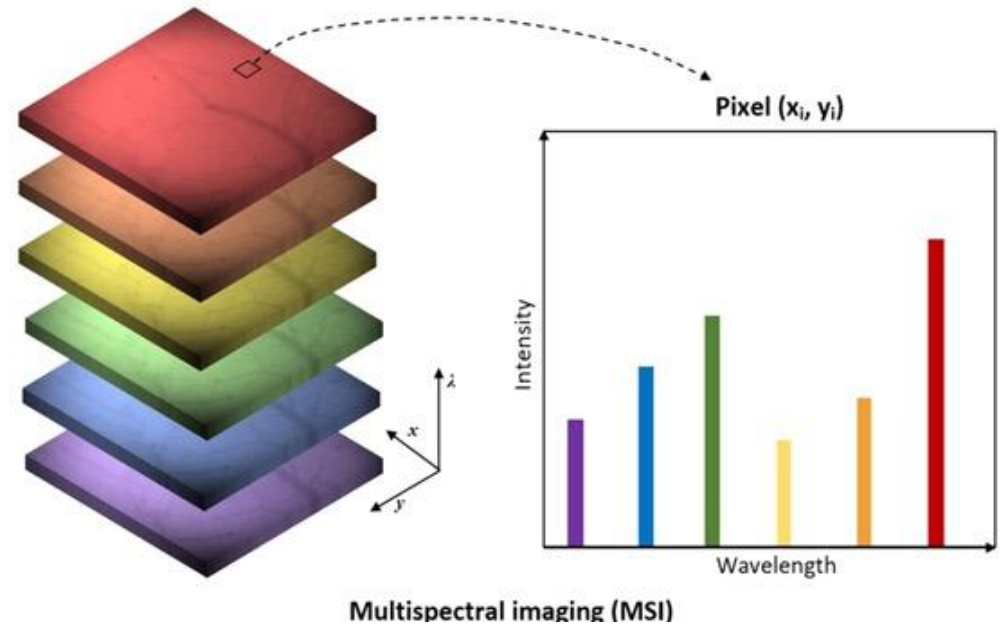
- Narrower bands (10-20 nm).
- Hundreds of bands



# Multi-spectral sensor

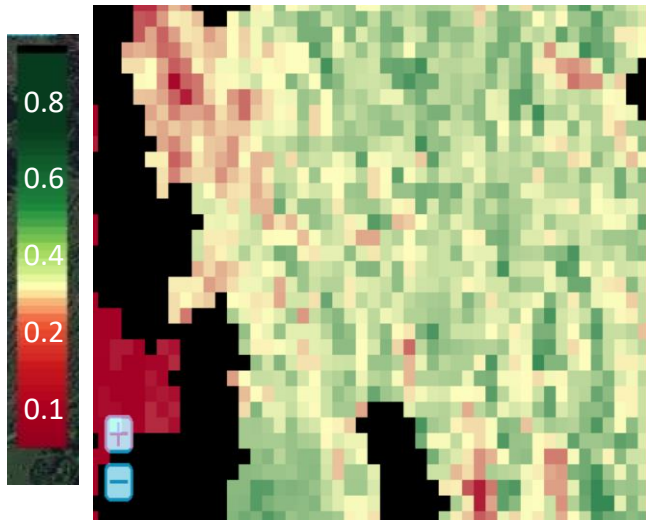


- Three to ten bands
- Eg. red, green, blue, near-infrared, and short-wave infrared.

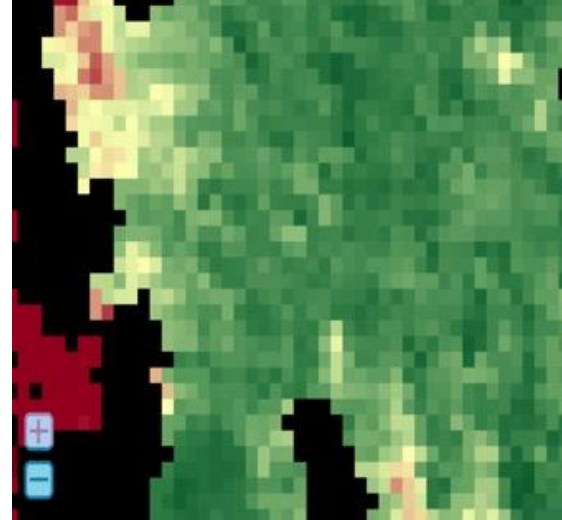


# Multispectral sensor on UAV

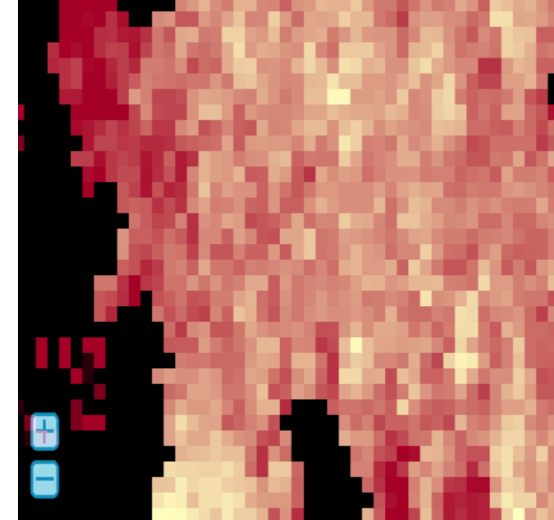
Green NDVI - 550 nm



Red NDVI - 650 nm



Red edge NDVI - 709 nm



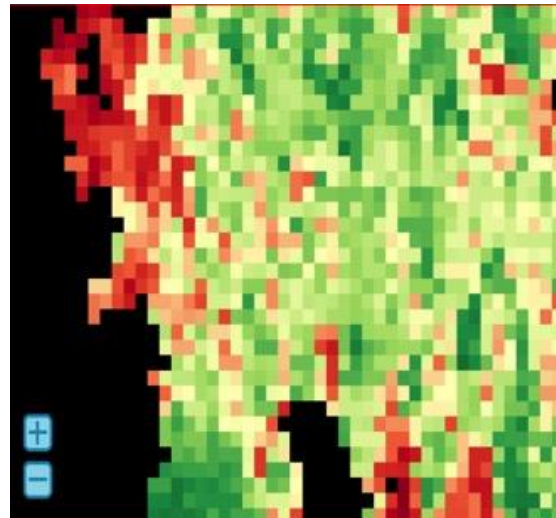
$$\text{GNDVI} = \frac{\text{NIR} - \text{Green}}{\text{NIR} + \text{Green}}$$

$$\text{RNDVI} = \frac{\text{NIR} - \text{Red}}{\text{NIR} + \text{Red}}$$

$$\text{RENDVI} = \frac{\text{NIR} - \text{Red edge}}{\text{NIR} + \text{Red edge}}$$



Stress Index



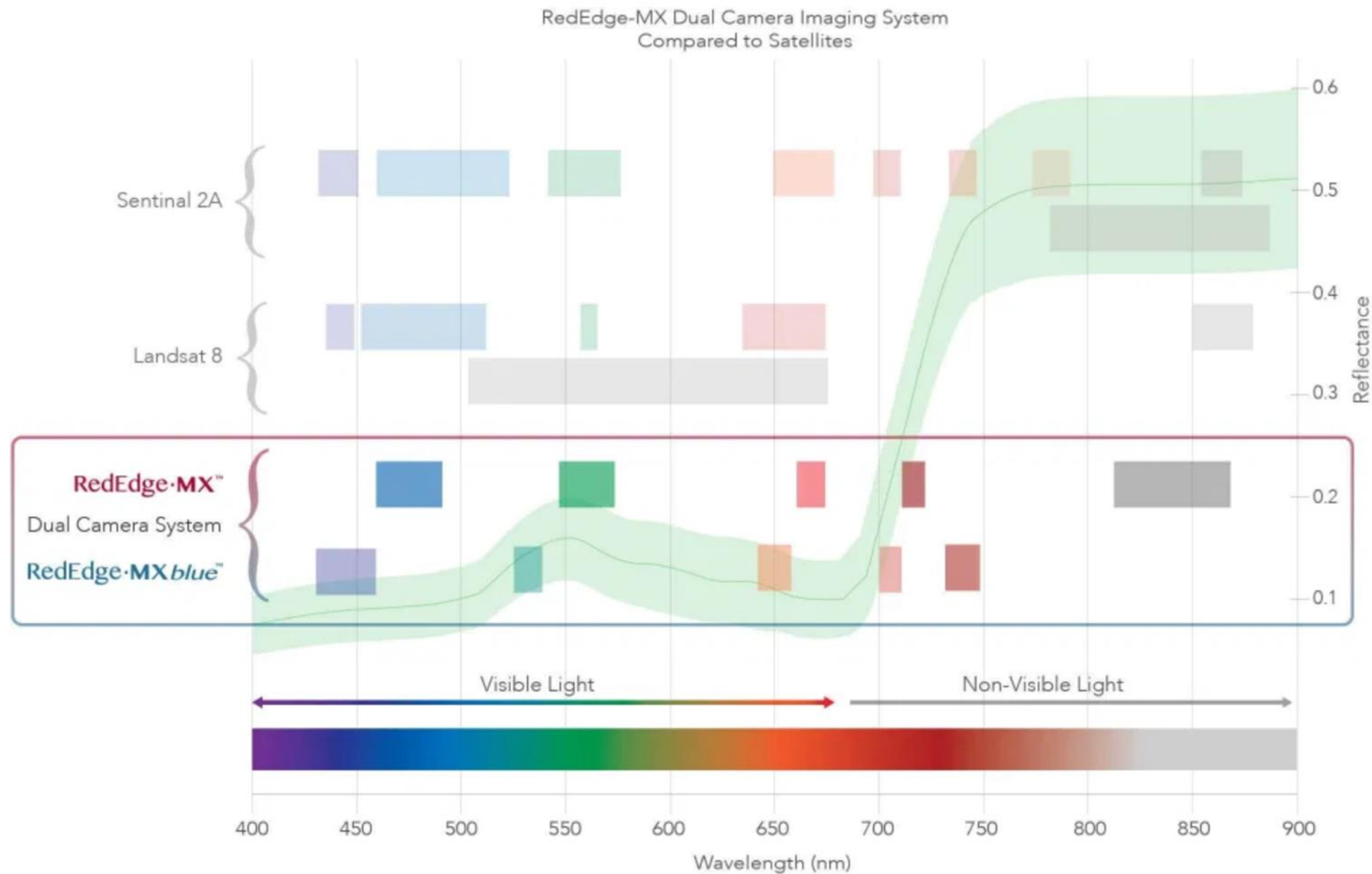
- Vegetation Fraction (Canopy Closure)
- Yield Potential
- and others

