New tools in plant disease assessments

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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

Slantrange 2p on a DJI Matrice 100
DIRECT DATA COMPARISON BETWEEN SATELLITE AND DRONE IMAGERY

RedEdge-MX Dual Camera Imaging System Compared to Satellites

- Sentinel 2A
- Landsat 8

RedEdge-MX
Dual Camera System

RedEdge-MX blue

Visible Light
Non-Visible Light

Wavelength (nm)

Reflectance

400 450 500 550 600 650 700 750 800 850 900
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 $P=0.05$. 
Raman spectroscopy

Energy

Rayleigh scattering: Elastic

Stokes Raman scattering: Inelastic

Anti-Stokes Raman scattering: Inelastic

virtual states

vibrational states

ground state
Raman spectroscopy as an early detection tool for rose rosette infection

Charles Farber¹ · Madalyn Shires² · Kevin Ong² · David Byrne³ · Dmitry Kurouski¹/⁴

Table 1 Vibrational band assignments for rose leaf spectra

<table>
<thead>
<tr>
<th>Band</th>
<th>Vibrational mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>520</td>
<td>ν(C–O–C) glycosidic</td>
</tr>
<tr>
<td>746–747</td>
<td>ν(C–O–H) of COOH</td>
</tr>
<tr>
<td>905–918</td>
<td>ν(C–O–C) in plane, symmetric</td>
</tr>
<tr>
<td>1000</td>
<td>In-plane C=O, rocking of polyene</td>
</tr>
<tr>
<td>1118</td>
<td>Sym ν(C=O–C), C–O–H bending</td>
</tr>
<tr>
<td>1186</td>
<td>C–C stretching; ν(C–O–C), ν(C–C) in glycosidic linkages, asymmetric ring breathing</td>
</tr>
<tr>
<td>1216</td>
<td>8(C–C–H)</td>
</tr>
<tr>
<td>1264</td>
<td>Guaiacyl ring breathing, C–O stretching (aromatic)</td>
</tr>
<tr>
<td>1287</td>
<td>8(C–C–H)</td>
</tr>
<tr>
<td>1327</td>
<td>8CH3 bending</td>
</tr>
<tr>
<td>1354</td>
<td>8(CH3) + 8(CH3)</td>
</tr>
<tr>
<td>1386</td>
<td>8CH2 bending</td>
</tr>
<tr>
<td>1441</td>
<td>8(CH3) + 8(CH3)</td>
</tr>
<tr>
<td>1488</td>
<td>8(CH3) + 8(CH3)</td>
</tr>
<tr>
<td>1526</td>
<td>–C=C– (in-plane)</td>
</tr>
<tr>
<td>1610</td>
<td>ν(C=C) aromatic ring + ν(CH)</td>
</tr>
<tr>
<td>1669</td>
<td>=O stretching, amide I</td>
</tr>
<tr>
<td>1720</td>
<td>C=O stretching</td>
</tr>
</tbody>
</table>

Agilent Resolve spectrometer - 830 nm laser
Raman Spectroscopy vs Quantitative Polymerase Chain Reaction In Early Stage Huanglongbing Diagnostics

Lee Sanchez1, Shankar Pant2,3, Kranthi Mandadi2,4,5, & Dmitry Kurowski2,4,5

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 CH2 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 utilizing a computational learning system and a network of functions to understand and translate a data input of one form into a desired output.

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.

**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. (o) Rain check. (p) Zebra stripe. (q) Zippering

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

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

 Partitioned from the complete dataset to test model performance when separated

- Fruit
- Leaf lower surface
- Leaf upper surface

Step 3: Building of CNN

Step 4: Data Augmentation

Convolutional Layers

Step 5: Training the Model

Step 6: External Models

- ResNet50
- VGG16

Step 7: Performance Measures

Kfold Cross Validation
CNN: Training Accuracy and Loss

Training Accuracy for All 4 d-CNN Models

Training Loss for All 4 d-CNN Models

- All
- Upperside of Leaf
- Underside of Leaf
- Fruit
### Qualitative Analysis: Confusion Matrices

#### KFold Average Scores for All CNN Models From Scratch

<table>
<thead>
<tr>
<th>Part of Plant</th>
<th>Accuracy</th>
<th>Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>90.46% (+- 2.12%)</td>
<td>0.64</td>
</tr>
<tr>
<td>Separated by part of Plant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit</td>
<td>97.05 (+- 1.04%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Under side leaf</td>
<td>96.50 %(+- 1.09%)</td>
<td>0.29</td>
</tr>
<tr>
<td>Upper side leaf</td>
<td>95.57 (+- 1.14%)</td>
<td>0.48</td>
</tr>
</tbody>
</table>

#### All Classes CNN From Scratch

The image contains a confusion matrix with predicted labels and true labels for different classes. The matrix is color-coded to indicate accuracy and loss. The colors range from blue (low accuracy) to red (high accuracy). The matrix is divided into sections for different parts of the plant: All, Separated by part, and Specific parts of the plant, such as fruit, under side leaf, and upper side leaf. The accuracy and loss values are provided for each category.
Recombinase Polymerase Amplification (RPA)

Recombinase polymerase amplification applied to plant virus detection and potential implications

Binoy Babu\textsuperscript{a,b,\textsuperscript{*}}, Francisco M. Ochoa-Corona\textsuperscript{a}, Mathews L. Pare\textsuperscript{a,b,\textsuperscript{*}}

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 µL of PD1 Buffer into the RPA pellet (Rehydration step – 1min)
Add 1 μL 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
### Summary

<table>
<thead>
<tr>
<th>Method</th>
<th>Approach</th>
</tr>
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<tbody>
<tr>
<td>Multi-spectral sensor</td>
<td>Reflectance from light for identifying areas with reflectance differences indicating hotspots of biotic and abiotic issues</td>
</tr>
<tr>
<td>Hyper-spectral sensor</td>
<td>Above + identifying areas with unique reflectance differences for certain diseases.</td>
</tr>
<tr>
<td>Raman spectroscopy</td>
<td>Laser induced vibrational spectra identifying chemical differences in plants that could be unique to certain diseases</td>
</tr>
<tr>
<td>Recombinase Polymerase Amplification</td>
<td>Very specific and sensitive field-based DNA/RNA detection of pathogens</td>
</tr>
<tr>
<td>Machine Learning and Artificial Intelligence (AI)</td>
<td>Neural network and image/object-based detection for disease identification</td>
</tr>
</tbody>
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