Imagery for Pest and Agronomic Evaluations

NAICC 2020 Annual Meeting
23 January 2020
Some place in Texas

Steve West
Research Designed for Agriculture
Yuma Arizona

FEAR NOT!!

- Our website under resources has a copy of this talk, the links and files.
- You do not need to scribble everything down… just watch and see what it can do…



RD4AG

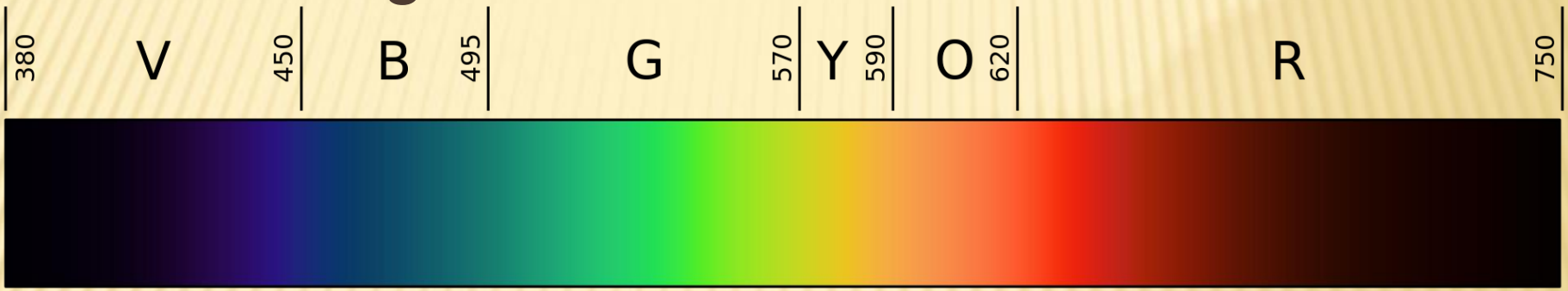
WHAT DEFINES IMAGERY FOR ANALYSIS

- Light is defined in Nanometers of wave lengths
- Radio Waves to Microwaves
- NASA says…
- The **visible light spectrum** is the segment of the electromagnetic **spectrum** that the human eye can view. Typically, the human eye can detect wavelengths from 380 to 700 nanometers

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THE SPECTRUM

- Visible Light



- In most cases, the light spectrum useful for plants is from 25-2500 nm

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SENSOR TYPES AND RANGES

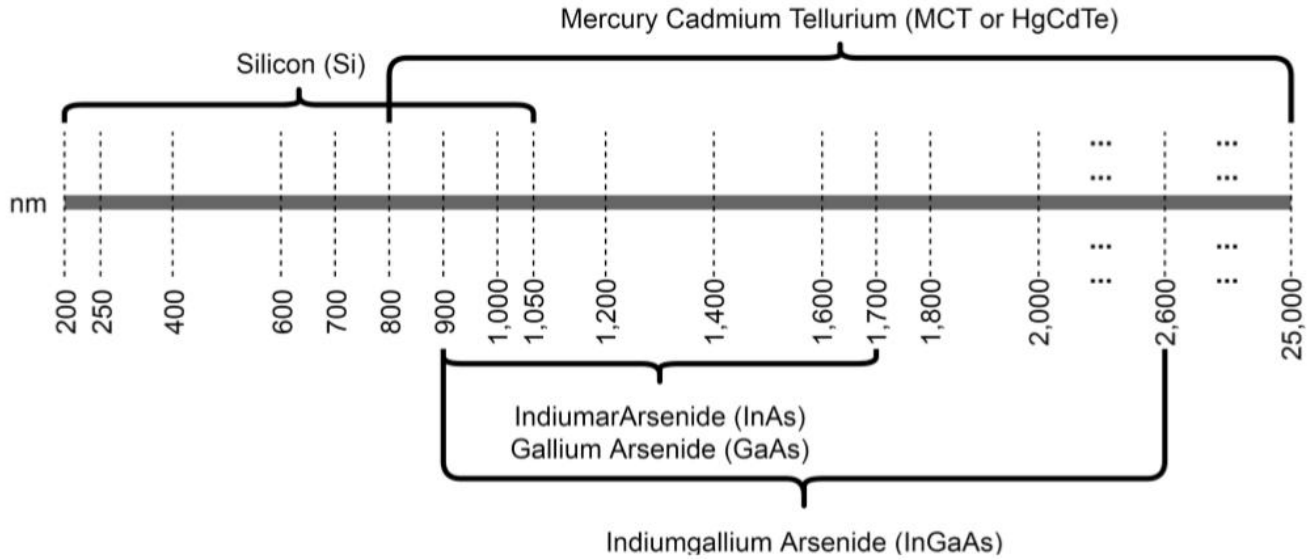


Figure 3. Materials involved in hyperspectral sensors fabrication (inspired by [43]): Silicon (Si) is used for acquiring ultraviolet, visible and shortwave NIR regions; indium arsenide (InAs) and gallium arsenide (GaAs) have a spectral response between 900–1700 nm; indium gallium arsenide (InGaAs) extends the previous range to 2600 nm; and mercury cadmium tellurium (MCT or HgCdTe) is characterized by a large spectral range and high quantum efficiency that enables reaching mid-infrared region (about 2500 to 25,000 nm) and NIR region (about 800–2500 nm).

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SILICON SENSORS

- Your phone, and most cameras are CMOS silicon chips. Some are CCD
  - Zillions made and low cost
- CCD and **CMOS** sensors are **sensitive** to **wavelengths** from approx 350 - 1050nm, although listed as from 400 - 1000nm.
- NDVI with Trimble Green Seeker is 656 and 774
- SPAD meters are normally 650 and 940nm

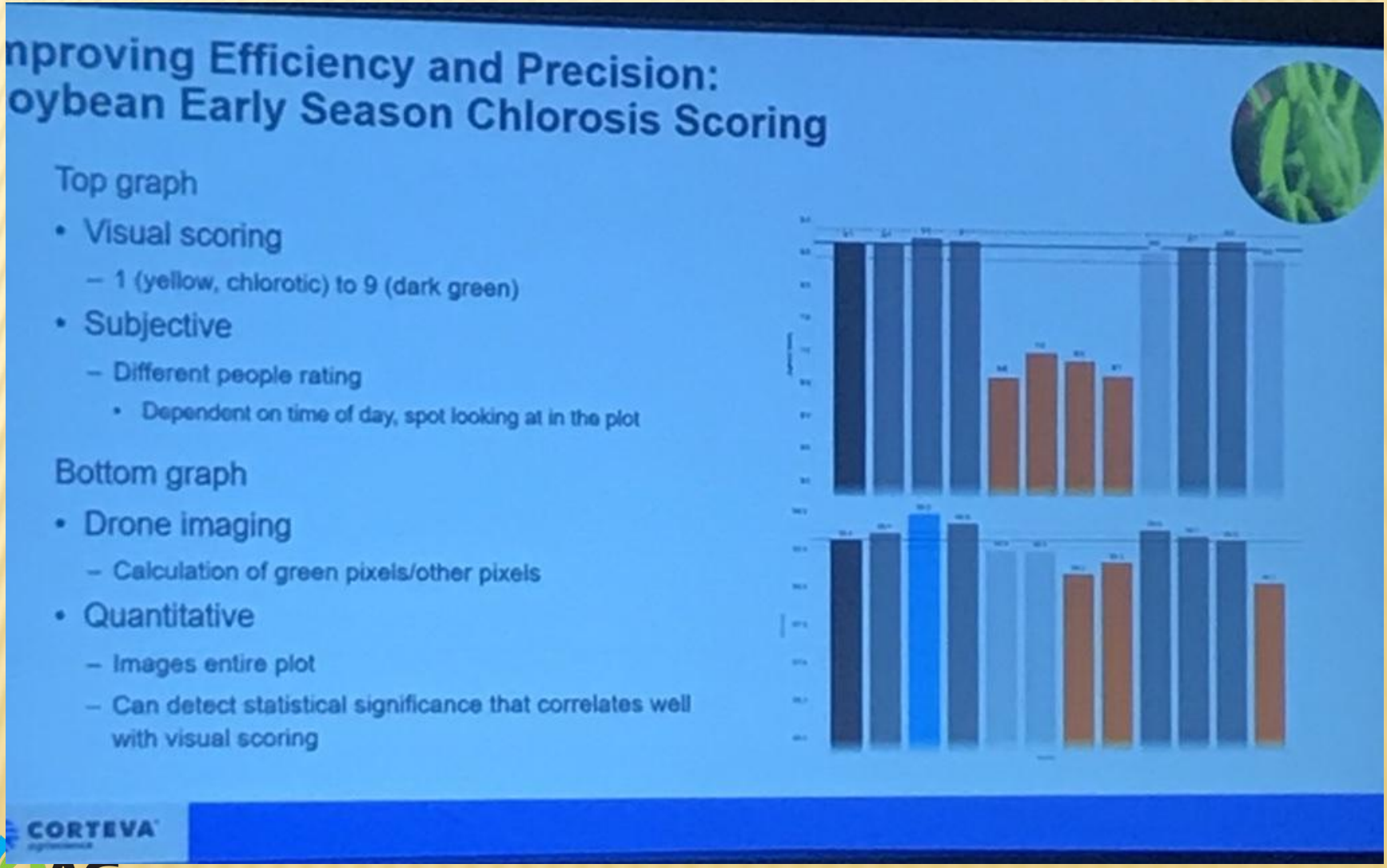
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RGB SPOKEN HERE

- For this talk we will be looking at how to utilize cameras with the standard CMOS or CCD sensors in the visible range, and generating RGB images
  - RGB is Red Green Blue
  - Translated…
  - Your Cell phone, digital cameras including GoPro's, and including digital SLRs

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THE ELECTRONIC EYES HAVE IT…



WE WANT OBJECTIVE, ACCURATE EVALUATIONS

- Steve, show us how you used to do it?
- Who thinks this will be the way we do these in 10 years??????????????
- We hear about and may know drones… what can we do with some imagination?
- And now for something completely different!

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EVERYTHING WE DO IS WHERE WE CAN GO

- Bugs
- Weeds
- Canopy Cover
- Diseases
- And???
- Following are some examples

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POWDERY MILDEW ON CUCURBITS



- Gather 40 leaves per plot and set them on a black back ground
- Take a picture
- Turn over and snap another
- Take to office and evaluate



POWDERY MILDEW ON MUSKMELON



EVALUATED



FOR THE SCORE

- We count the number of each class
  - 3 severity four is 12
  - 6 severity three is 18
  - 8 severity two is 16
  - 12 severity one is 12
  - Total is 58
  - Number of leaves is 40, infected is 29, clean is 11
  - So average of all is 1.45
  - Incidence is 72.5%, severity of infected is 2.0

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BUGS

- Took a picture of a Bell Pepper Leaf with an iPhone and enlarged it.
- Opened Paint and picked a fine pen
- Using a touch screen computer, marked off the aphids on a leaf and counted them
- Anyone can go back and check the counts…



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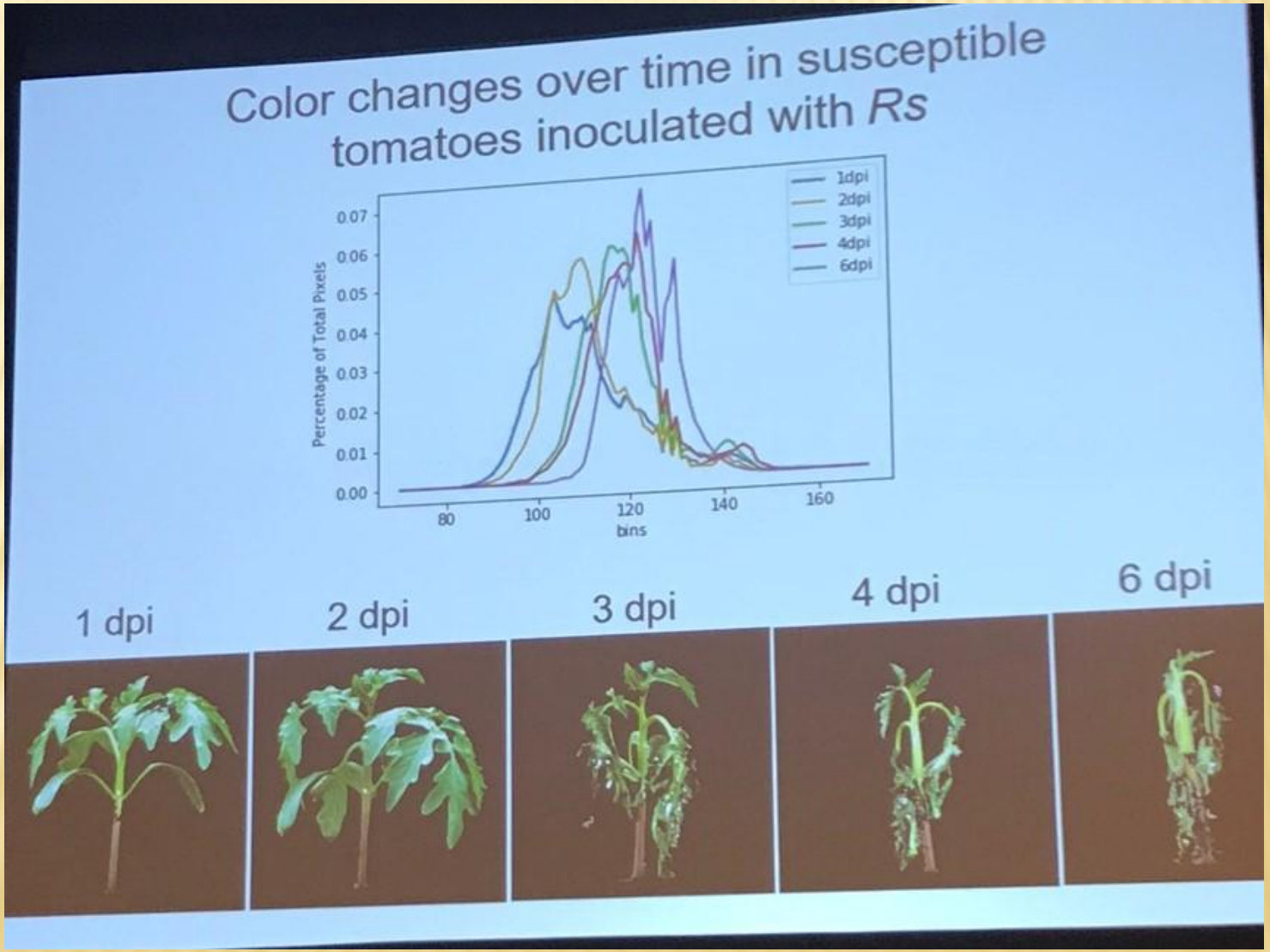
SOME CONSIDERATIONS

- Take samples at the right time of the day
- Knowing what pests there are is key as this is just one view
- You can tag leaves and count them again