Slanrange 2p on a DJI Matrice 100

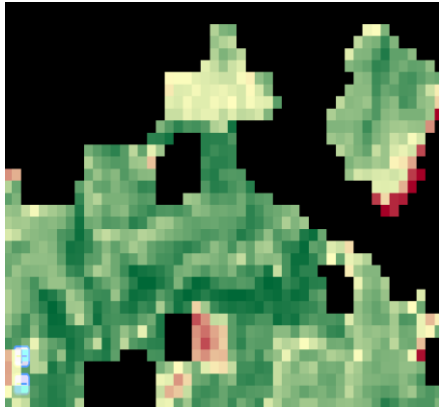




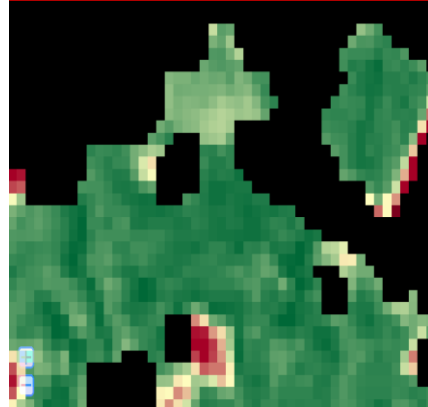
# DIRECT DATA COMPARISON BETWEEN SATELLITE AND DRONE IMAGERY



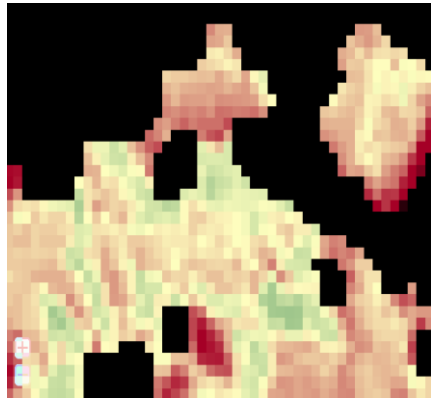
## Healthy



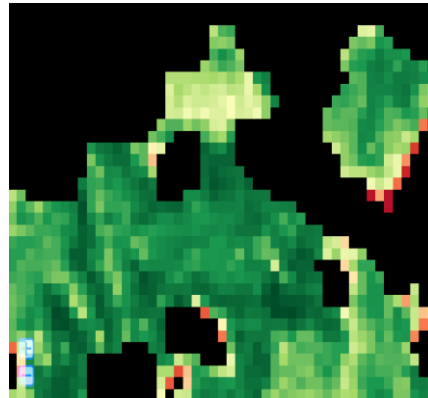
GNDVI



RNDVI

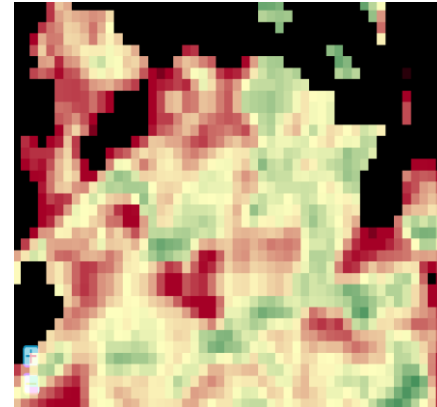


RENDVI

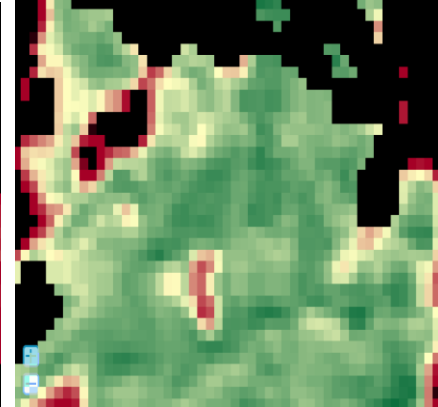


Stress Index

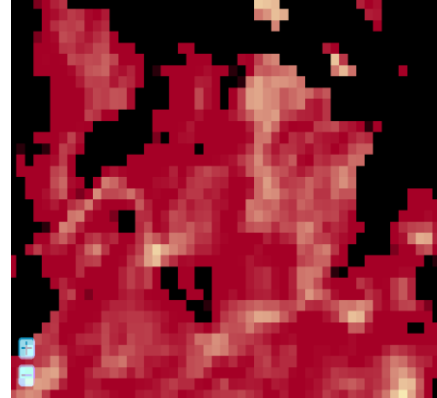
## Stressed



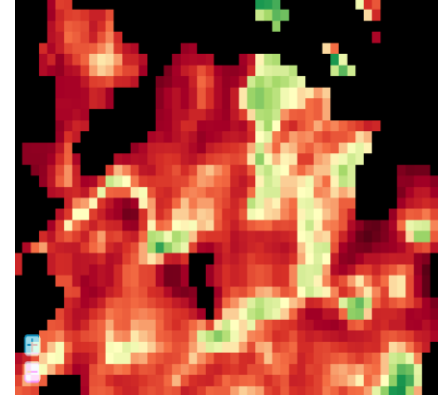
GNDVI



RNDVI



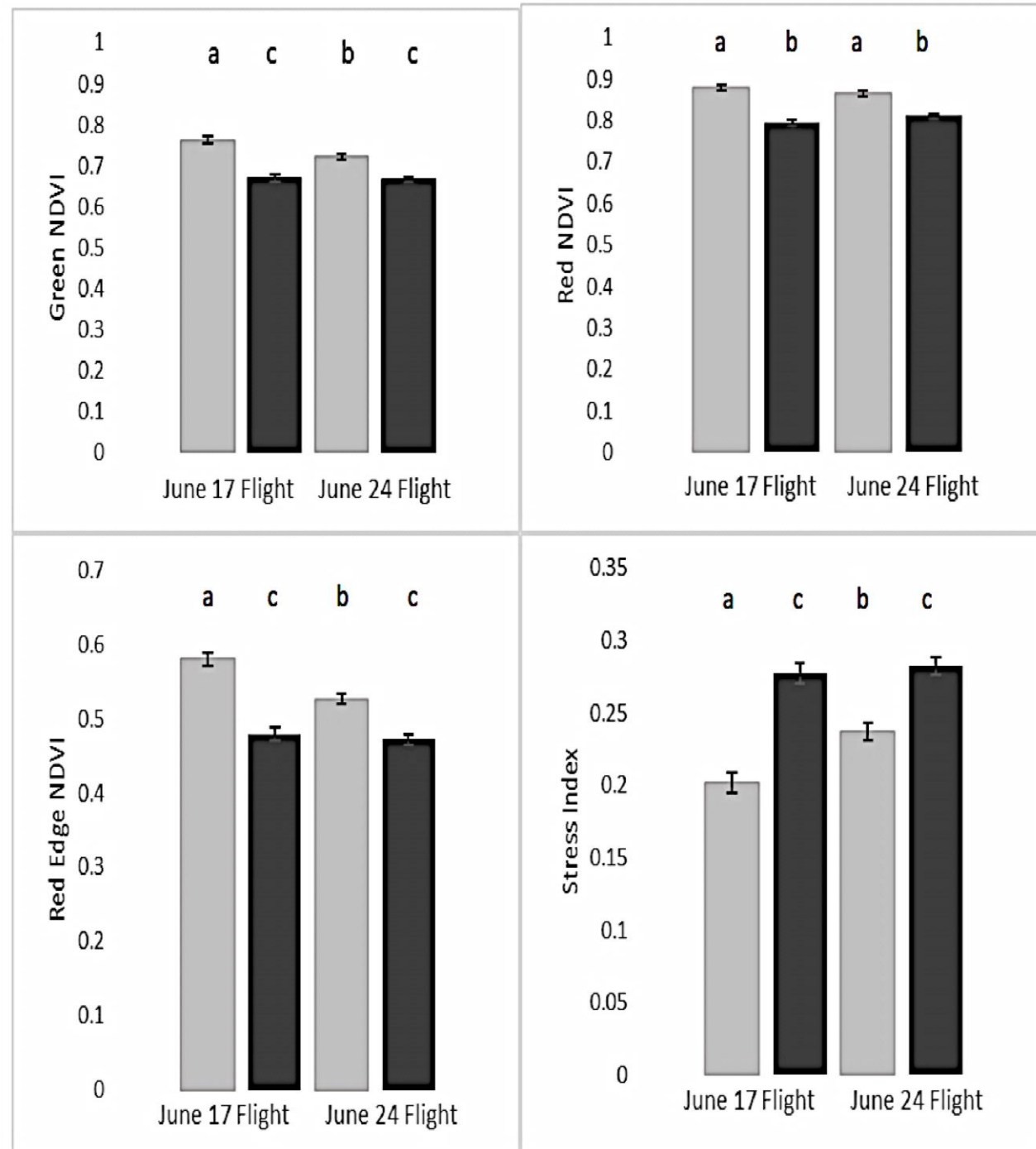
RENDVI



Stress NDVI

Green NDVI, Red NDVI, Red edge NDVI and Stress Index for **conventional (grey)** and **UAV-assisted (black)** scouting at two flight dates.

Different letter above the bar indicates significant difference at  $P=0.05$ .

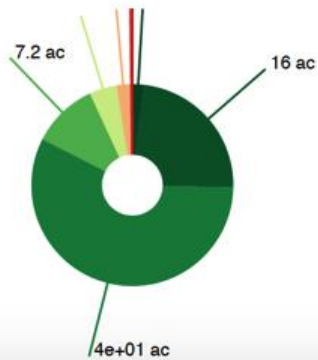
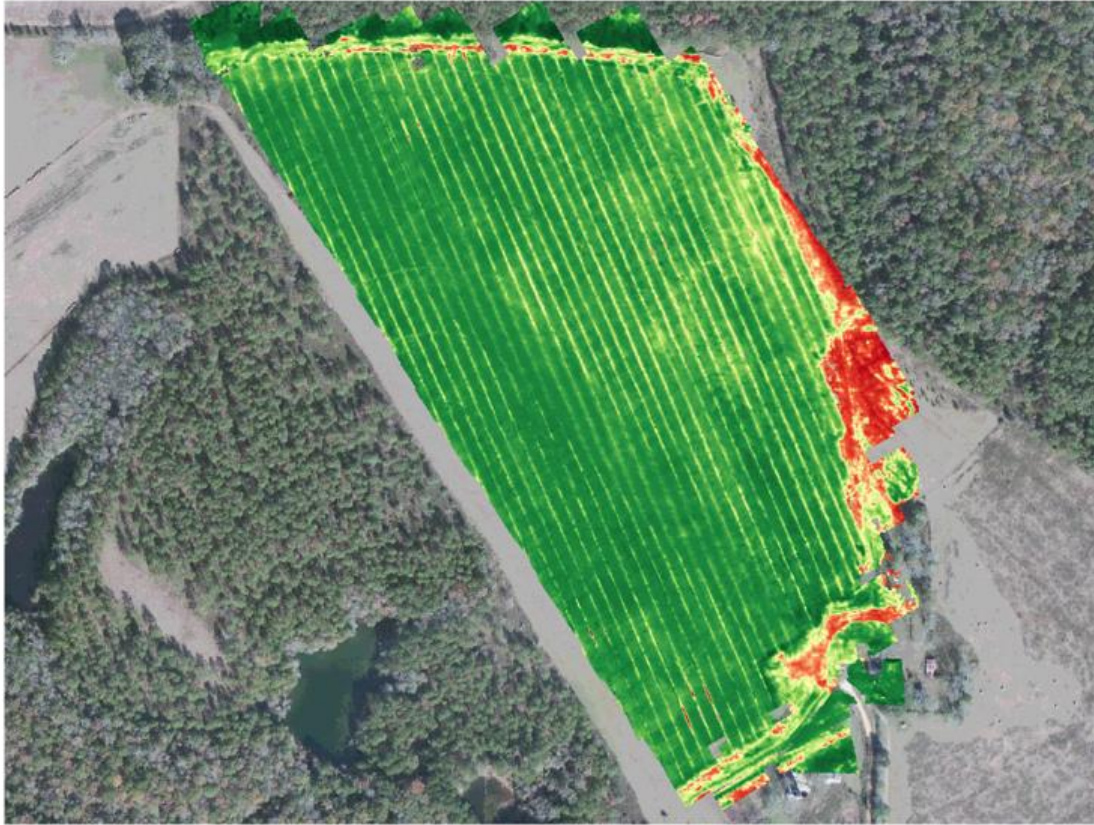




06/09/2017

STRESS

Total map area: 130.0 acres, Vegetated area: 68.4 acres.