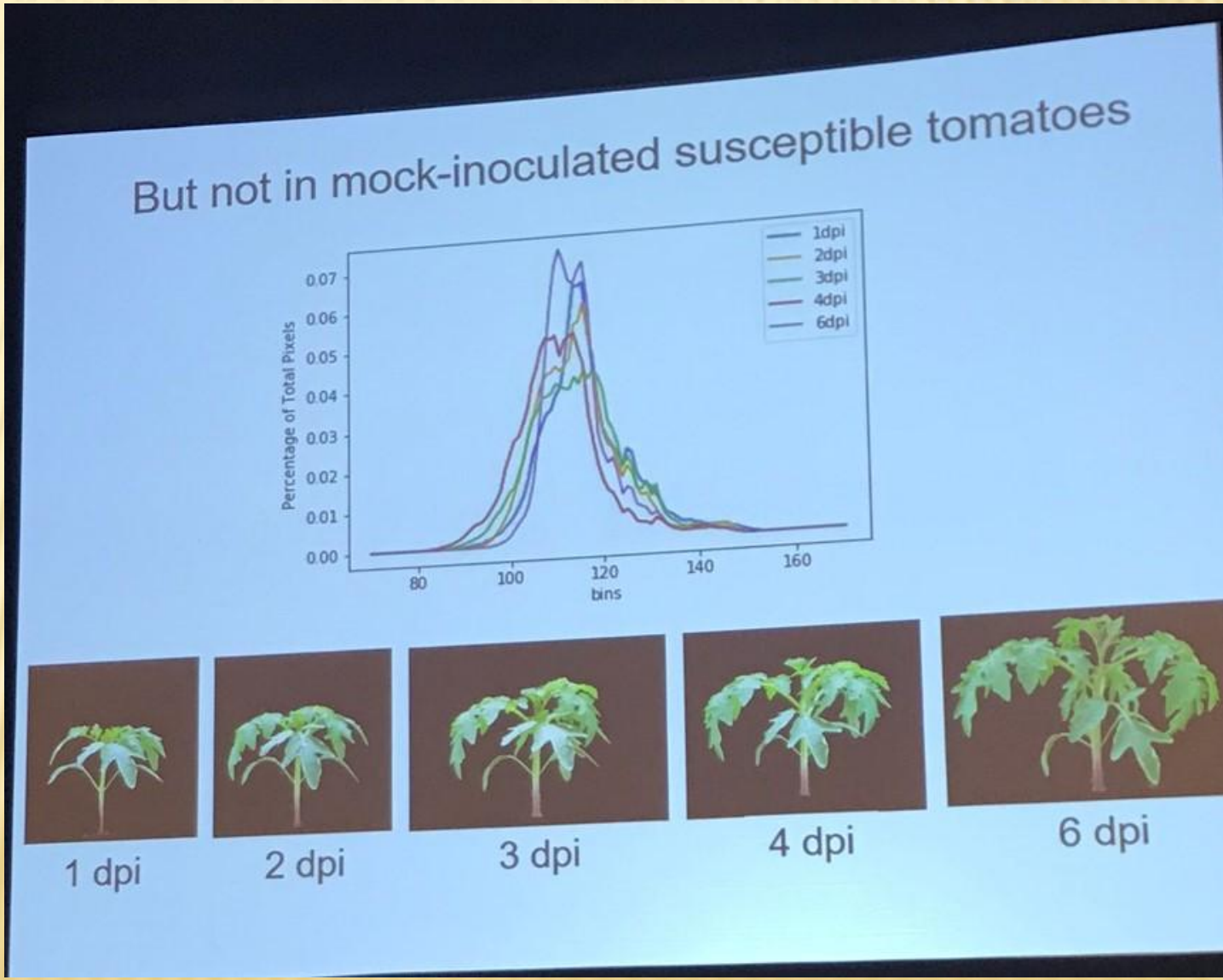
COLOR PROGRESSION OF DISEASED TOMATOES

The Histogram shifts to the right as the plants die



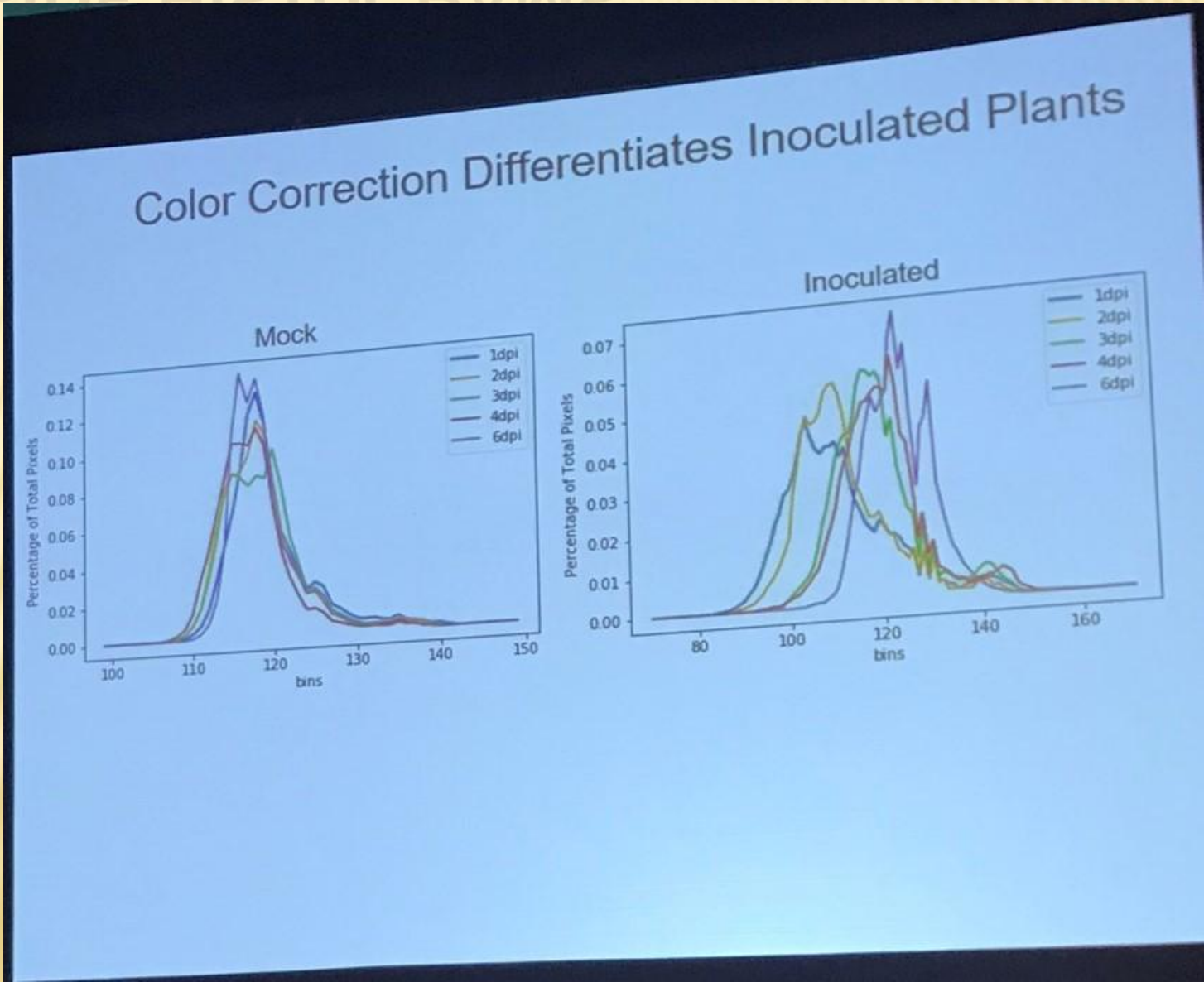
NON DISEASED PLANTS STAY THE SAME

Tomato plants not dying from Bacterial Wilt



SIDE BY SIDE HISTOGRAMS

Easy to see which one is changing and which one is not



THRESHOLDING SPINACH DOWNY MILDEW TOP

Top view of Yellow areas do not separate well in bright sunlight

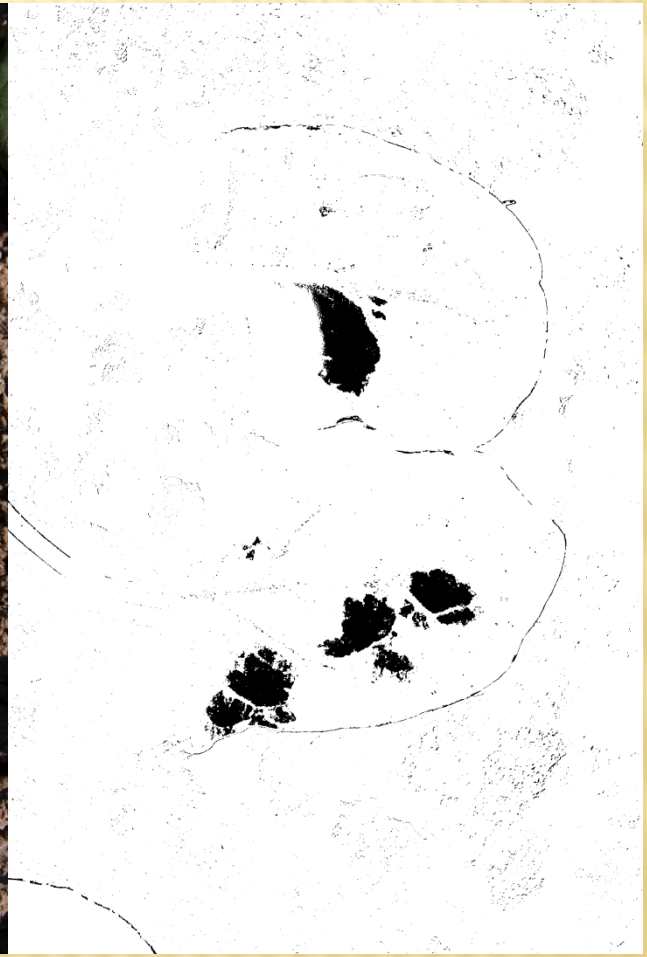




SPINACH DOWNY MILDEW BOTTOM

Diseased areas are easy to isolate on bottom and an actual % of leaf infected can be determined





RUST ON BARLEY USING PLANT CV FROM DANFORTH CENTER

PlantCV

Search docs

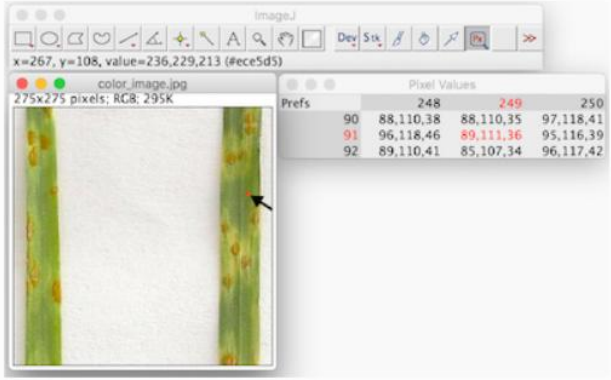
- Home
- Documentation
  - Installation
  - Updating PlantCV
  - Frequently Asked Questions
  - Analysis Approaches
  - Using Jupyter Notebooks
  - Summary of Output Measurements
- Contributing
  - Contributing to PlantCV
  - Code of Conduct
  - Adding/Editing Documentation
- Tutorials
  - VIS Workflow
  - NIR Workflow
  - PSII Workflow
  - VIS/NIR Dual Workflow
  - Multi Plant Workflow
  - Machine Learning

GitHub « Previous Next »

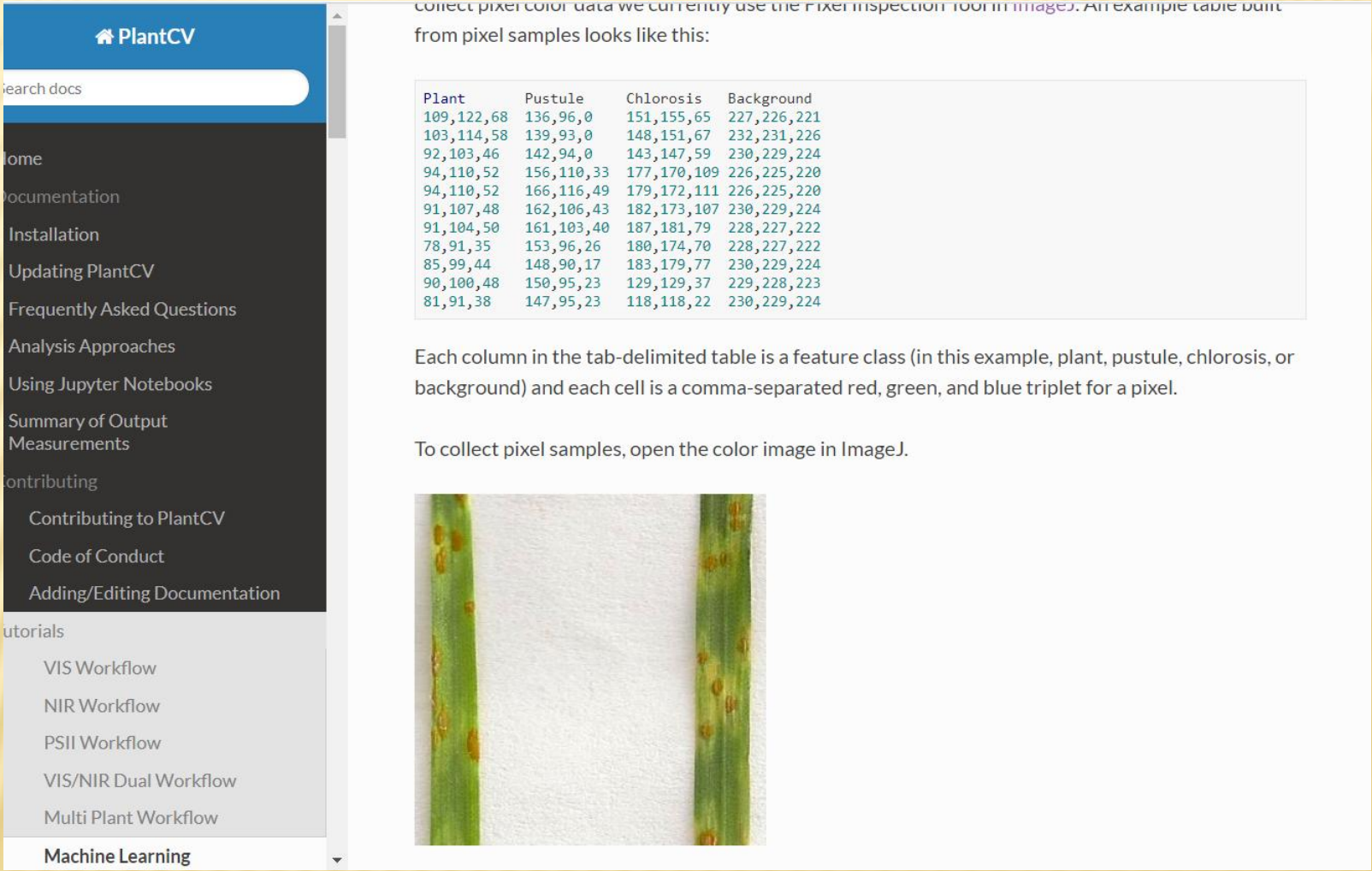
To collect pixel samples, open the color image in ImageJ.



Use the Pixel Inspector Tool to select regions of the image belonging to a single class. Copy and paste the pixel red, green, and blue values into a spreadsheet or text editor and reformat into a single column per class. In this example, nine pixels are sampled with one click but the radius is adjustable.

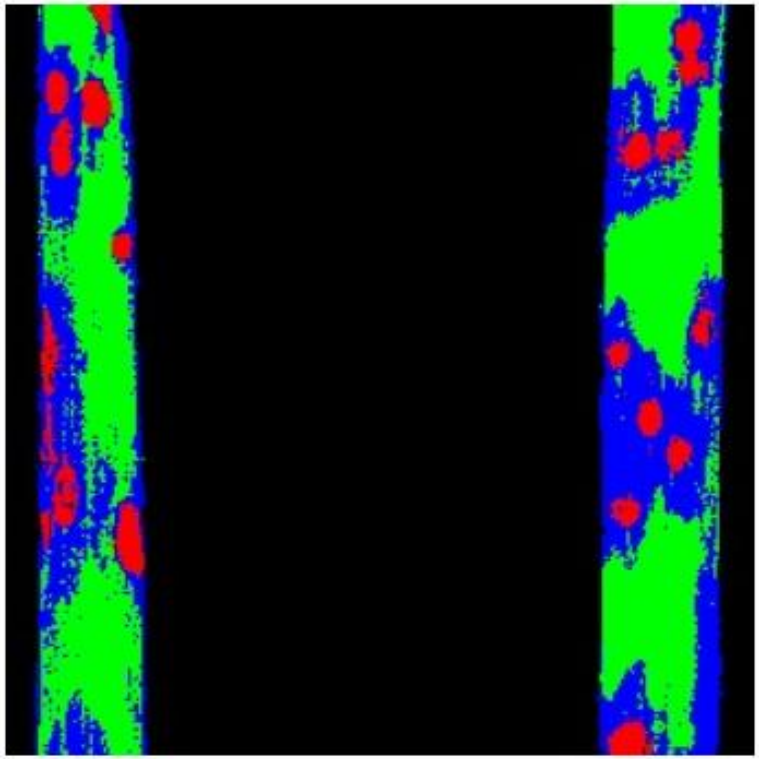


THIS IS MACHINE LEARNING



AND THE END PRODUCT

- We determined the color of the various parts of the image…
- The RGB numbers for the orange spots, the yellow areas
- The green leaf and background
- Ran it through the Naive Bayes Multiclass tool
- Return was # orange spots
- % or leaf, % Yellow…
- No guess,
- Real Data



IT ALL STARTS WITH THE IMAGE

- This checklist is from the Danforth Center's Plant CV guide
- It is a good idea to capture a test image and process it before capturing a full set of data. It is ALWAYS best to try to reduce potential image processing problems up front, rather than to try to process 'bad' / inconsistent images. Things to think about:
  - If color is analyzed, do you need a color card for color correction in each image?
  - If lighting is changing do you need a color card / white balance card to 'normalize' lighting across images?
  - Is the camera set at exactly the same vantage point in each image or do you need a size marker?
  - Is there enough contrast between the target object (plant) and the background or do you need to add materials to increase contrast (blue material for example).
  - If you are going to image more than one plant in an image, how long before the plants overlap each other? Is this long enough for the trait you are interested in?

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THE PROCESS OF COLOR THRESHOLDING

- Image J… it is your friend
- The version you want is Fiji
- https://imagej.net/Fiji
- Note this is from the National Institute of Health
- It runs on Java

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CANOPY COVER

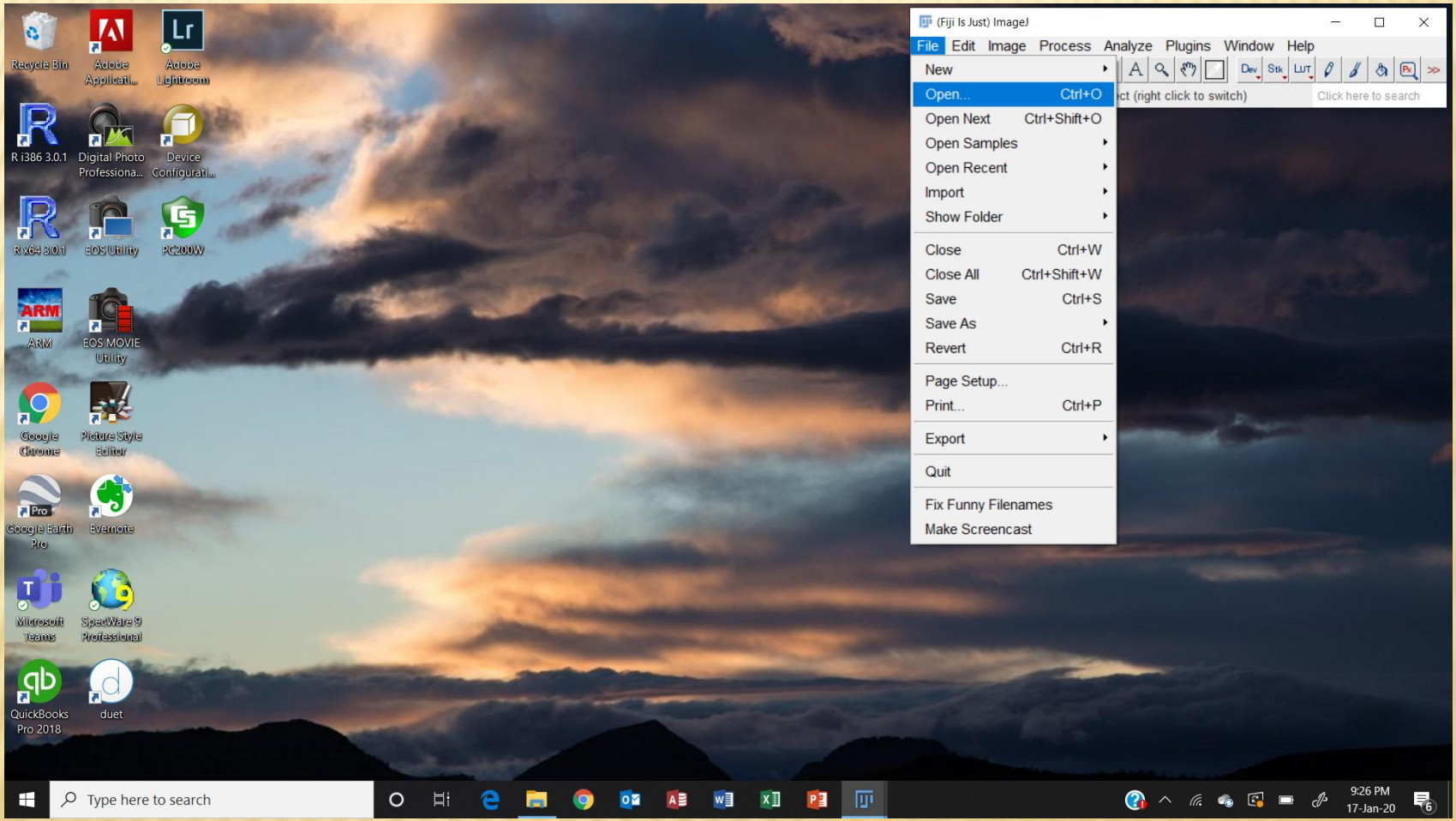
- We use Canopy cover in a few ways:
  - To determine growth increases
    - Fertilizers
    - Plant Health Products
  - To determine canopy loss
    - Herbicides
    - Some diseases

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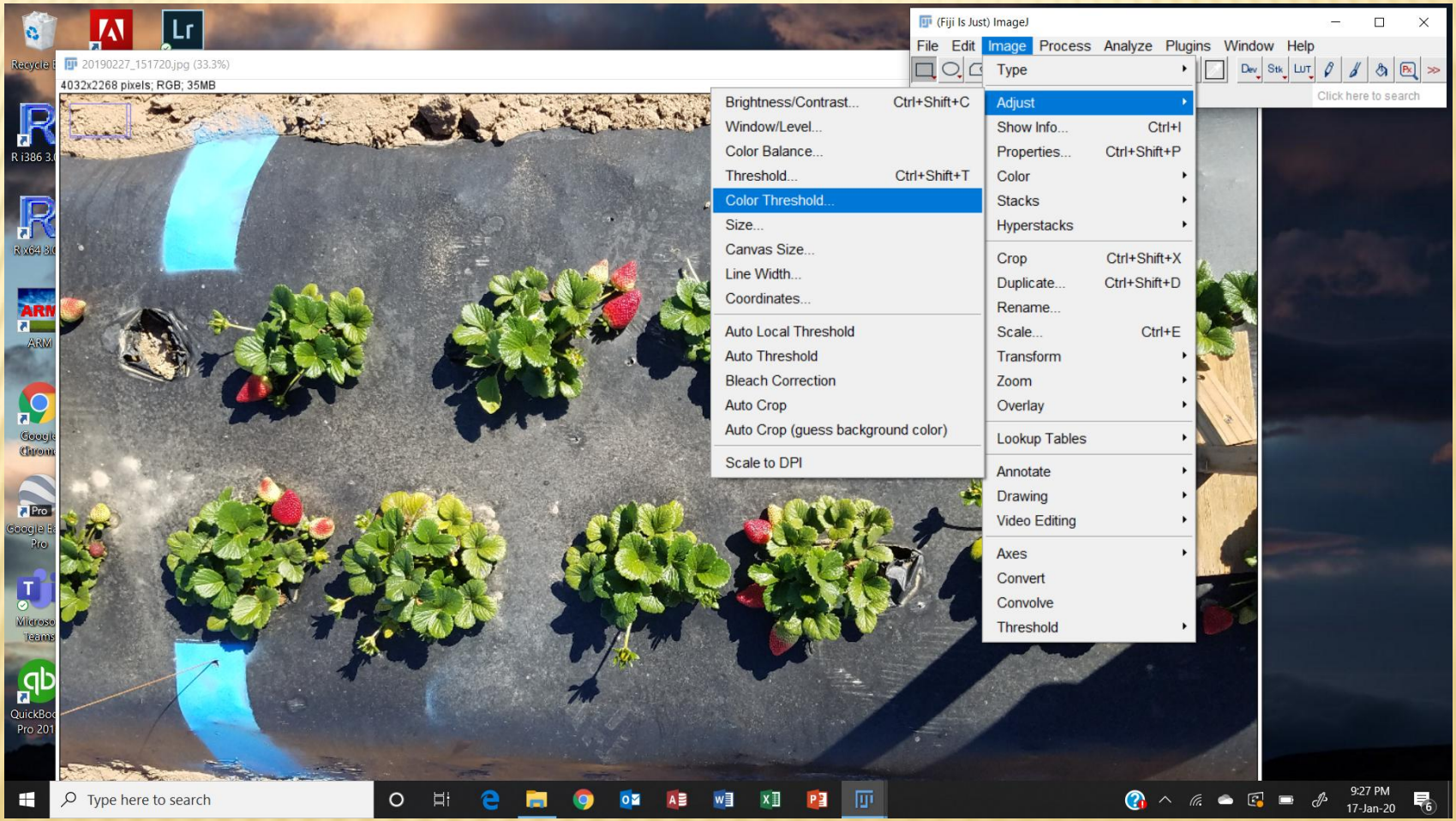
THE IMAGE TAKING PROCEDURE

- Paint spots on the ground or plastic
- Make a simple selfie stick with wood so it is durable with a platform to put camera on in the same place
- Put stick on painted spot
- Take a picture…
- Repeat later– Same height, same view… repeatable…

OPEN A FILE FROM IMAGE J

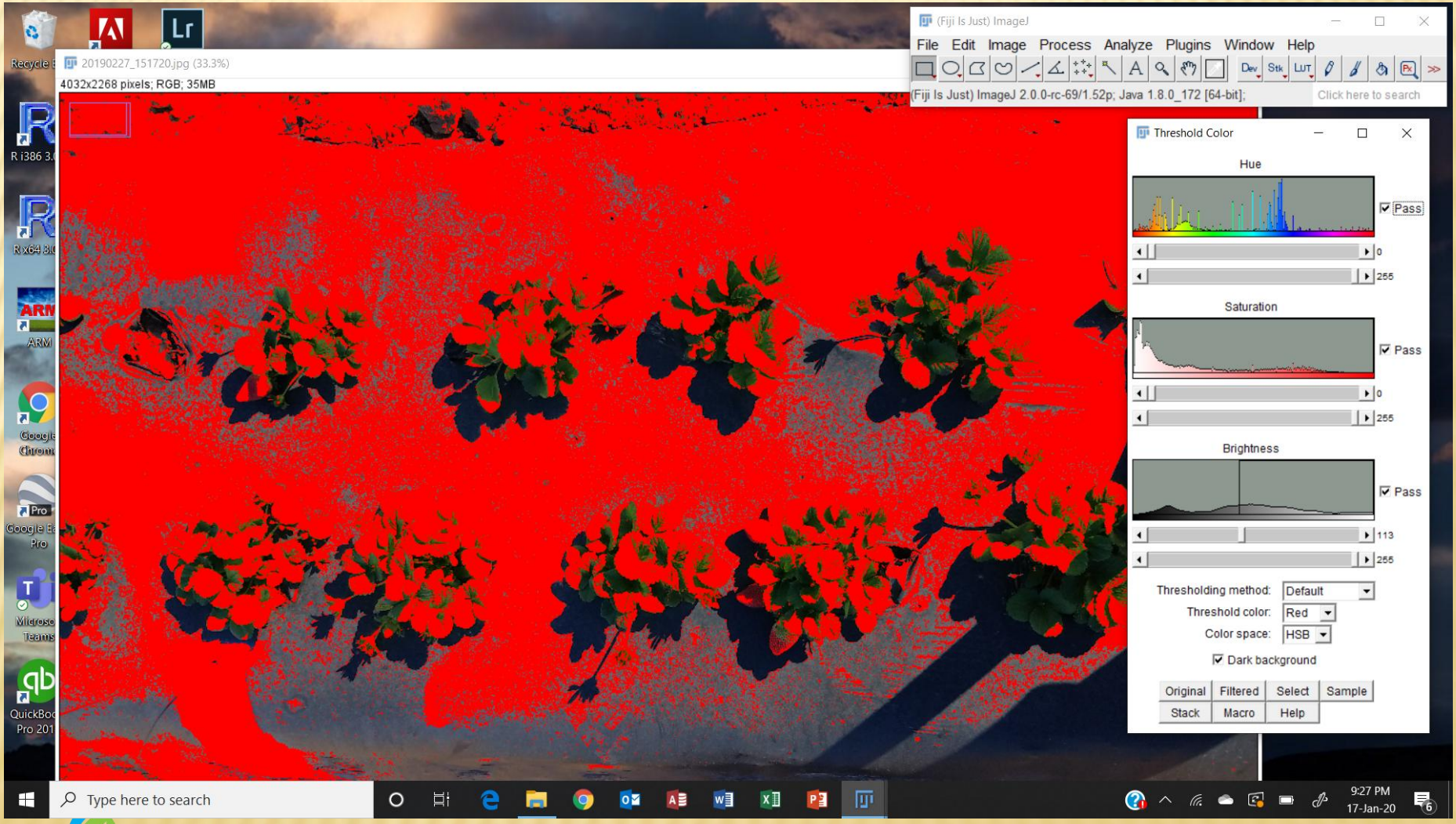


OPEN IMAGE–ADJUST—COLOR THRESHOLD



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YOU START HERE



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START WITH HUE TO SELECT DESIRED RANGE