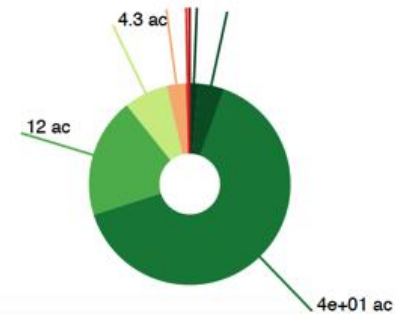
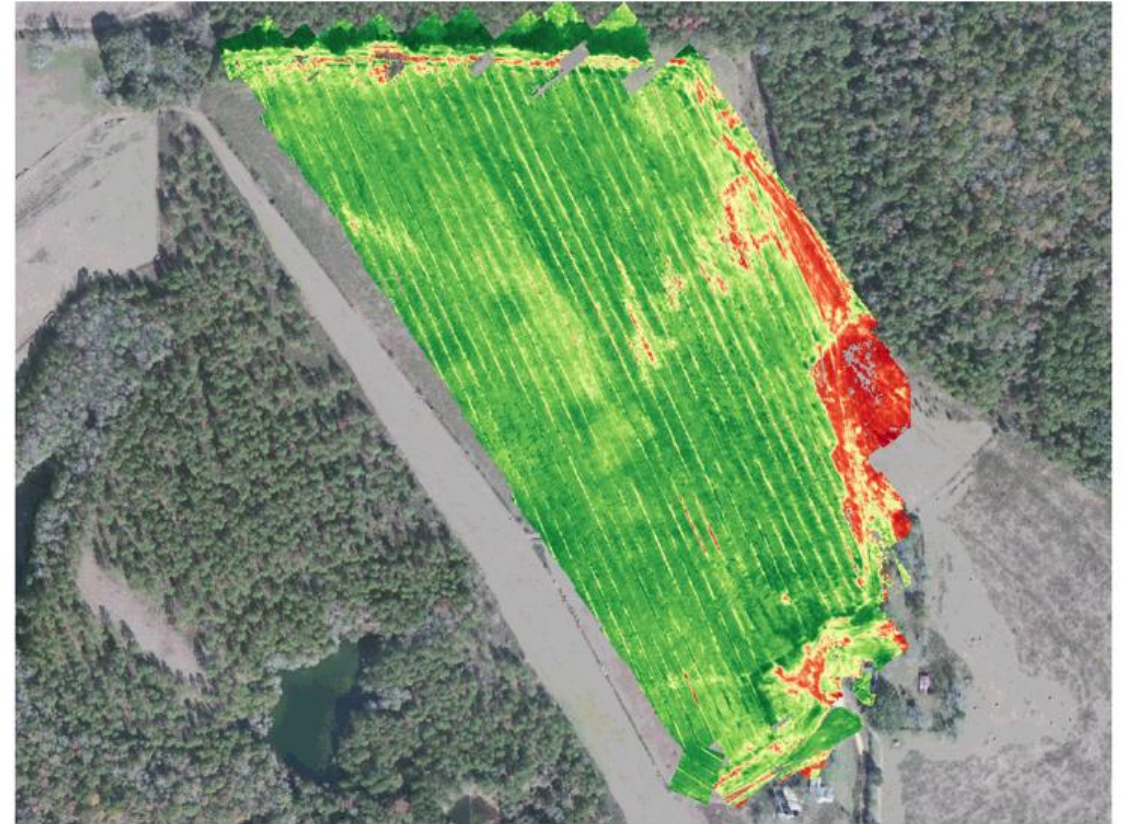
min - max , Area : acres, in %

0.001 - 0.050	Area : 0.02	0.04 %
0.050 - 0.100	Area : 1.22	1.78 %
0.100 - 0.150	Area : 16.10	23.55 %
0.150 - 0.200	Area : 39.08	57.16 %
0.200 - 0.250	Area : 7.23	10.58 %
0.250 - 0.300	Area : 3.00	4.39 %
0.300 - 0.350	Area : 1.45	2.13 %
0.350 - 0.400	Area : 0.25	0.37 %
0.400 - 0.450	Area : 0.01	0.01 %

06/17/2017

STRESS

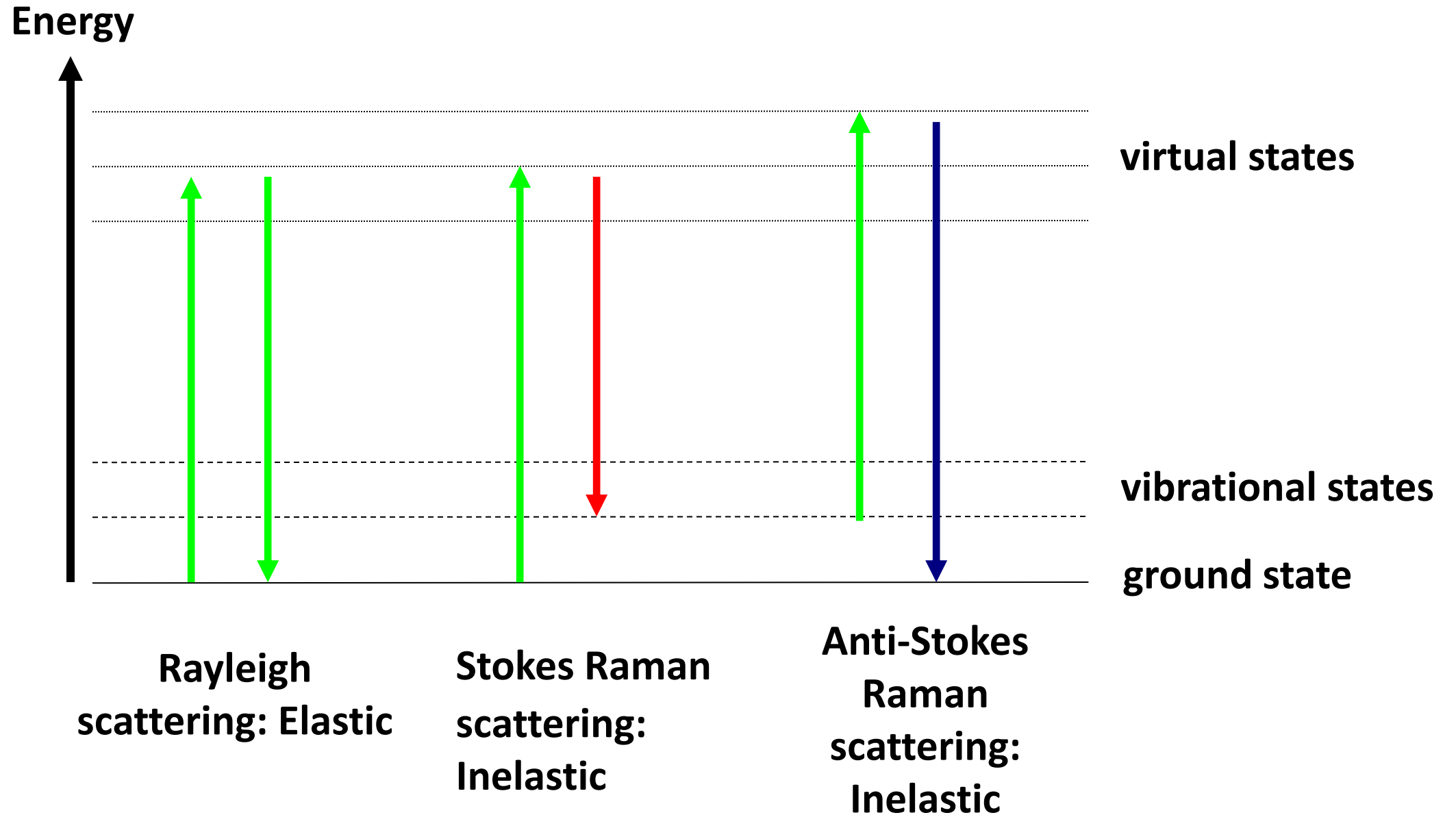
Total map area: 118.8 acres, Vegetated area: 60.3 acres.



min - max , Area : acres, in %

0.001 - 0.050	Area : 0.00	0.00 %
0.050 - 0.100	Area : 0.79	1.30 %
0.100 - 0.150	Area : 2.54	4.22 %
0.150 - 0.200	Area : 38.92	64.54 %
0.200 - 0.250	Area : 11.59	19.22 %
0.250 - 0.300	Area : 4.28	7.09 %
0.300 - 0.350	Area : 1.82	3.02 %
0.350 - 0.400	Area : 0.34	0.56 %
0.400 - 0.450	Area : 0.02	0.04 %

# Raman spectroscopy





# Raman spectroscopy as an early detection tool for rose rosette infection

Charles Farber<sup>1</sup> · Madalyn Shires<sup>2</sup> · Kevin Ong<sup>2</sup> · David Byrne<sup>3</sup> · Dmitry Kurouski<sup>1,4</sup>



Agilent  
Resolve  
spectrometer  
- 830 nm  
laser

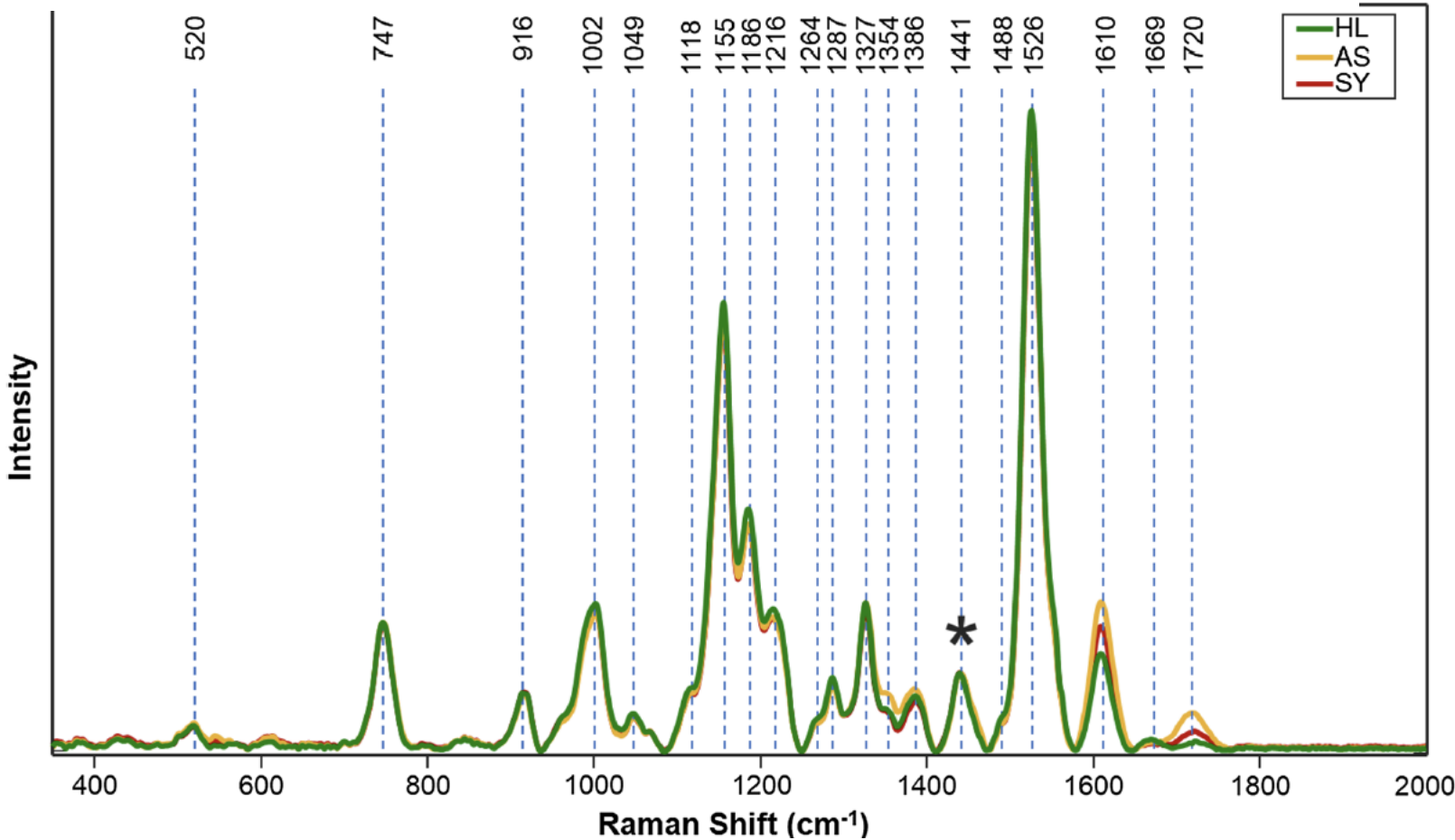
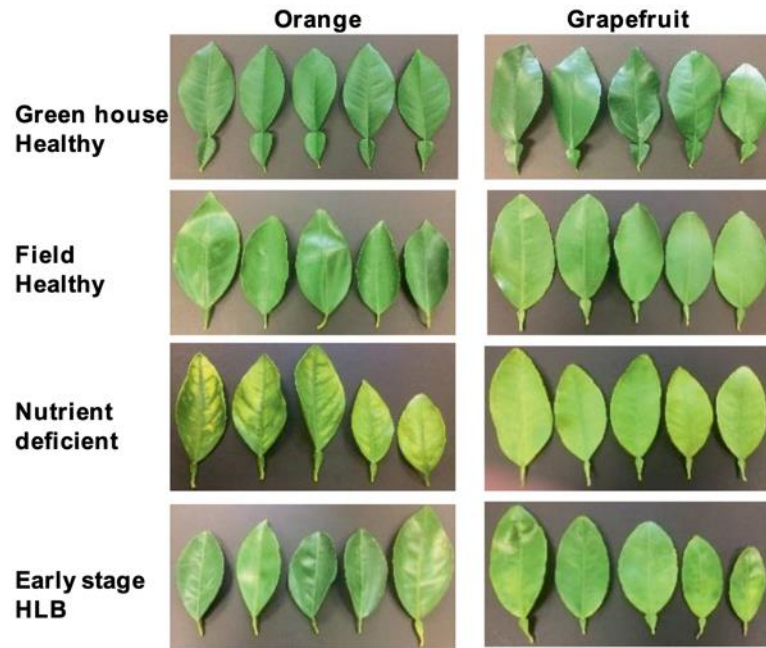


Table 1 Vibrational band assignments for rose leaf spectra

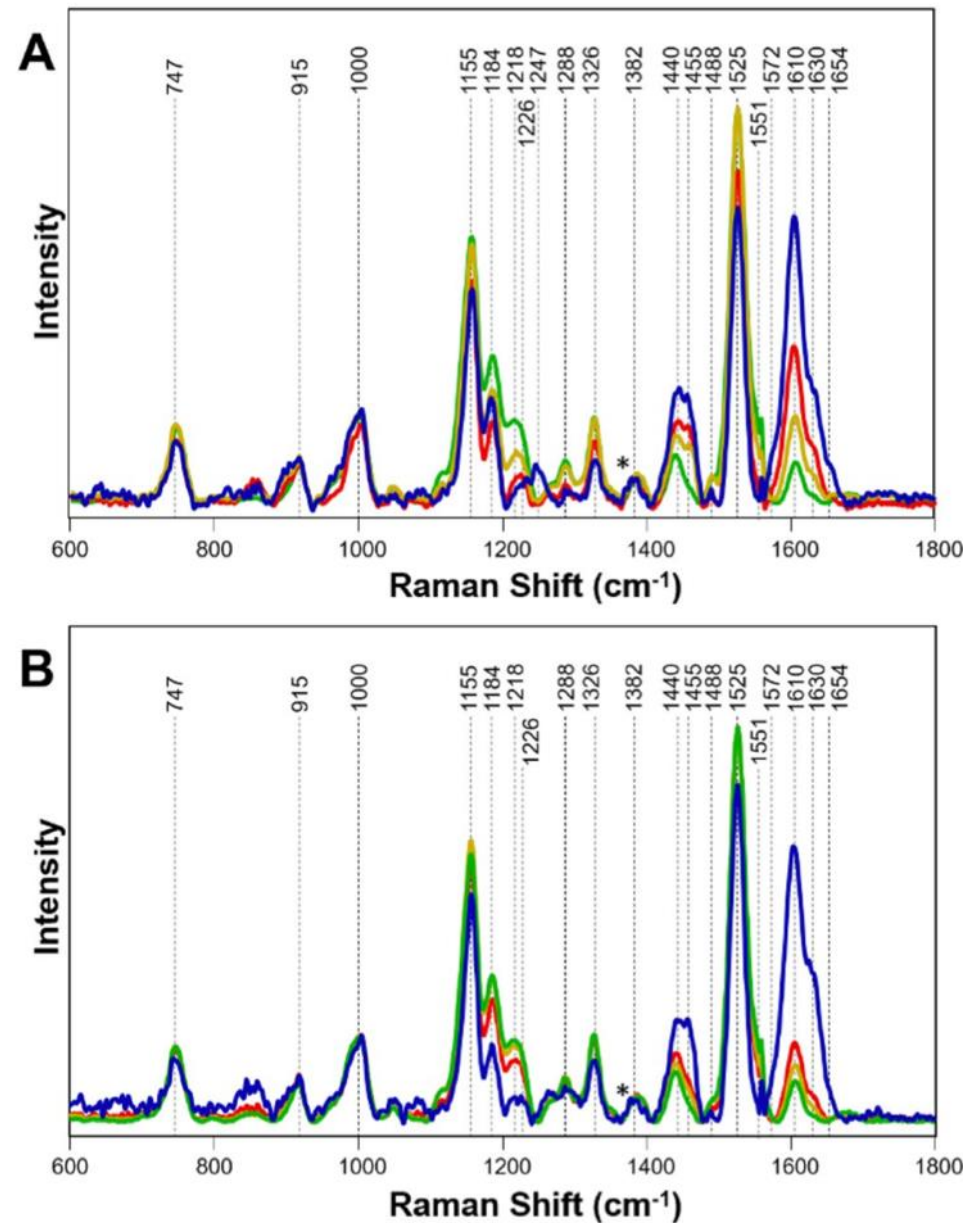
Band	Vibrational mode
520	$\nu(\text{C-O-C})$ glycosidic
740–747	$\gamma(\text{C-O-H})$ of COOH
905–918	$\nu(\text{C-O-C})$ in plane, symmetric
1000	In-plane $\text{CH}_3$ rocking of polyene
1048	$\nu(\text{C-O}) + \nu(\text{C-C}) + \delta(\text{C-O-H})$
1118	Sym $\nu(\text{C-O-C})$ , C–O–H bending
1157	C–C stretching; $\nu(\text{C-O-C})$ , $\nu(\text{C-C})$ in glycosidic linkages, asymmetric ring breathing
1186	$\nu(\text{C-O-H})$ next to aromatic ring + $\sigma(\text{CH})$
1216	$\delta(\text{C-C-H})$
1264	Guaiacyl ring breathing, C–O stretching (aromatic)
1287	$\delta(\text{C-C-H})$
1327	$\delta\text{CH}_2$ bending
1354	$\delta(\text{CH}_2) + \delta(\text{CH}_3)$
1386	$\delta\text{CH}_2$ bending
1441	$\delta(\text{CH}_2) + \delta(\text{CH}_3)$
1488	$\delta(\text{CH}_2) + \delta(\text{CH}_3)$
1526	$\text{C}=\text{C}$ (in-plane)
1610	$\nu(\text{C-C})$ aromatic ring + $\sigma(\text{CH})$
1669	$=\text{O}$ stretching, amide I
1720	$\text{C}=\text{O}$ stretching

# Raman Spectroscopy vs Quantitative Polymerase Chain Reaction In Early Stage Huanglongbing Diagnostics

Lee Sanchez<sup>1</sup>, Shankar Pant<sup>2,5</sup>, Kranthi Mandadi<sup>2,3</sup> & Dmitry Kurouski<sup>1,4</sup>