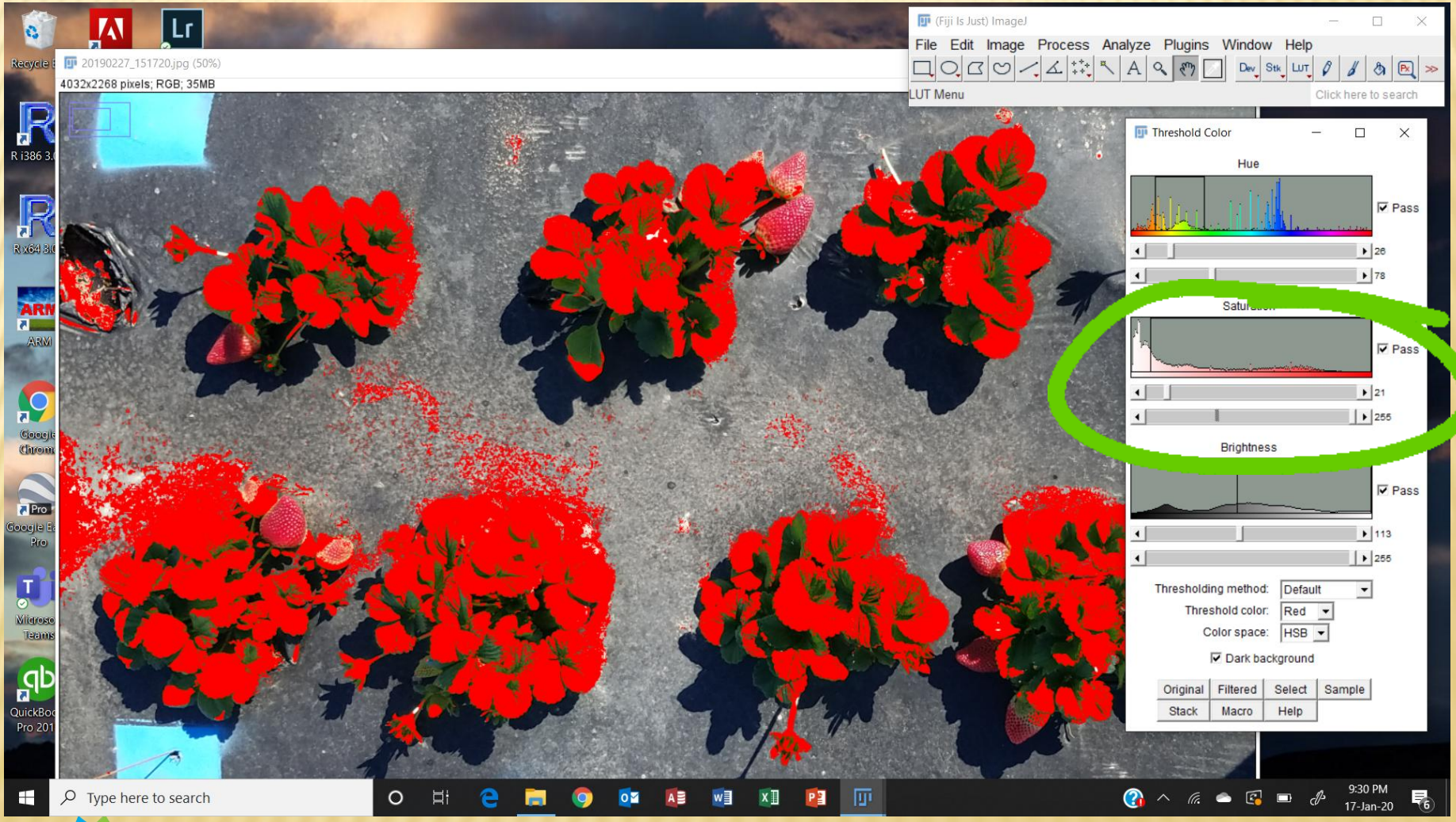
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BETTER



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PLAY WITH SATURATION & BRIGHTNESS



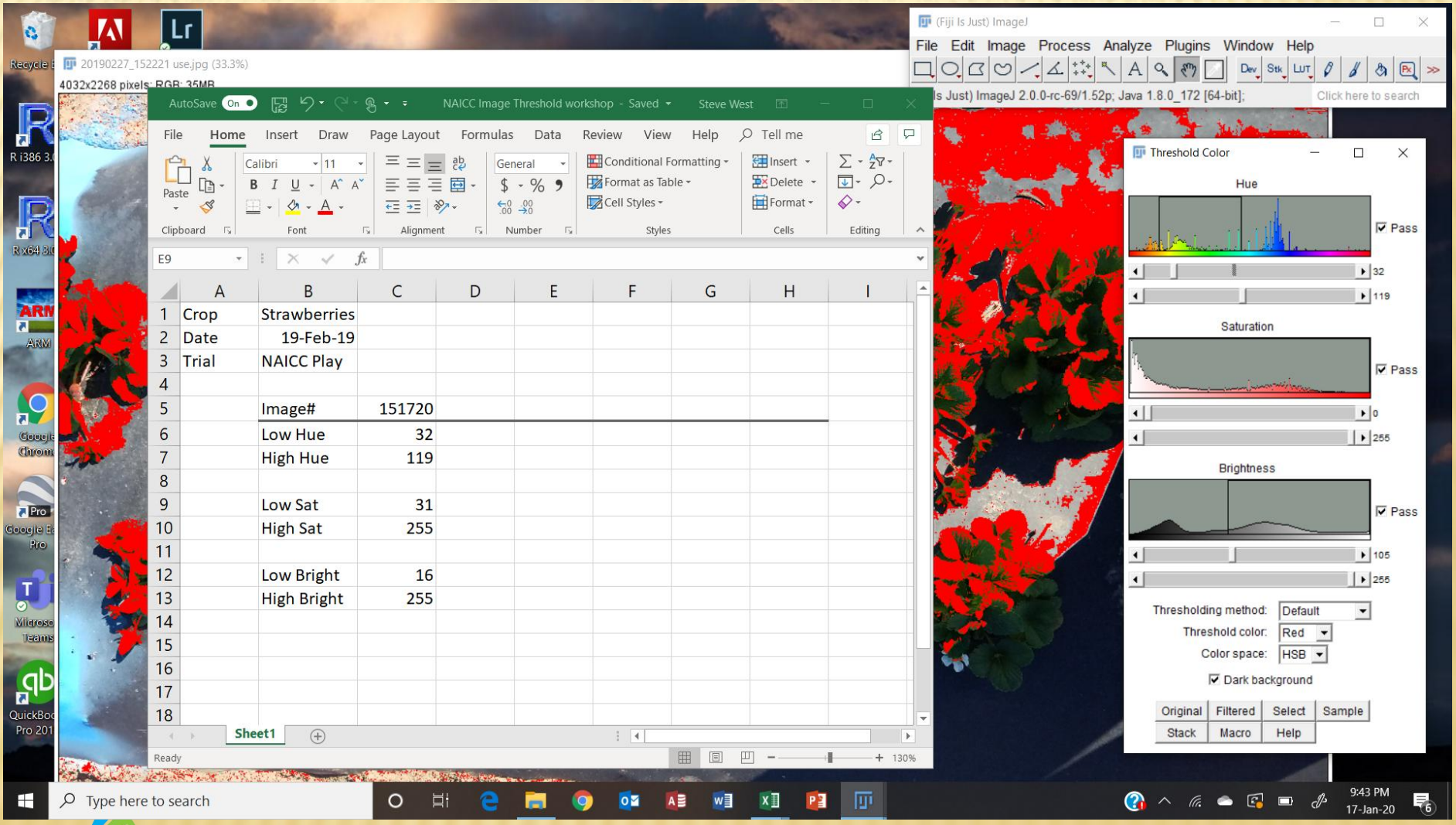
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ABOUT RIGHT



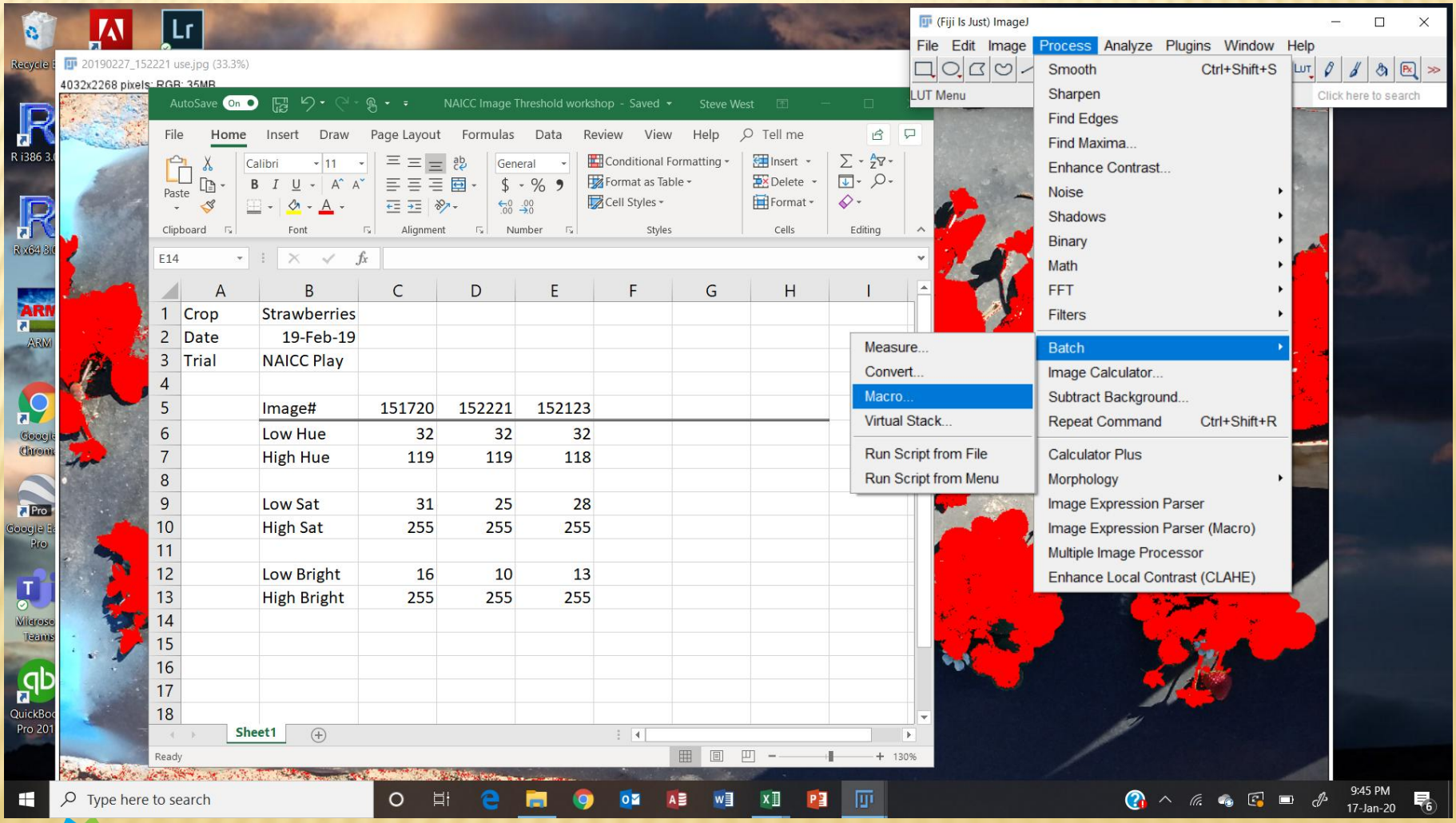
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MAKE AN EXCEL FILE WITH SETTINGS



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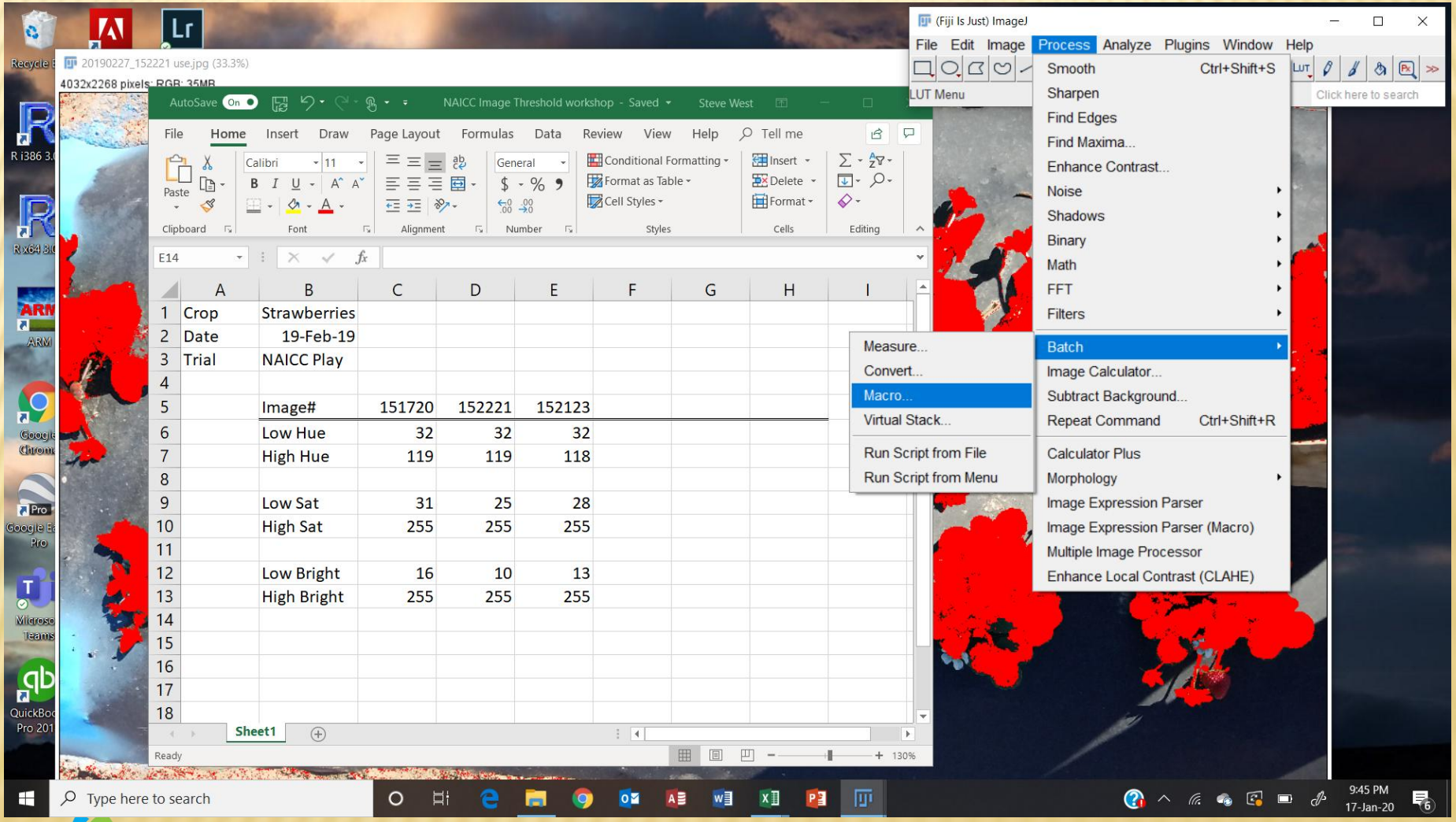
DO SEVERAL IMAGES TO GET A FINAL AVE.