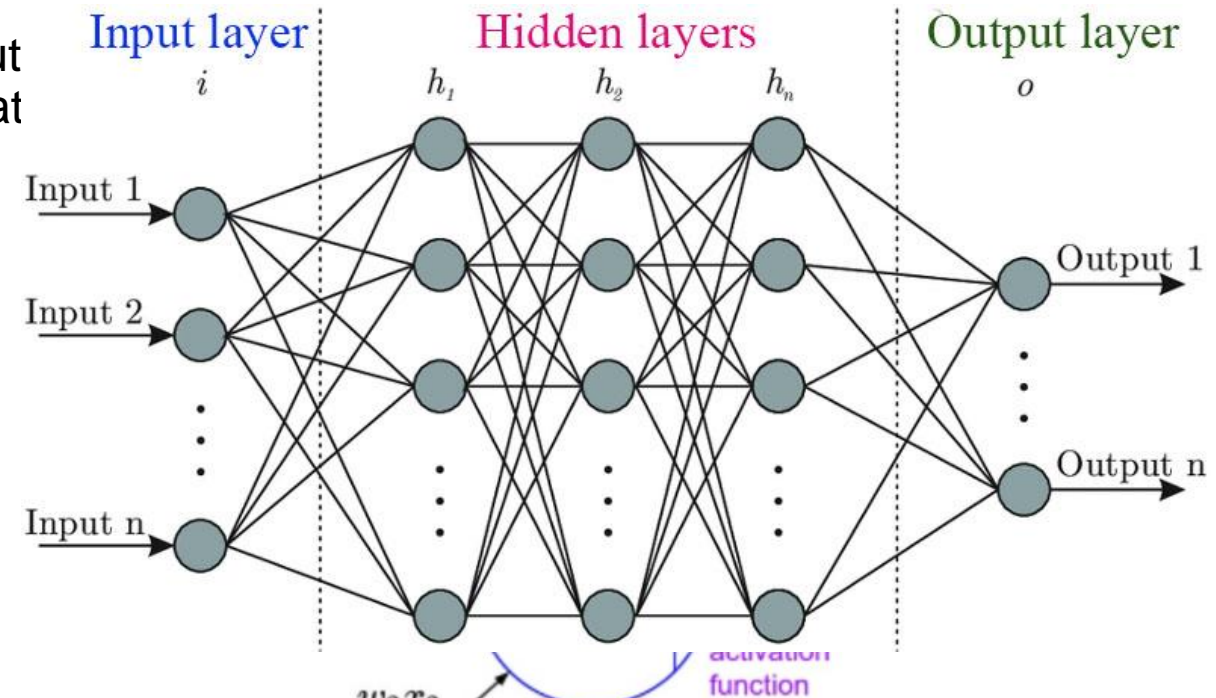
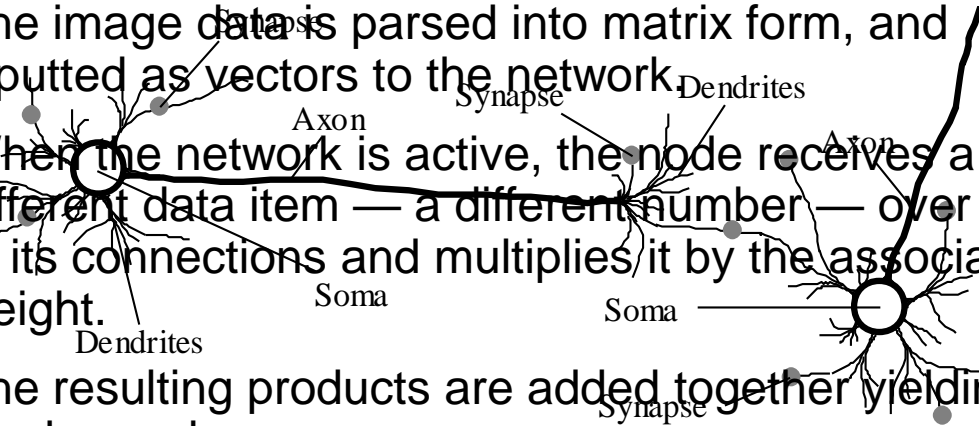
**Figure 1.** Leaf samples collected from greenhouse healthy (GHH) and field healthy leaves (IFH), as well leaves from both orange and grapefruit trees with nutrient deficit (ND) symptoms and asymptomatic HLB (Figure panels for ND and asymptomatic HLB were adapted from Sanchez *et al.*, 2019, Anal. Bioanal. Ch




**Figure 4.** Raman spectra collected from leaves of GHH (green), IFH (gold), asymptomatic HLB infection (red), and nutrient-deficit (blue) symptoms in (A) grapefruit and (B) orange trees. Spectra are normalized on the CH<sub>2</sub> vibrational band that is present in nearly all classes in biological molecules (marked by asterisks (\*)).

# Introduction to Neural Networks

- Neural Network:** a type of machine learning model that uses a network of functions to understand and translate data
1. **Nodes** form connections based on the respective layers the connections are being established for
  2. To each of these connections, the node randomly assigns a number (weight) from biological neurons
  3. The image data is parsed into matrix form, and inputted as vectors to the network
  4. When the network is active, the node receives a different data item — a different number — over each of its connections and multiplies it by the associated weight.
  5. The resulting products are added together yielding a single number
  6. The weights and thresholds are continually adjusted during each **epoch** until training data with the same labels consistently yield similar outputs.



**node:** receives a different data item over each of its connections and multiplies it by the associated weight

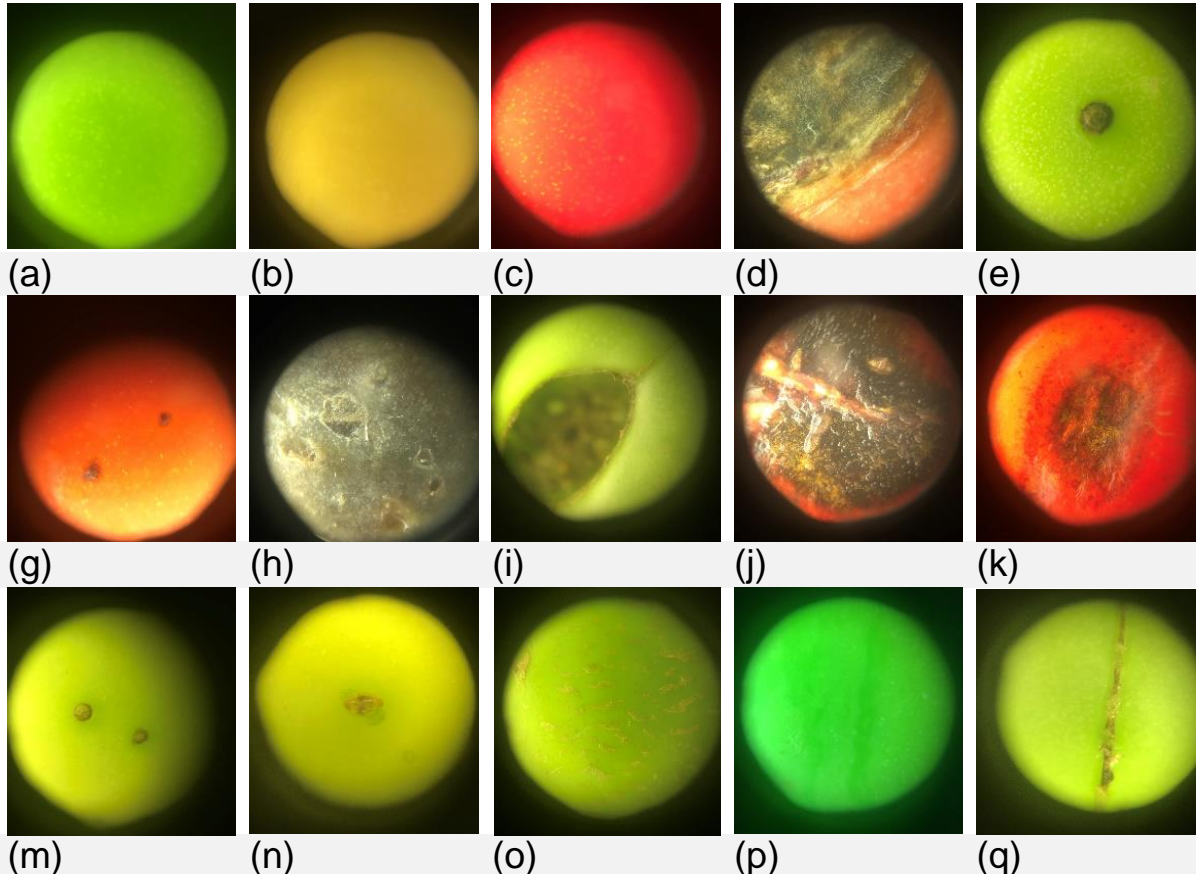
- denoted by the symbol,  in the diagram above

**weight:** the parameter within a neural network that transforms input data within the network's hidden layers

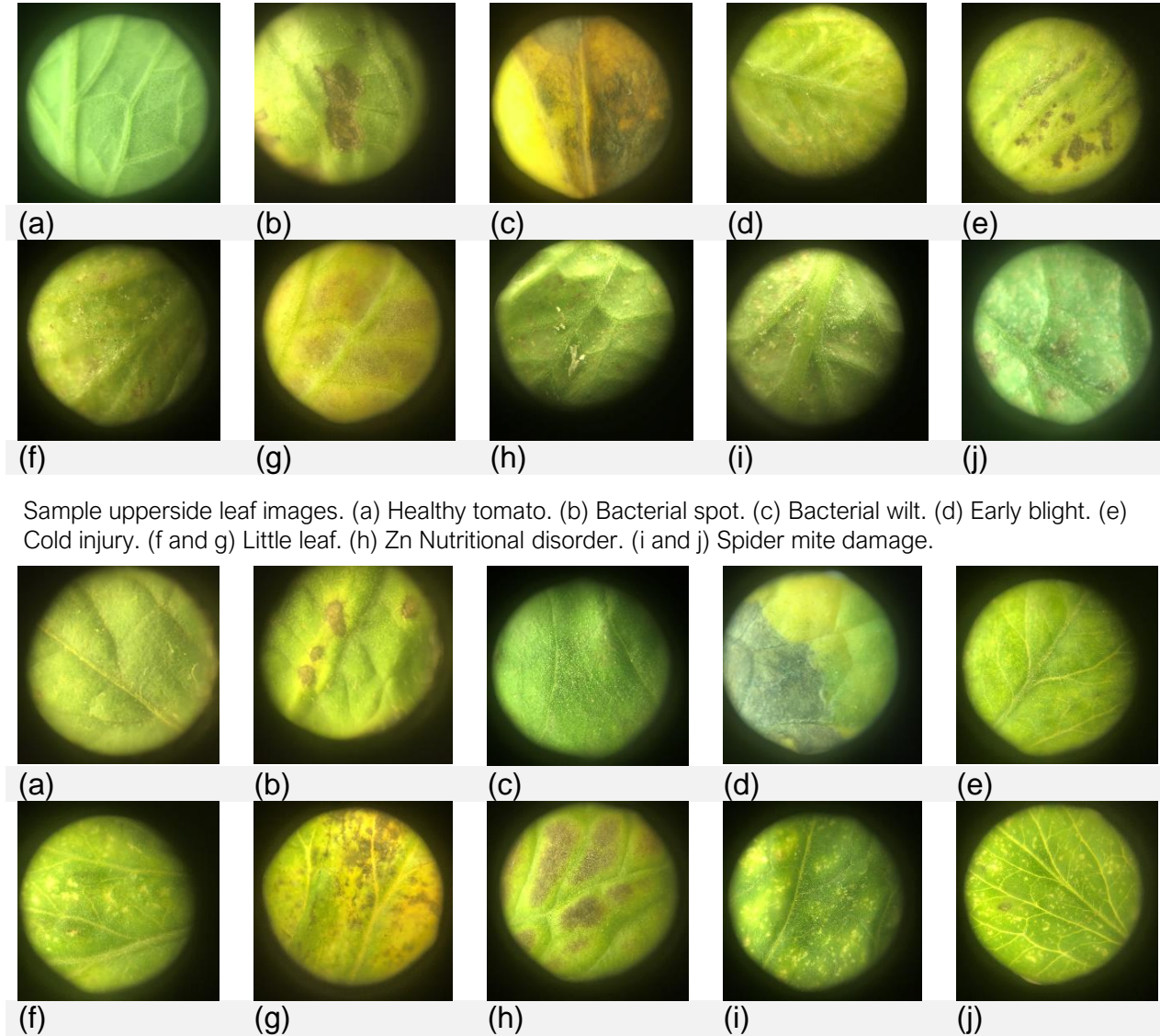
**epoch:** A full training pass over the entire dataset such that each example has been seen once