	A	B	C	D	E
1	Crop	Strawberries			
2	Date	19-Feb-19			
3	Trial	NAICC Play			
4					
5		Image#	151720	152221	152123
6		Low Hue	32	32	32
7		High Hue	119	119	118
8					
9		Low Sat	31	25	28
10		High Sat	255	255	255
11					
12		Low Bright	16	10	13
13		High Bright	255	255	255
14					
15					
16					
17					
18					

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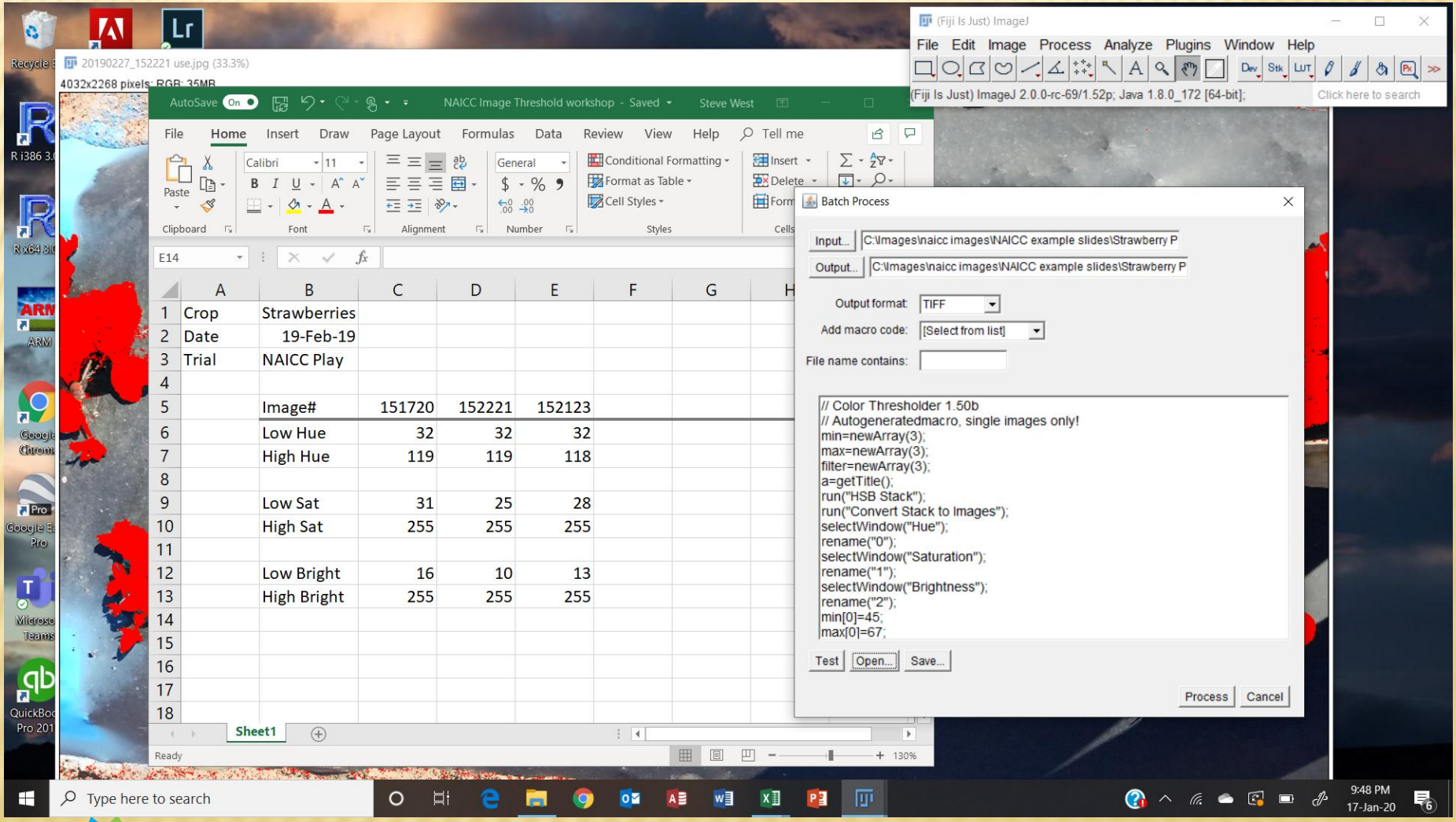
OPEN PROCESS—BATCH–MACRO



	A	B	C	D	E
1	Crop	Strawberries			
2	Date	19-Feb-19			
3	Trial	NAICC Play			
4					
5		Image#	151720	152221	152123
6		Low Hue	32	32	32
7		High Hue	119	119	118
8					
9		Low Sat	31	25	28
10		High Sat	255	255	255
11					
12		Low Bright	16	10	13
13		High Bright	255	255	255
14					
15					
16					
17					
18					

RD4AG

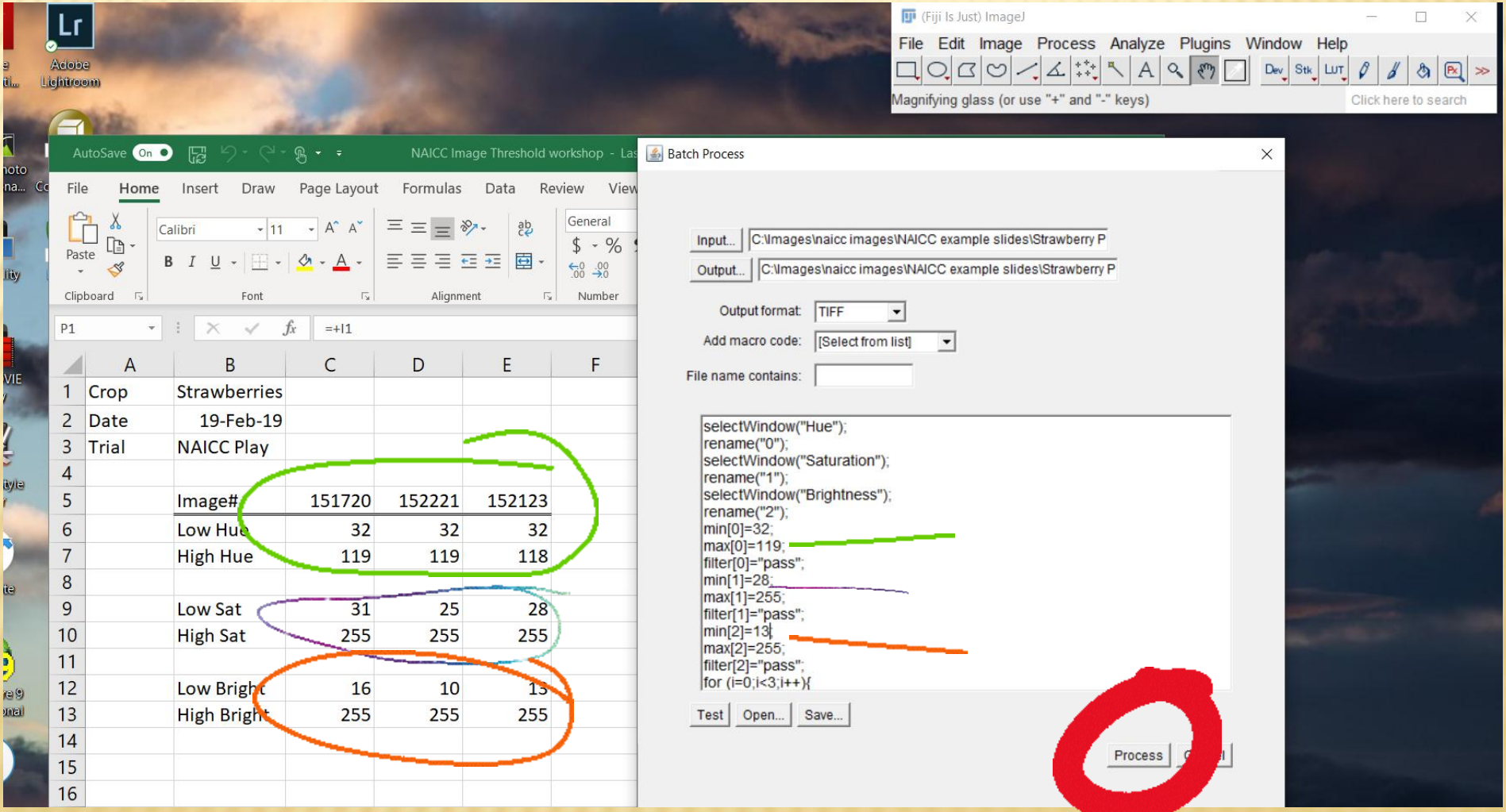
SET INPUT, OUTPUT AND LOAD MACRO



	A	B	C	D	E
1	Crop	Strawberries			
2	Date	19-Feb-19			
3	Trial	NAICC Play			
4					
5		Image#	151720	152221	152123
6		Low Hue	32	32	32
7		High Hue	119	119	118
8					
9		Low Sat	31	25	28
10		High Sat	255	255	255
11					
12		Low Bright	16	10	13
13		High Bright	255	255	255
14					
15					
16					
17					
18					

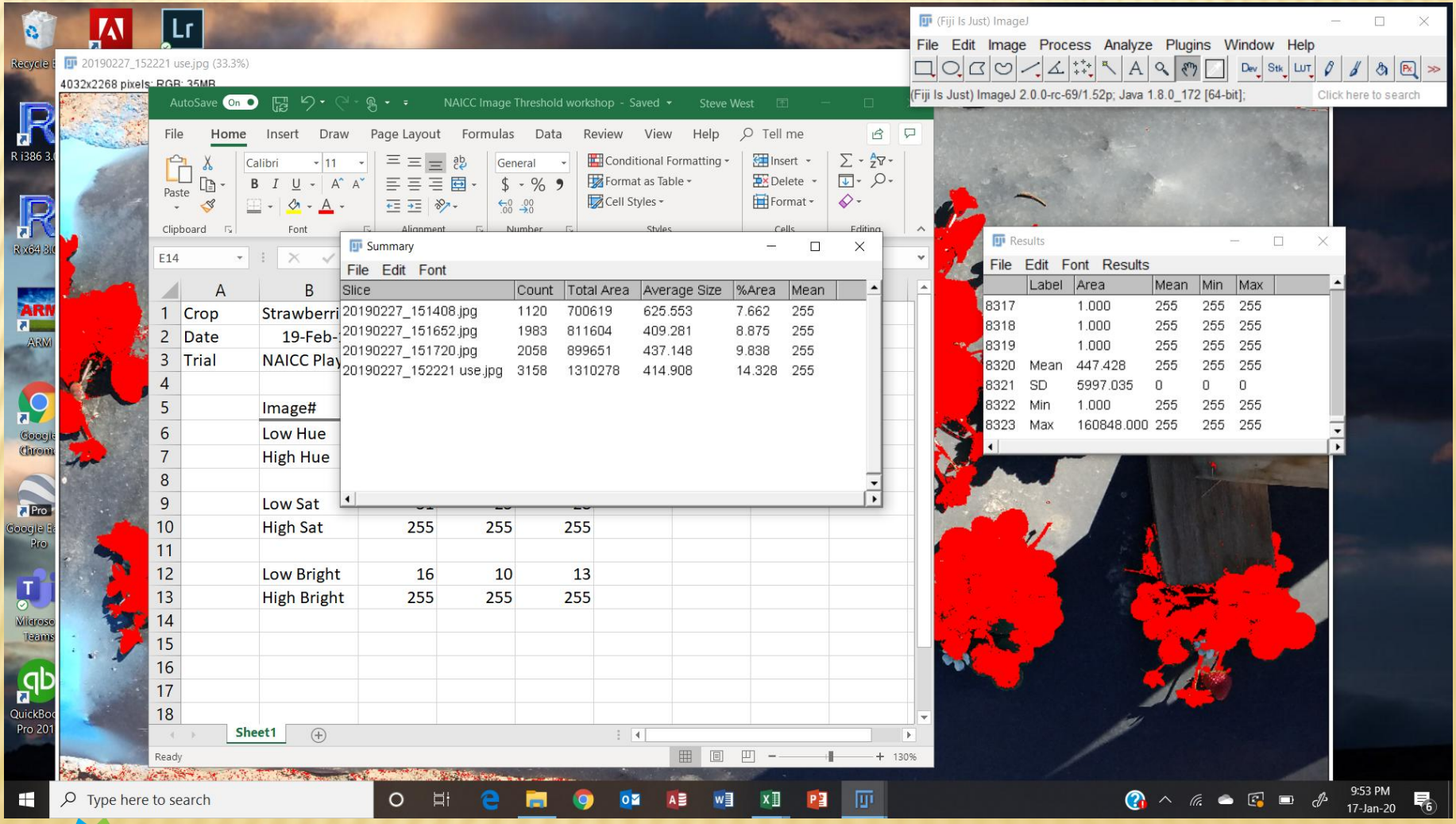
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MOVE SETTINGS INTO MACRO AND PROCESS