# Raw Dataset



Sample fruit images. (a-c) Healthy tomato. (d) Anthracnose. (e-g) Bacterial spot. (h) Buckeye rot. (i) Catface-zippering. (j and k) Cladosporium fruit rot. (m) Pox-fleck. (n) Catface-Zippering-Zebra Stripe. (p) Rain check. (q) Zippering



Sample underside leaf images. (a) Healthy tomato. (b) Bacterial spot. (c) Early blight. (d) Cold injury. (e and f) Little leaf. (g) Zn Nutritional disorder. (h and i) Spider mite damage. (j) Tomato yellow leaf curl.

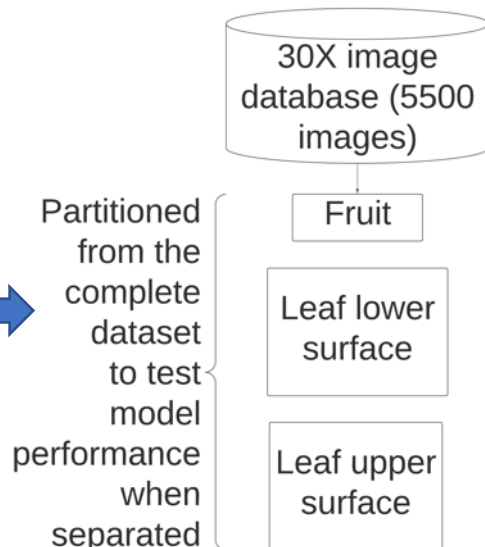


# Methods

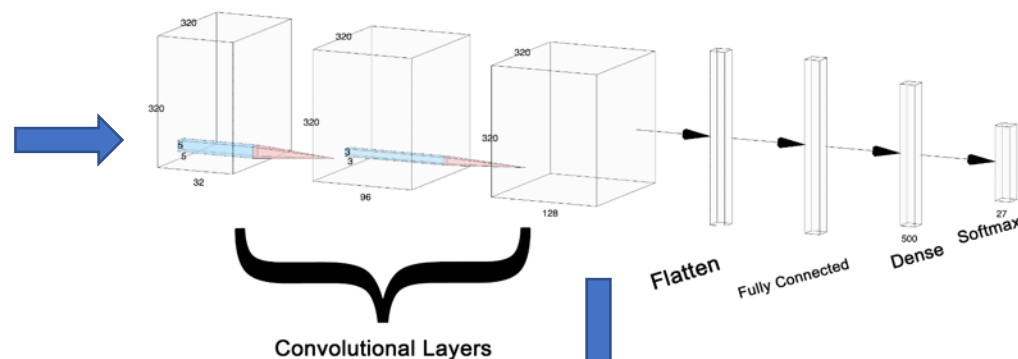
## Step 1: Imaging with 30X Smartphone Microscope



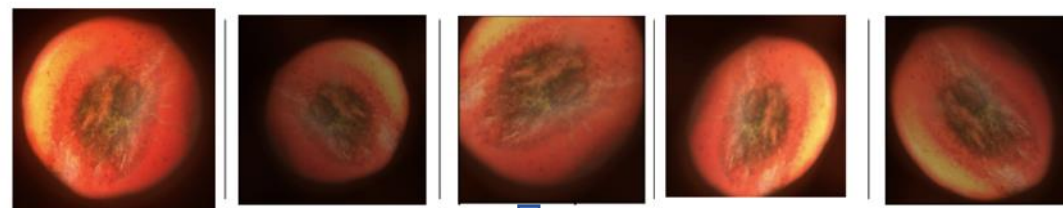
## Step 2: Dataset



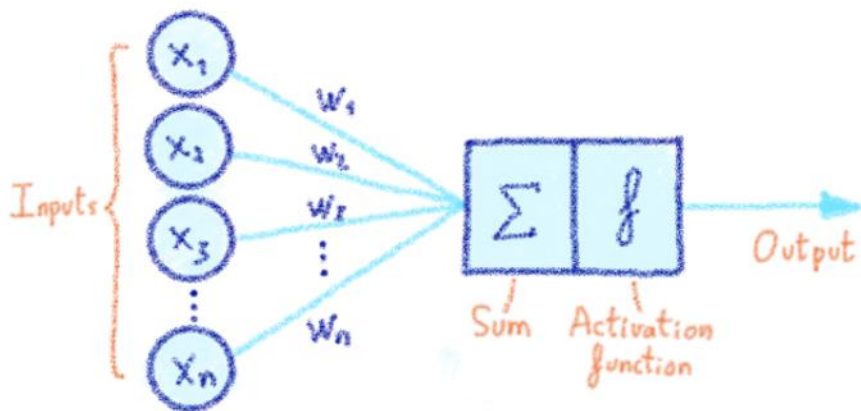
## Step 3: Building of CNN



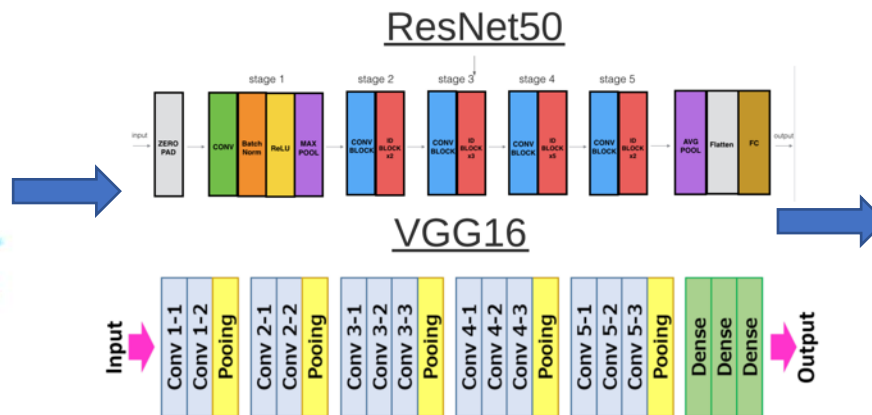
## Step 4: Data Augmentation



## Step 5: Training the Model

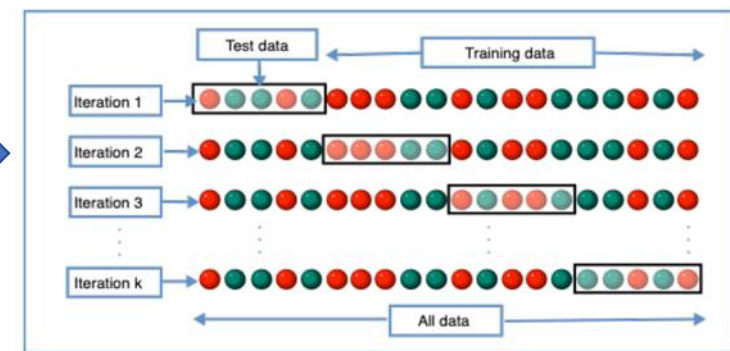


## Step 6: External Models

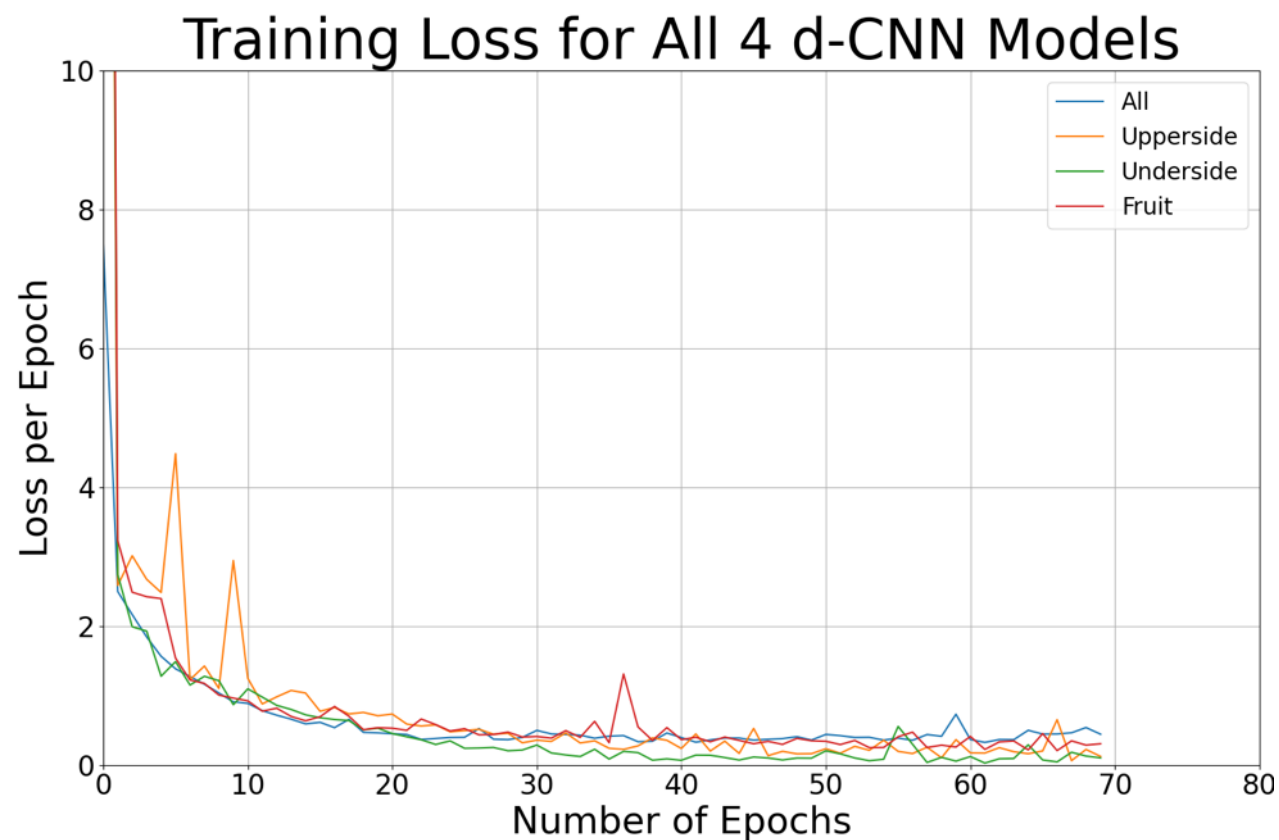
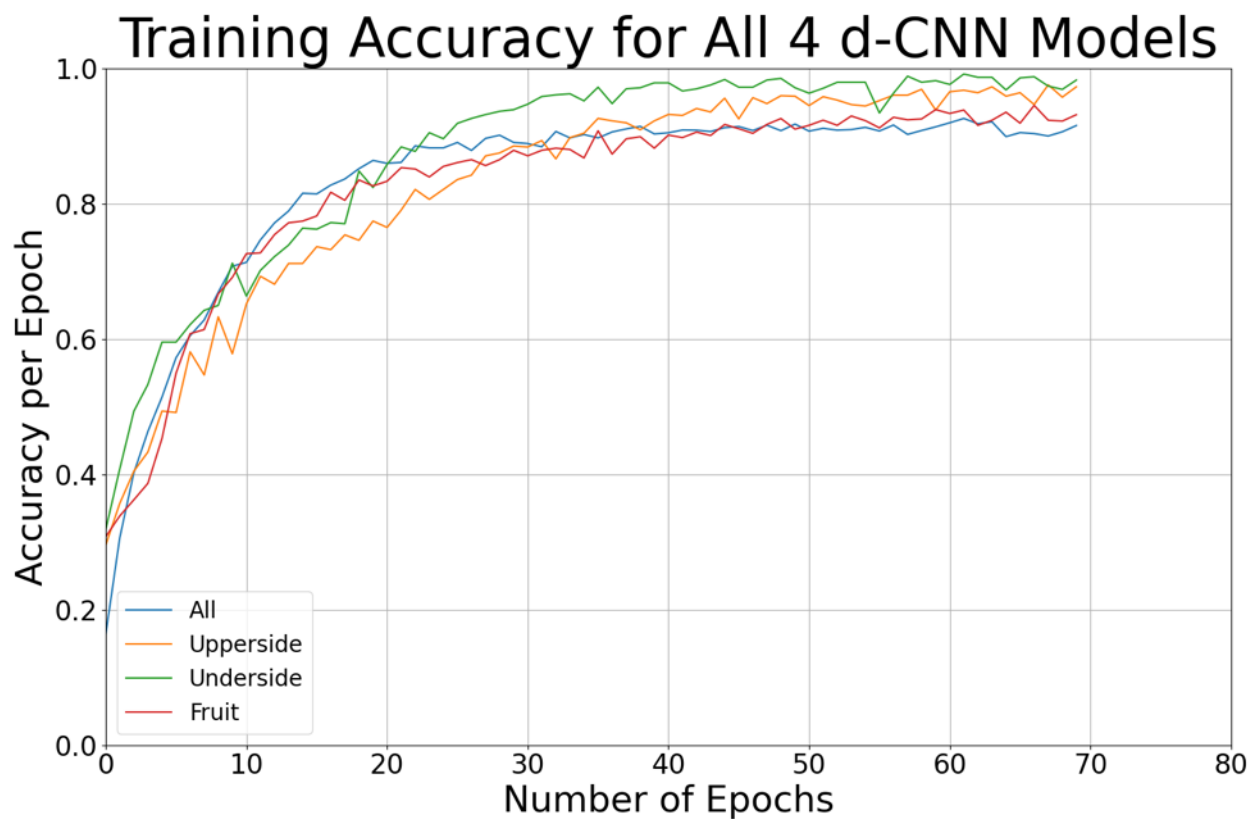
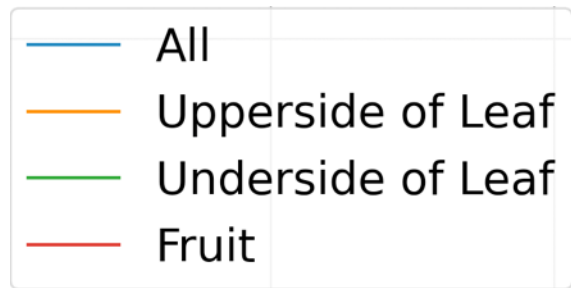


## Step 7: Performance Measures

### KFold Cross Validation

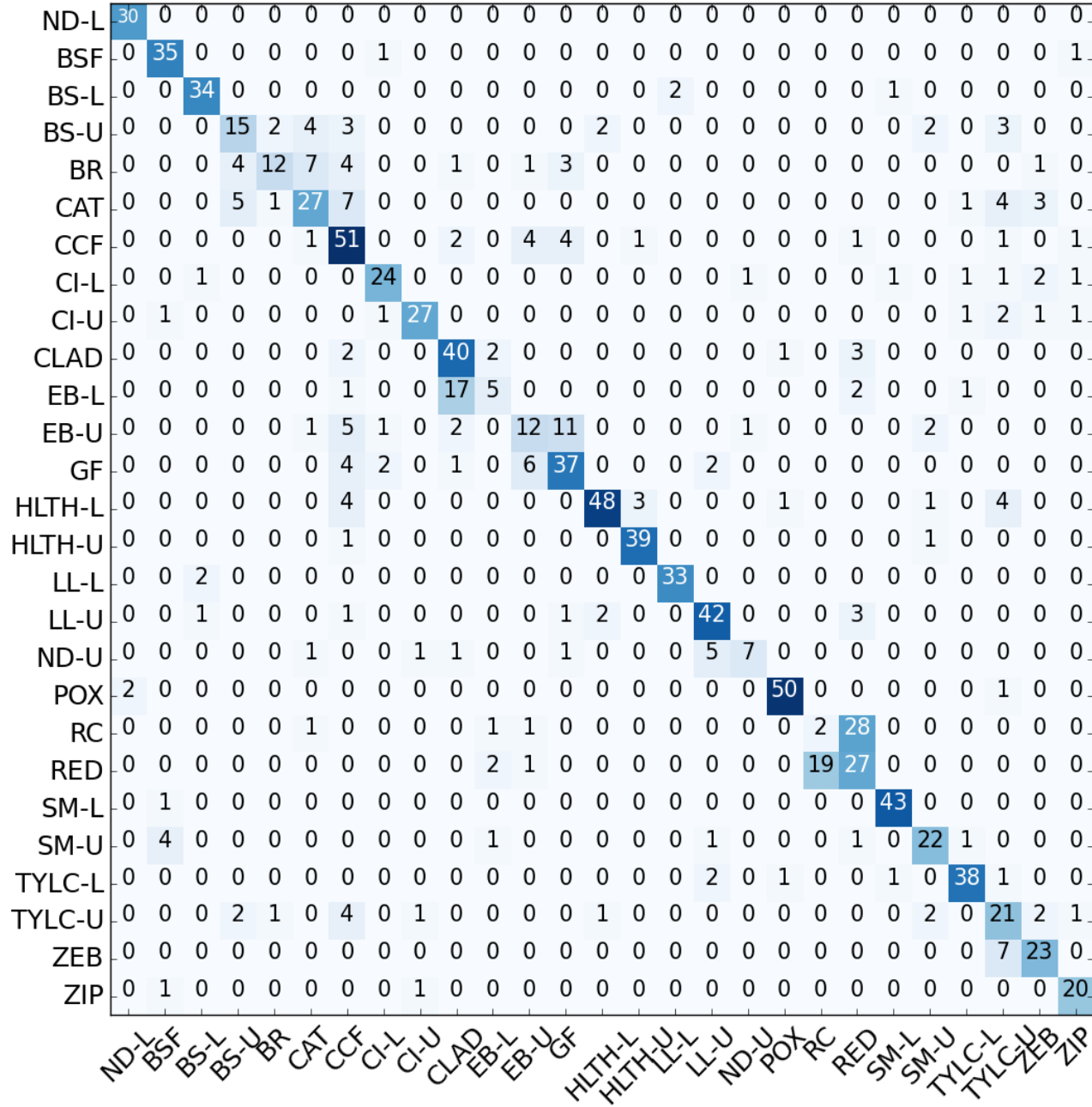


# CNN: Training Accuracy and Loss



## All Classes CNN From Scratch

True label



Predicted label

## Qualitative Analysis: Confusion Matrices

Part of Plant		Accuracy	Loss
All		90.46% (+- 2.12%)	0.64
Separated by part of Plant	Fruit	97.05 (+- 1.04%)	0.26
	Under side leaf	96.50 %(+- 1.09%)	0.29
	Upper side leaf	95.57% (+- 1.14%)	0.48

KFold Average Scores for All CNN Models From Scratch

# Recombinase Polymerase Amplification (RPA)



ELSEVIER

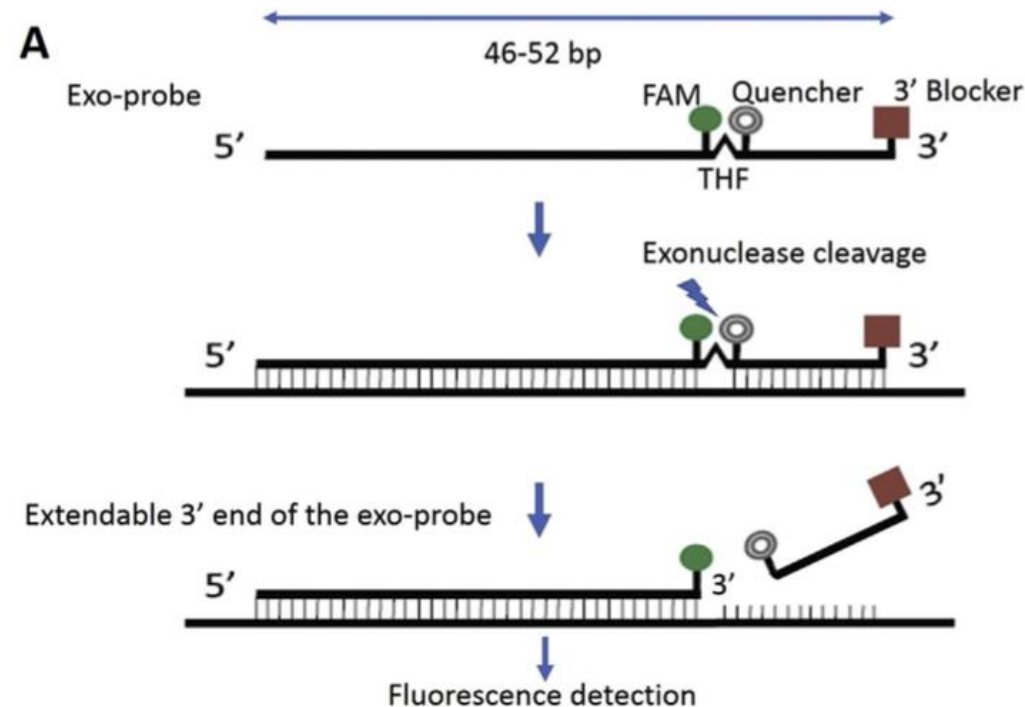


## Recombinase polymerase amplification applied to plant virus detection and potential implications

Binoy Babu<sup>a,b,\*\*</sup>, Francisco M. Ochoa-Corona<sup>c</sup>, Mathews L. Paret<sup>a,d,\*</sup>