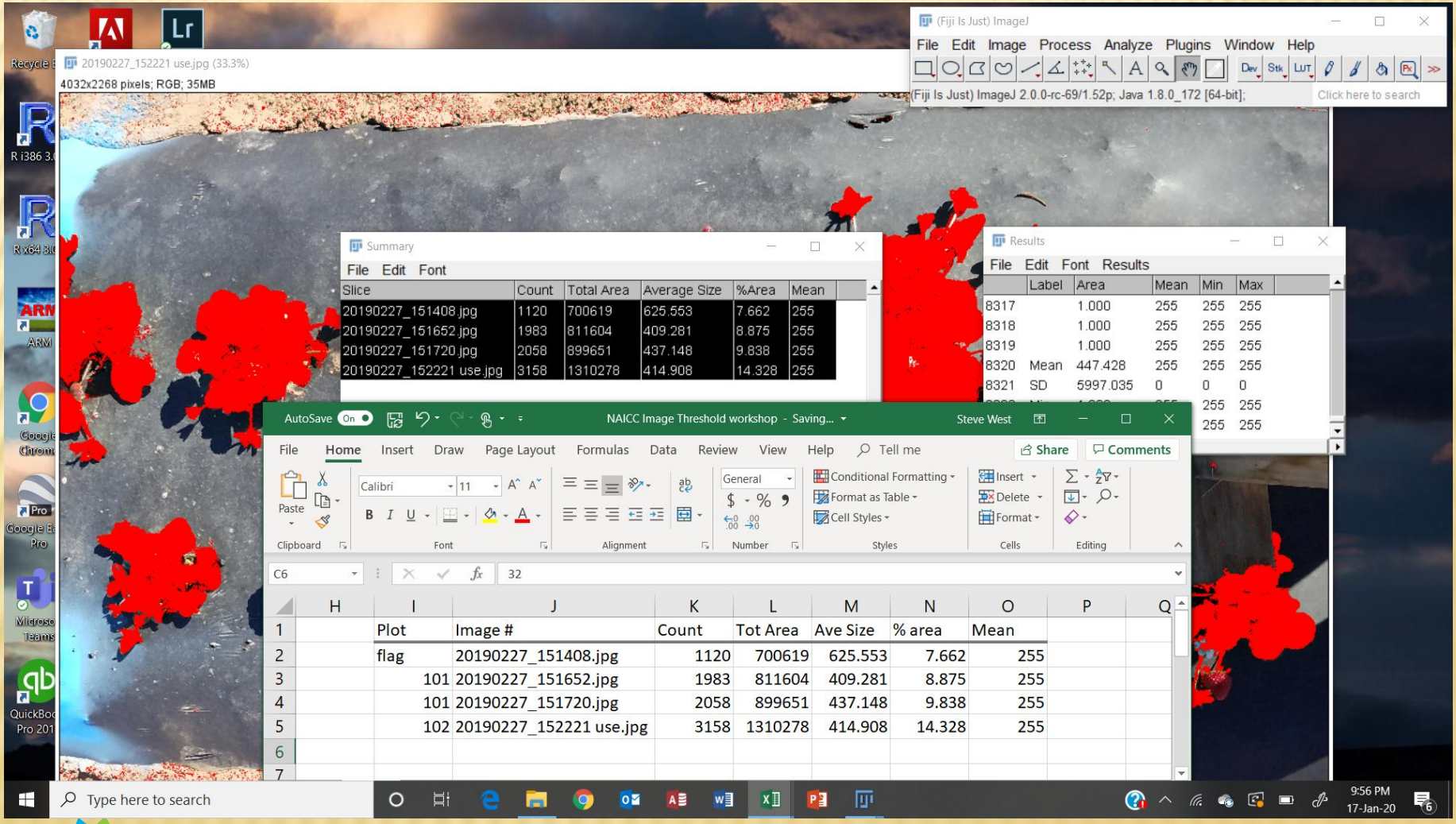
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THE OUTPUTS… CSV YOU CAN PASTE



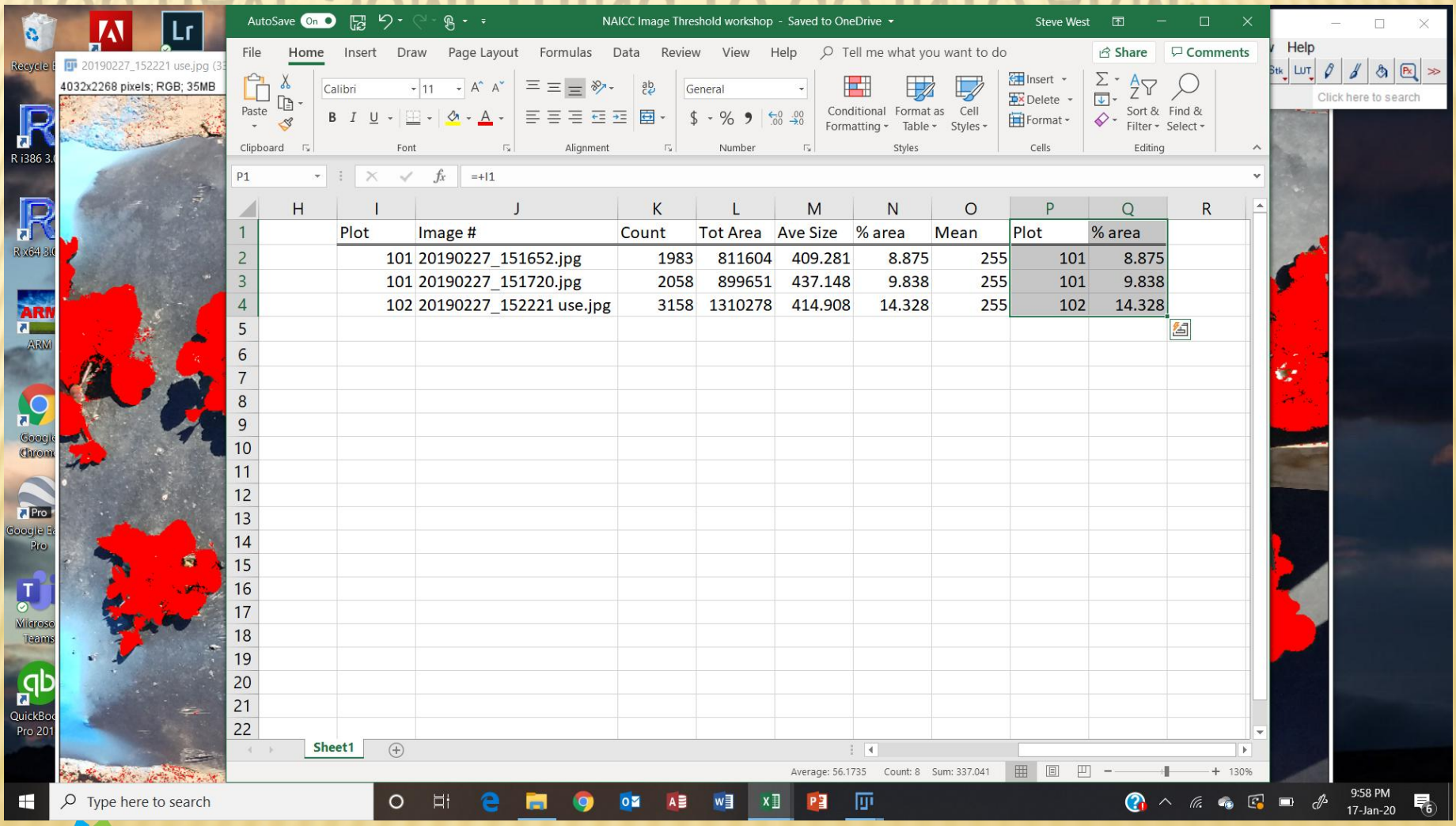
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COPY SUMMARY TO EXCEL



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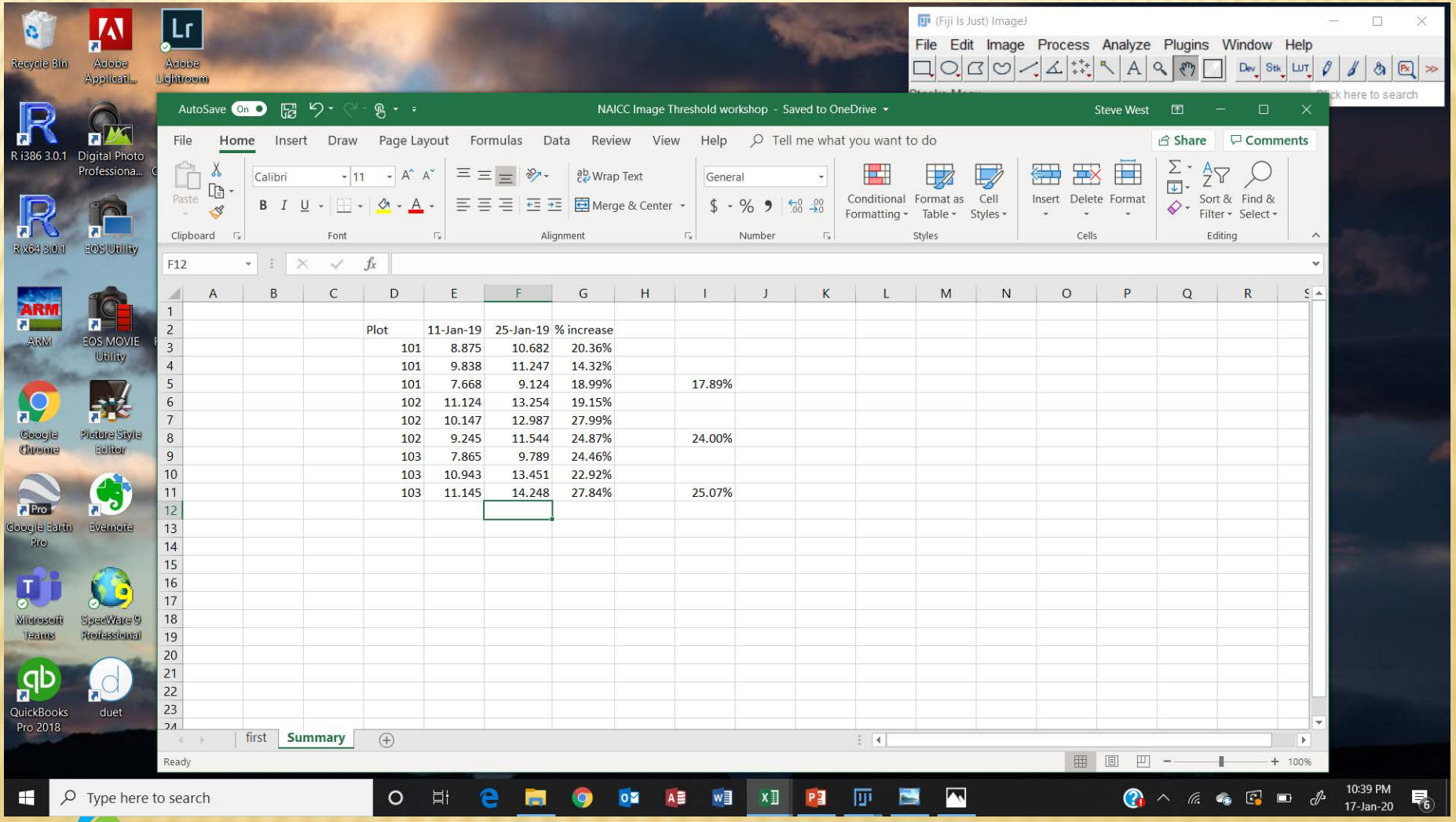
ADD PLOT NUMBERS, PULL THE MARKERS AND YOU HAVE SOMETHING TO GO INTO ARM!



	H	I	J	K	L	M	N	O	P	Q	R
1		Plot	Image #	Count	Tot Area	Ave Size	% area	Mean	Plot	% area	
2		101	20190227_151652.jpg	1983	811604	409.281	8.875	255	101	8.875	
3		101	20190227_151720.jpg	2058	899651	437.148	9.838	255	101	9.838	
4		102	20190227_152221 use.jpg	3158	1310278	414.908	14.328	255	102	14.328	
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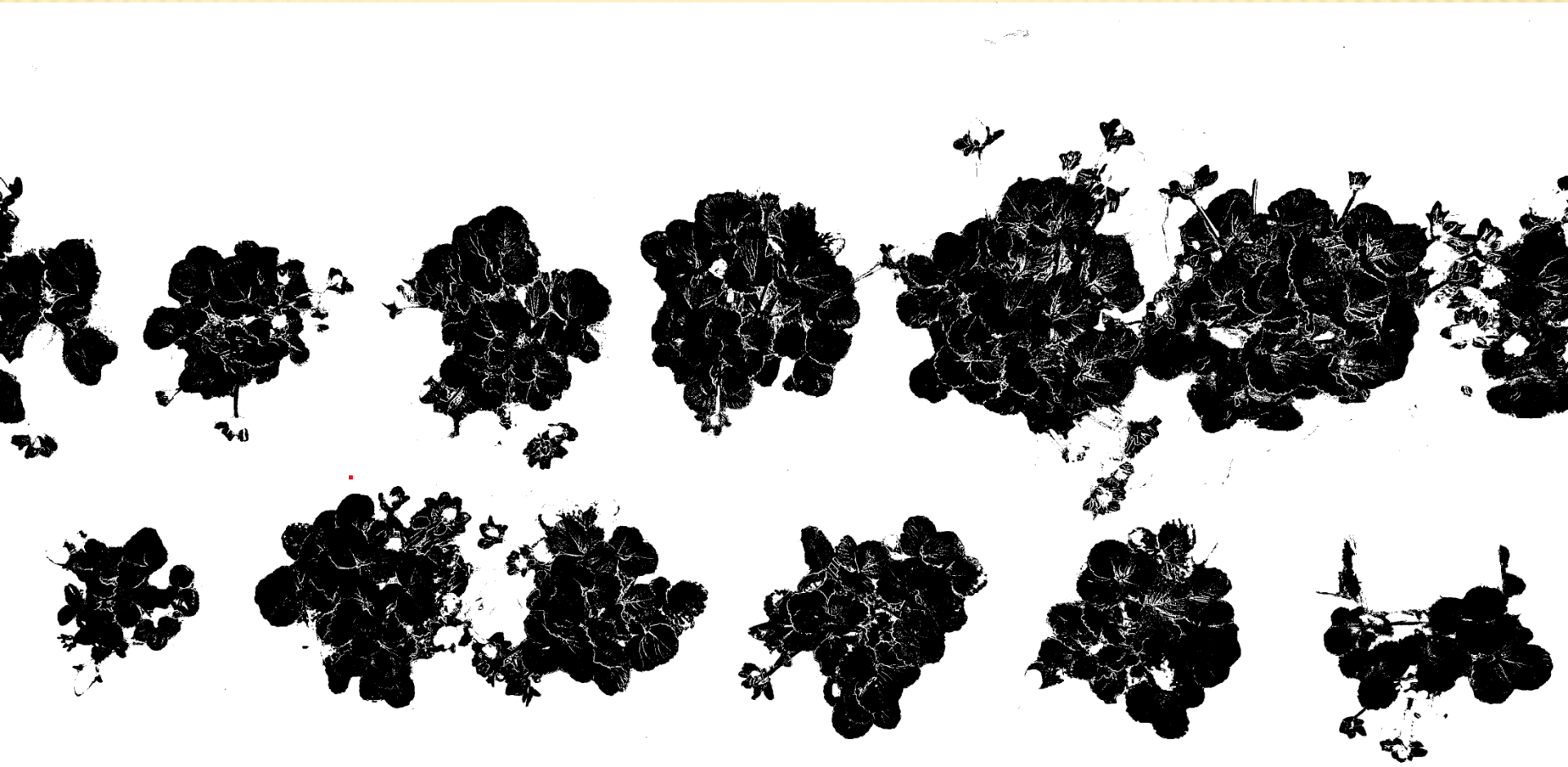
POWER IS CHANGE OVER TIME



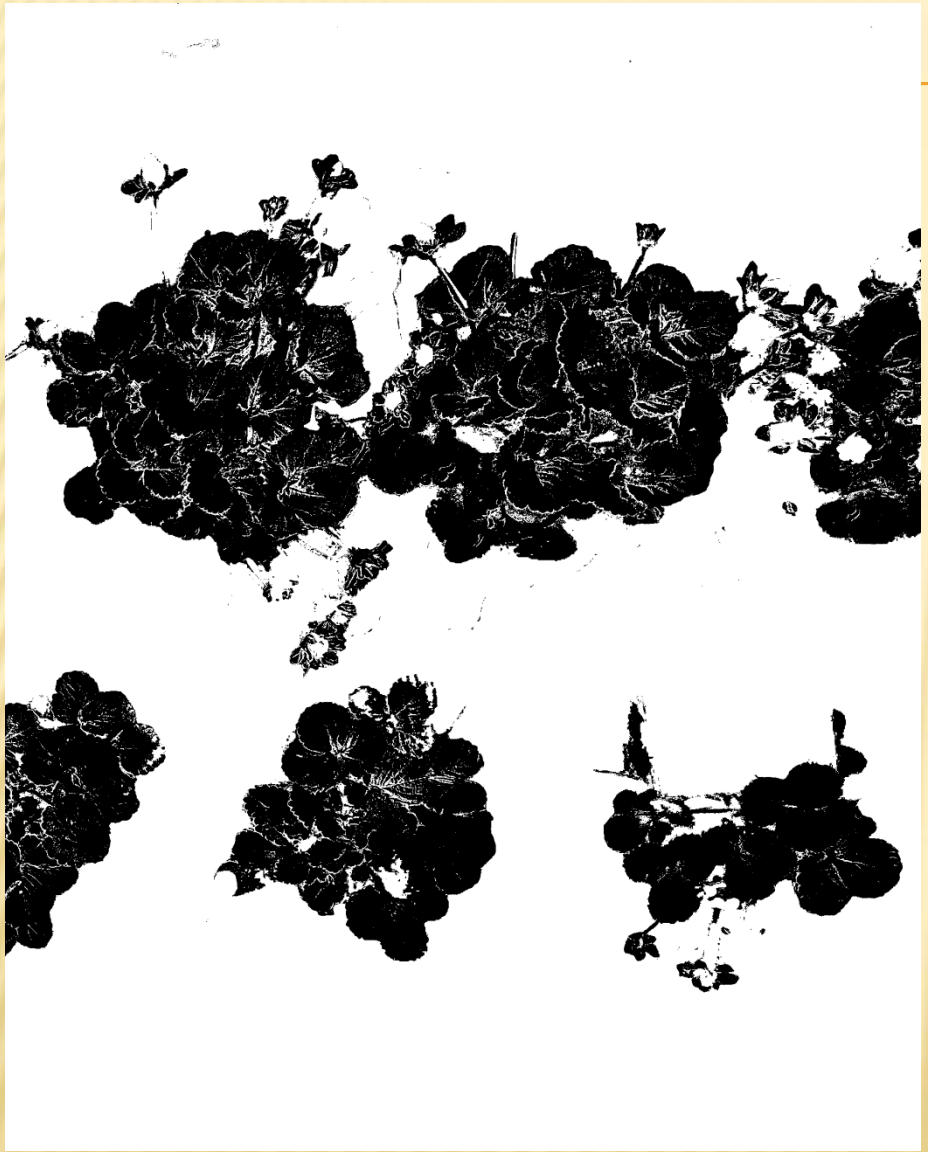
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1																		
2				Plot	11-Jan-19	25-Jan-19	% increase											
3				101	8.875	10.682	20.36%											
4				101	9.838	11.247	14.32%											
5				101	7.668	9.124	18.99%		17.89%									
6				102	11.124	13.254	19.15%											
7				102	10.147	12.987	27.99%											
8				102	9.245	11.544	24.87%		24.00%									
9				103	7.865	9.789	24.46%											
10				103	10.943	13.451	22.92%											
11				103	11.145	14.248	27.84%		25.07%									
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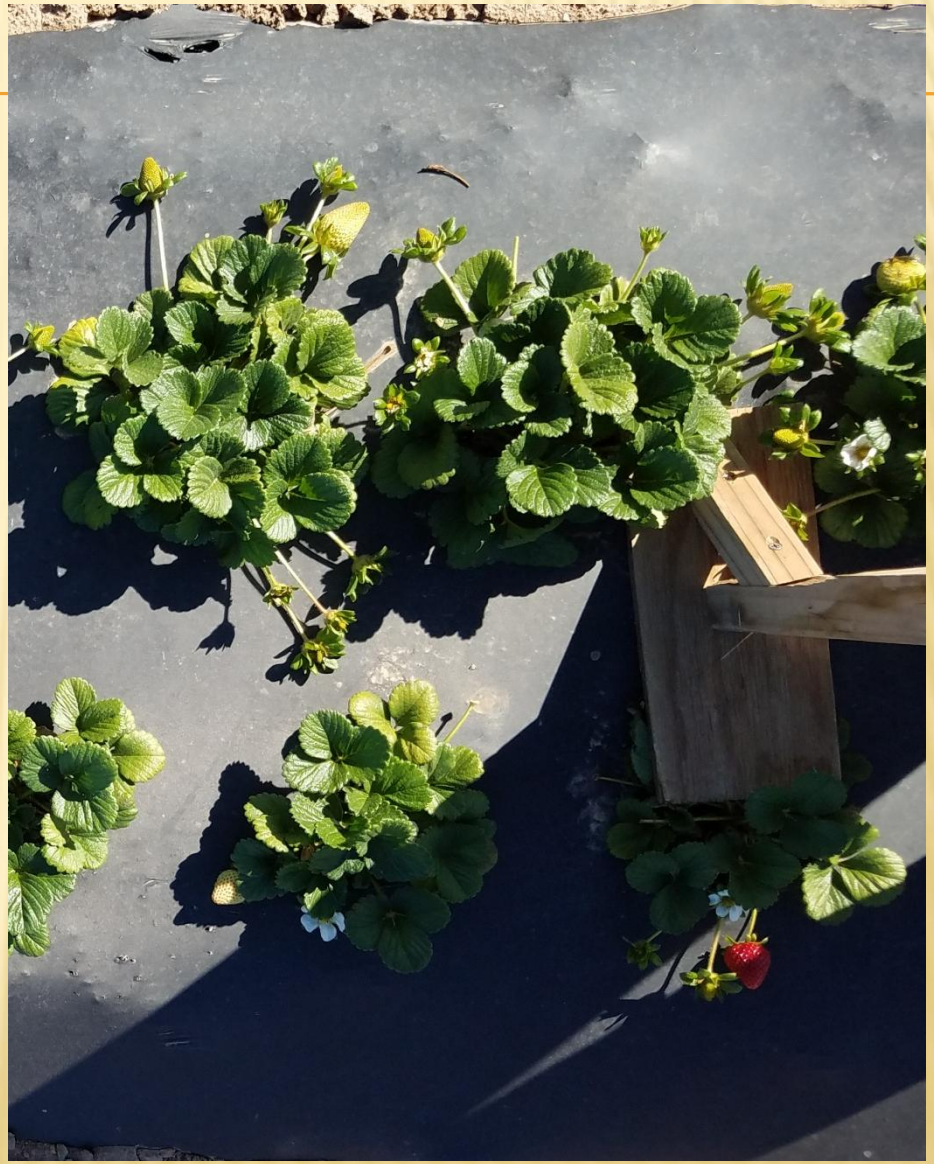
NOTE SHADOWS





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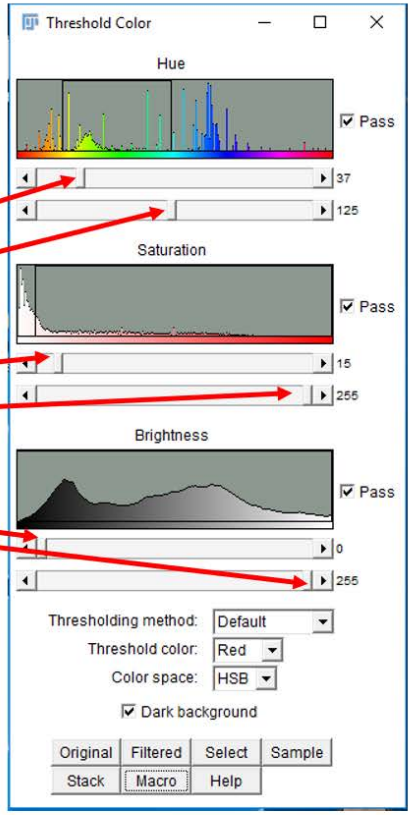


A SUMMARY OF THE PROCESS FOR HSB

Basic HSB Thresholding

Splits input image into three channels (hue, saturation and brightness) and processes each separately

```
// Color Thresholder 2.0.0-rc-69/1.52s
// Autogenerated macro, single images only!
min=newArray(3);
max=newArray(3);
filter=newArray(3);
a=getTitle();
run("HSB Stack");
run("Convert Stack to Images");
selectWindow("Hue");
rename("0");
selectWindow("Saturation");
rename("1");
selectWindow("Brightness");
rename("2");
min[0]=37;
max[0]=125;
filter[0]="pass";
min[1]=15;
max[1]=255;
filter[1]="pass";
min[2]=0;
max[2]=255;
filter[2]="pass";
for (i=0;i<3;i++){
  selectWindow(""+i);
  setThreshold(min[i], max[i]);
  run("Convert to Mask");
  if (filter[i]=="stop")  run("Invert");
}
imageCalculator("AND create", "0","1");
imageCalculator("AND create", "Result of 0","2");
for (i=0;i<3;i++){
  selectWindow(""+i);
  close();
}
selectWindow("Result of 0");
close();
selectWindow("Result of Result of 0");
rename(a);
// Colour Thresholding-------------
run("Analyze Particles...", "size=20-Infinity summarize");
```



Min/max values for thresholding HSB values can be changed by replacing with any value 0-255

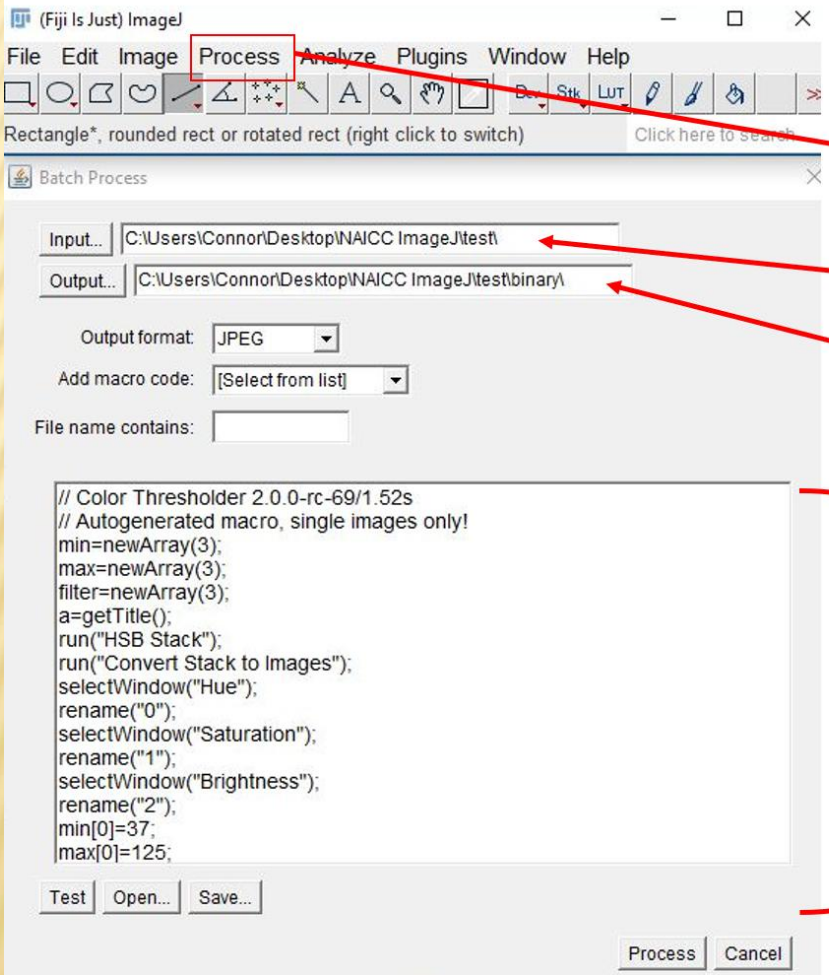
Best practice is to load one image from the set before analysis, run color threshold manually to determine best HSB values, then use these values in the script

Size range determines what size pixels to include/exclude from analysis; helps reduce noise

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SOME DETAILS

Automated batch processing in FIJI



Either rename plot photos with treatment/replicate identities, or keep track of the image sequence

Process > Batch > Macro

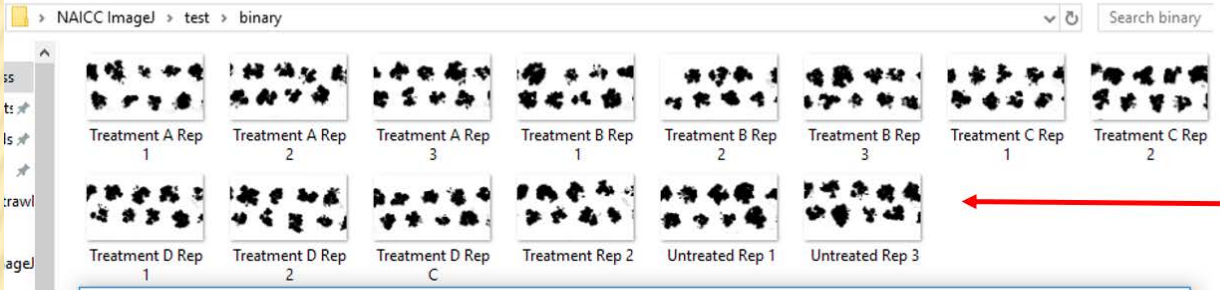
Location of input plot photos

Output location of processed photos

Copy and paste canopy analysis (or any other) script here

Results summary

Save summary table as .csv for export to Excel



Saving binary output images is important to confirm the macro is working properly

Summary

File Edit Font

Slice	Count	Total Area	Average Size	%Area	Mean	Perim.	Major	Minor	Angle
Treatment A Rep 1.jpg	242	1717732	7098.066	18.784	255	219.275	39.667	28.821	96.352
Treatment A Rep 2.jpg	263	1824808	6938.433	19.955	255	195.935	35.540	19.684	97.201
Treatment A Rep 3.jpg	242	1868228	7719.950	20.430	255	208.888	39.062	22.066	99.982
Treatment B Rep 1.jpg	212	1960380	9247.075	21.438	255	225.626	41.164	24.104	88.404
Treatment B Rep 2.jpg	133	1550118	11655.023	16.951	255	273.085	50.561	31.261	100.772
Treatment B Rep 3.jpg	219	1910643	8724.397	20.894	255	218.173	36.984	24.102	101.372
Treatment C Rep 1.jpg	174	1768242	10162.310	19.337	255	277.773	44.381	28.869	86.357
Treatment C Rep 2.jpg	142	2212998	15584.493	24.200	255	330.567	59.650	34.328	93.797
Treatment C Rep 3.jpg	163	1800254	11044.503	19.687	255	248.497	47.985	28.606	93.162
Treatment D Rep 1.jpg	156	1784209	11437.237	19.511	255	295.507	49.776	31.896	99.374
Treatment D Rep 2.jpg	133	1807069	13586.985	19.761	255	319.982	56.330	33.099	99.779
Treatment D Rep C.jpg	229	1742959	7611.175	19.060	255	203.427	37.948	22.556	97.989
Treatment Rep 2.jpg	148	1763746	11917.203	19.287	255	280.712	53.942	32.084	89.580
Untreated Rep 1.jpg	222	2116317	9532.959	23.143	255	213.732	38.634	25.148	107.896
Untreated Rep 3.jpg	201	2101117	10453.318	22.977	255	248.030	43.304	27.077	96.350