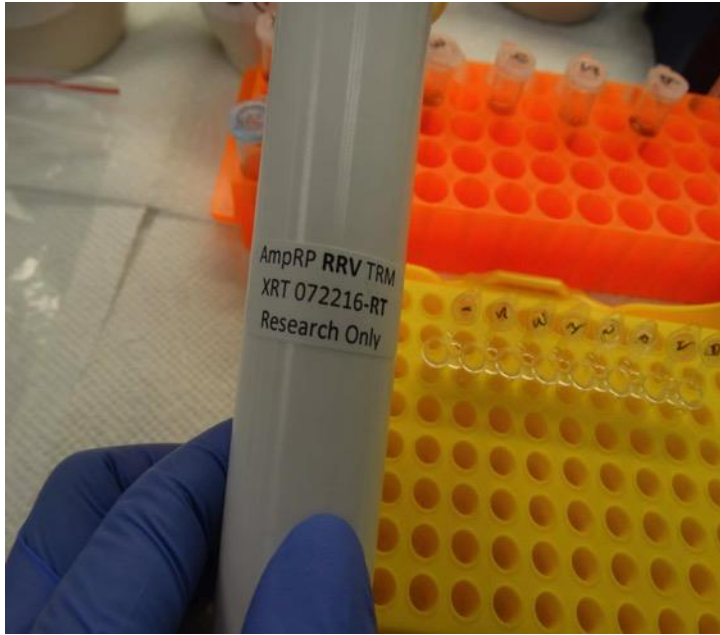


*B. Babu et al.*

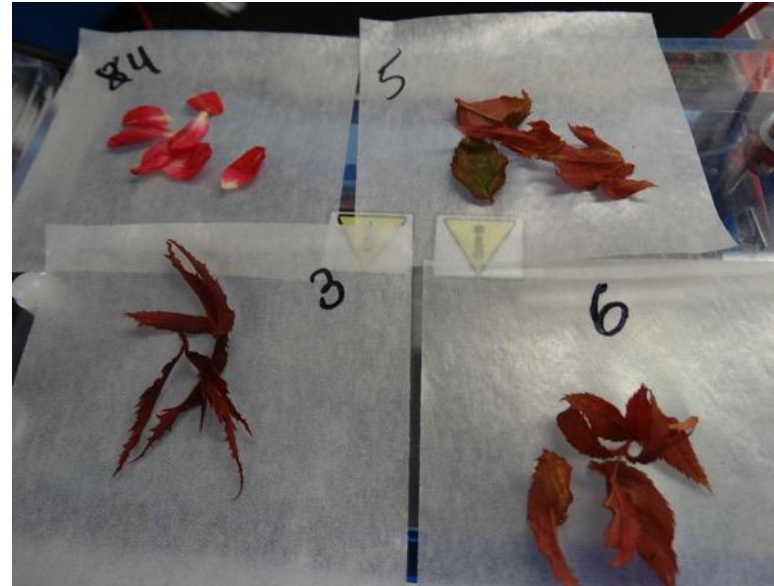




# Sample Preparation for RPA



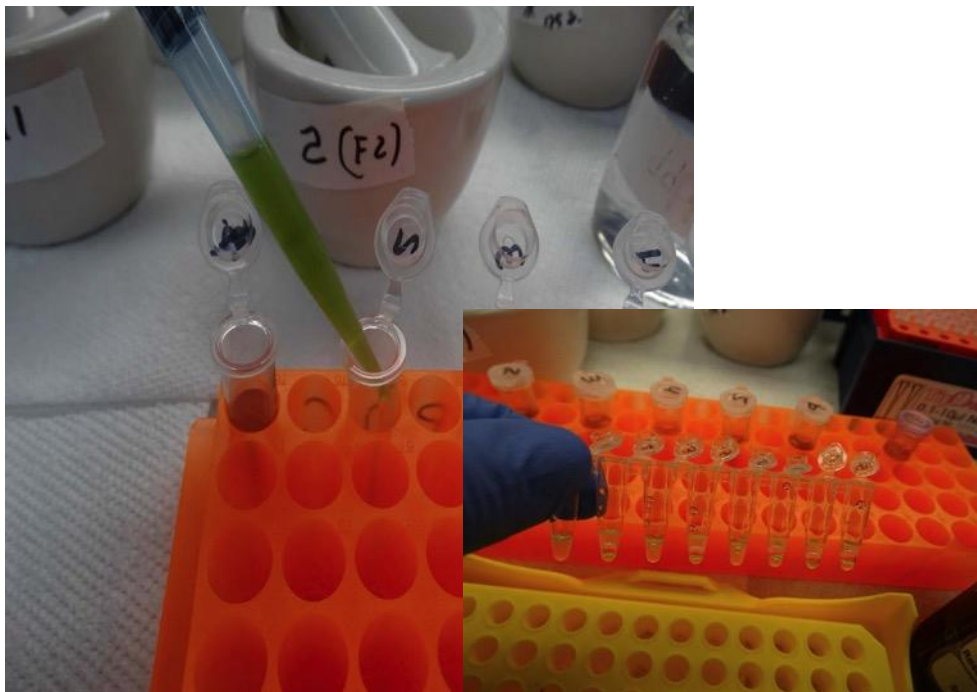
Ready to use Agdia “AmplifyRP XRT” RRV Pellet which includes all amplification reagents



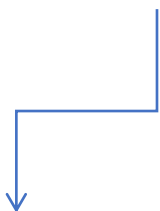
Grind plant samples (0.1 g) with 1:1 (w:v) GE ELISA Buffer. Extract diluted 1:4 in sterile deionized water



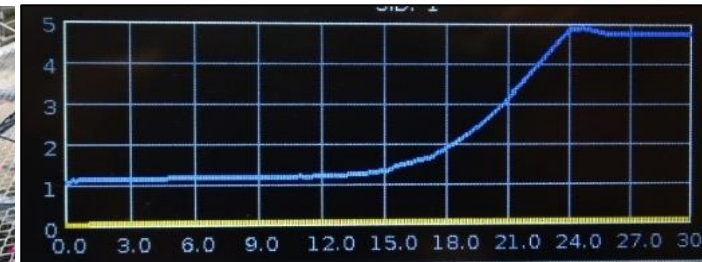
Add 23  $\mu$ L of PD1 Buffer into the RPA pellet (Rehydration step – 1min)



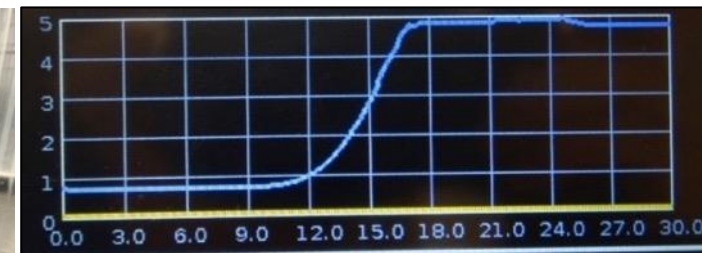
Add 1  $\mu$ L of diluted sample to the PCR tubes with RPA reaction.



Run RPA (39 C, 15-30 min)  
Battery powered to last 4 h and portable  
8 samples at a time



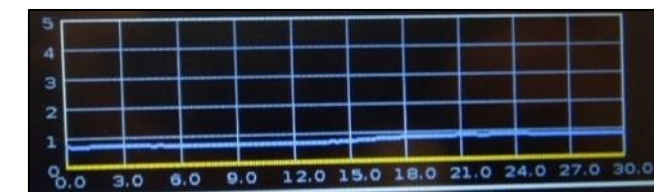
RRV infected plant 1



RRV infected plant 2



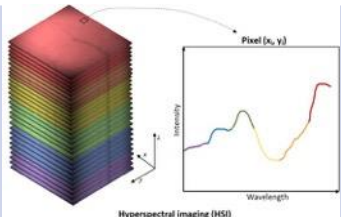
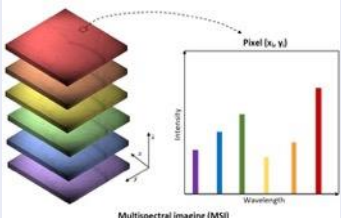

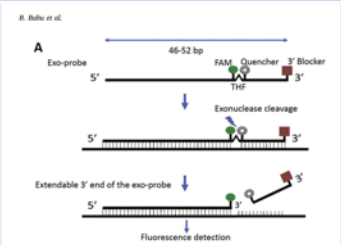
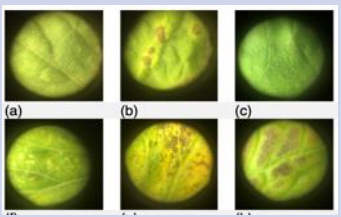
Healthy plant control



Negative control



# Summary

Method	Approach	
Multi-spectral sensor	Reflectance from light for identifying areas with reflectance differences indicating hotspots of biotic and abiotic issues	 <p>Hyperspectral imaging (HSI)</p>
Hyper-spectral sensor	Above + identifying areas with unique reflectance differences for certain diseases.	 <p>Multispectral imaging (MSI)</p>
Raman spectroscopy	Laser induced vibrational spectra identifying chemical differences in plants that could be unique to certain diseases	
Recombinase Polymerase Amplification	Very specific and sensitive field-based DNA/RNA detection of pathogens	 <p>B. Bibe et al.</p> <p>A</p> <p>46-52 bp</p> <p>Exo-probe 5' 3'</p> <p>FAM Quencher 3' Blocker</p> <p>Exonuclease cleavage</p> <p>Extendable 3' end of the exo-probe</p> <p>Fluorescence detection</p>
Machine Learning and Artificial Intelligence (AI)	Neural network and image/object-based detection for disease identification	 <p>(a) (b) (c)</p> <p>(d) (e) (f)</p>