% Area column calculates what portion of the image is occupied by plant canopy

Can use total/average size (measured here in pixels) to approximate absolute area (m^2) if a reliable correlation from pixels to meters is known

Add in the plot numbers for the data, and paste to ARM

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ANOTHER COLOR SPACE TO USE

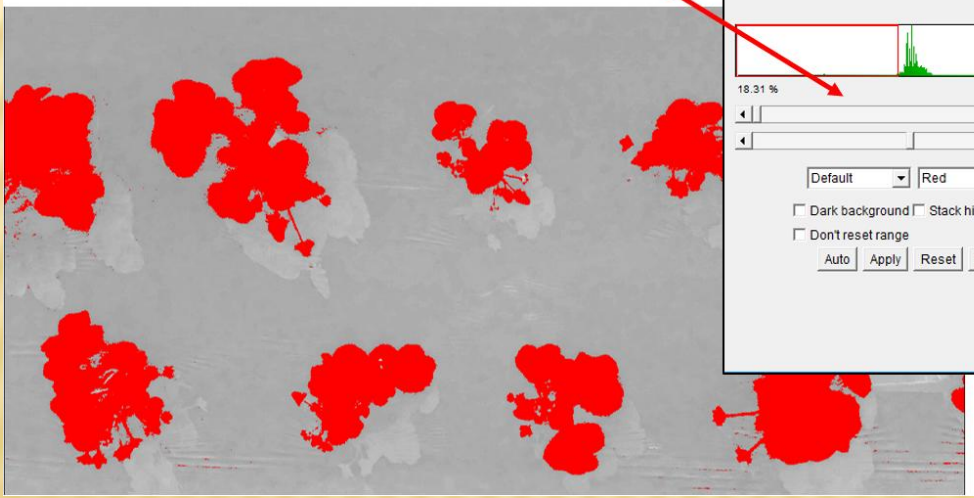
L*a*b* (Lab) Colorspace

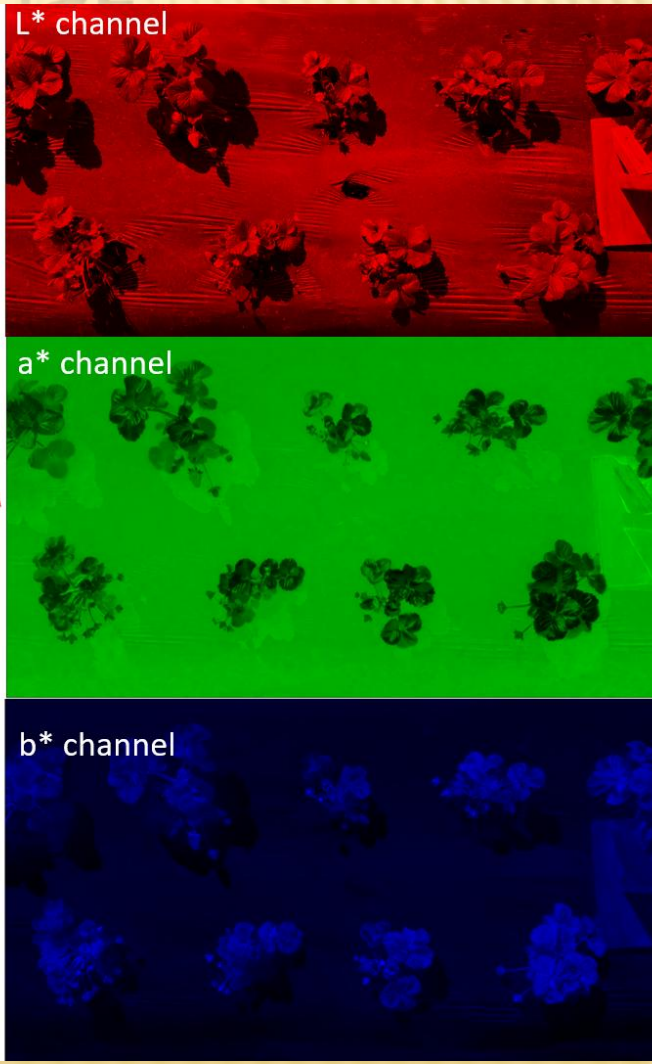
Can be used for better isolation of green on some images

- L*: lightness (white to black)
- a*: red-green range
- b*: blue-yellow range

Independent channels represented in grayscale, thresholding only uses high/low points of range

Note contrast between plants and background without any manipulation





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DIFFERENT METHODS CAN IMPROVE

Improving accuracy

Lighting conditions will affect image processing accuracy. Strong shadows or highlights cause loss of data

Certain filters can condition the image for better results during thresholding:

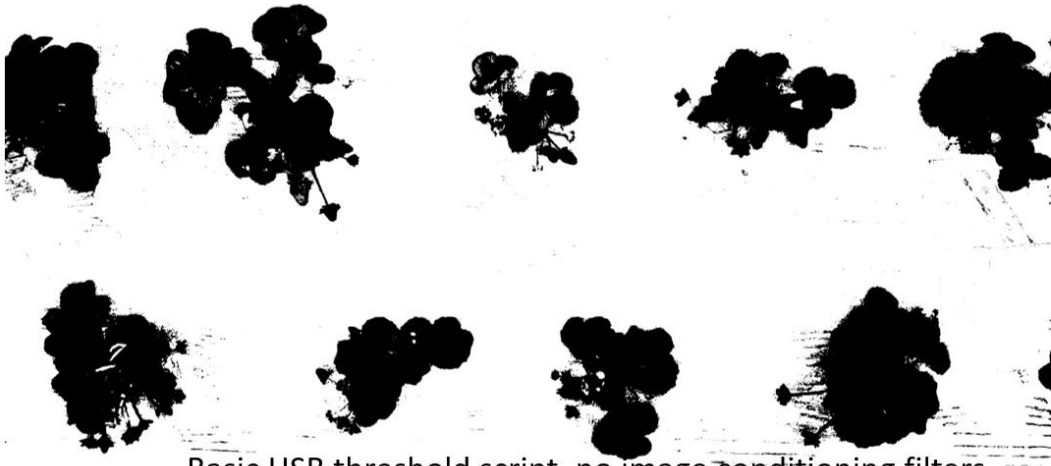
- Gaussian blur
- Median filter
- Kuwahara filter
- Bandpass filter (helps with shadows and specular light)

Overexposure, glare from sun

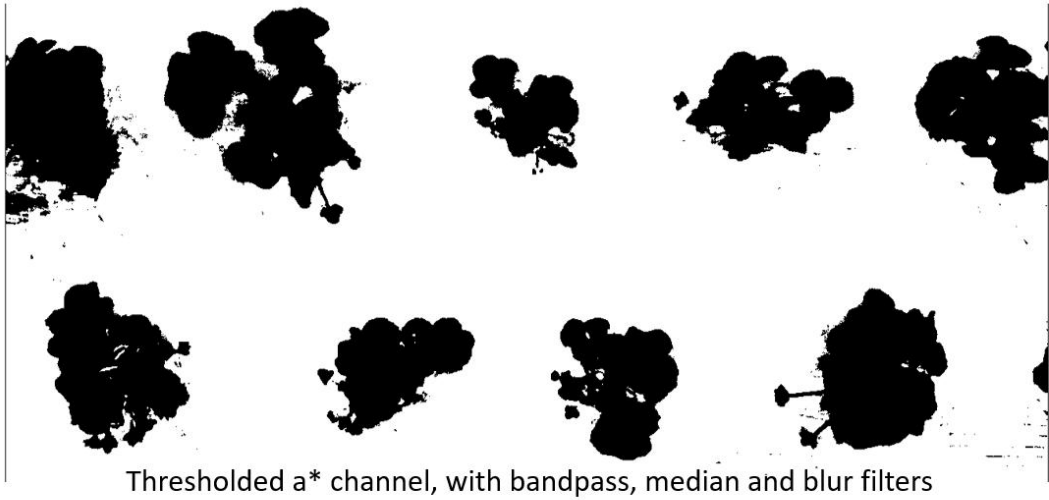


Loss of green pixels captured in analysis





Basic HSB threshold script, no image conditioning filters



Thresholded a* channel, with bandpass, median and blur filters applied before channel separation

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THE MACROS TO USE

Scripts

HSB

```
// Color Thresholder 2.0.0-rc-69/1.52s
// Autogenerated macro, single images only!
min=newArray(3);
max=newArray(3);
filter=newArray(3);
a=getTitle();
run("HSB Stack");
run("Convert Stack to Images");
selectWindow("Hue");
rename("0");
selectWindow("Saturation");
rename("1");
selectWindow("Brightness");
rename("2");
min[0]=37;
max[0]=125;
filter[0]="pass";
min[1]=15;
max[1]=255;
filter[1]="pass";
min[2]=0;
max[2]=255;
filter[2]="pass";
for (i=0;i<3;i++){
  selectWindow(""+i);
  setThreshold(min[i], max[i]);
  run("Convert to Mask");
  if (filter[i]=="stop")  run("Invert");
}
imageCalculator("AND create", "0","1");
imageCalculator("AND create", "Result of 0","2");
for (i=0;i<3;i++){
  selectWindow(""+i);
  close();
}
selectWindow("Result of 0");
close();
selectWindow("Result of Result of 0");
rename(a);
// Colour Thresholding-------------
run("Analyze Particles...", "size=20-Infinity summarize");
```

Can copy/paste these lines at beginning of HSB script to add filters. Change values to affect intensity of filter

To use other filters or actions, use the "record macro" option in FIJI while manually applying a filter

LAB

Threshold range, determine manually with one test image

```
x=getTitle();
run("Lab Stack");
run("Stack to Images");
selectWindow("a*");
rename(x);
selectWindow("L*");
close();
selectWindow("b*");
close();
//run("Threshold...");
setThreshold(-1000000000000000000000000000000.0000, -2.6113);
run("Make Binary");
run("Convert to Mask");
run("Analyze Particles...", "size=20-Infinity summarize");
```

LAB (With filters)

```
x=getTitle();
run("Bandpass Filter...", "filter_large=100 filter_small=3 suppress=None tolerance=5 autoscale saturate");
run("Median...", "radius=5");
run("Gaussian Blur...", "sigma=2");
run("Lab Stack");
run("Stack to Images");
selectWindow("a*");
rename(x);
selectWindow("L*");
close();
selectWindow("b*");
close();
//run("Threshold...");
setThreshold(-1000000000000000000000000000000.0000, -2.6113);
run("Make Binary");
run("Convert to Mask");
run("Analyze Particles...", "size=20-Infinity summarize");
```

RD4AG

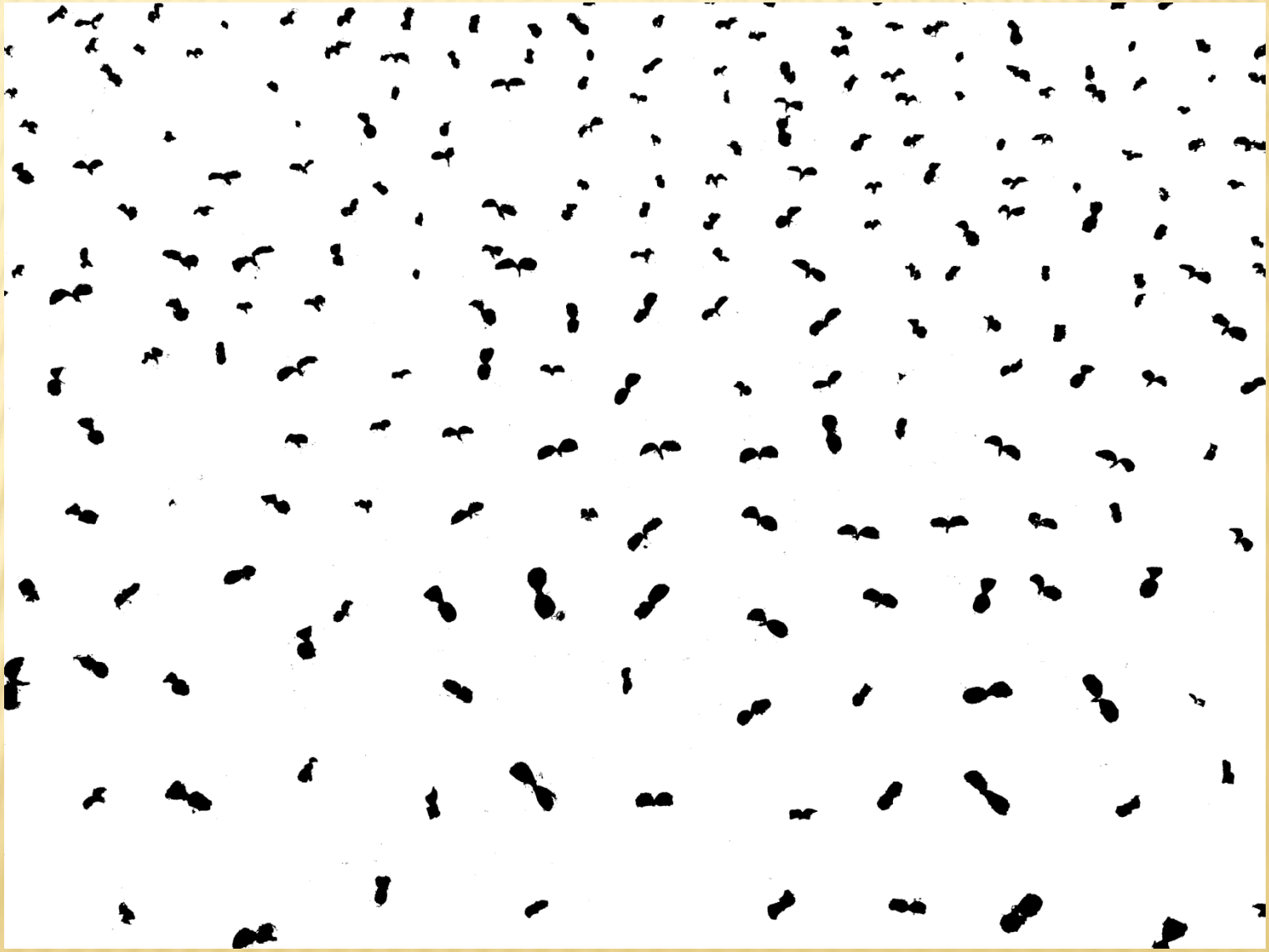
RADISH IN GREENHOUSE–COTYLEDON



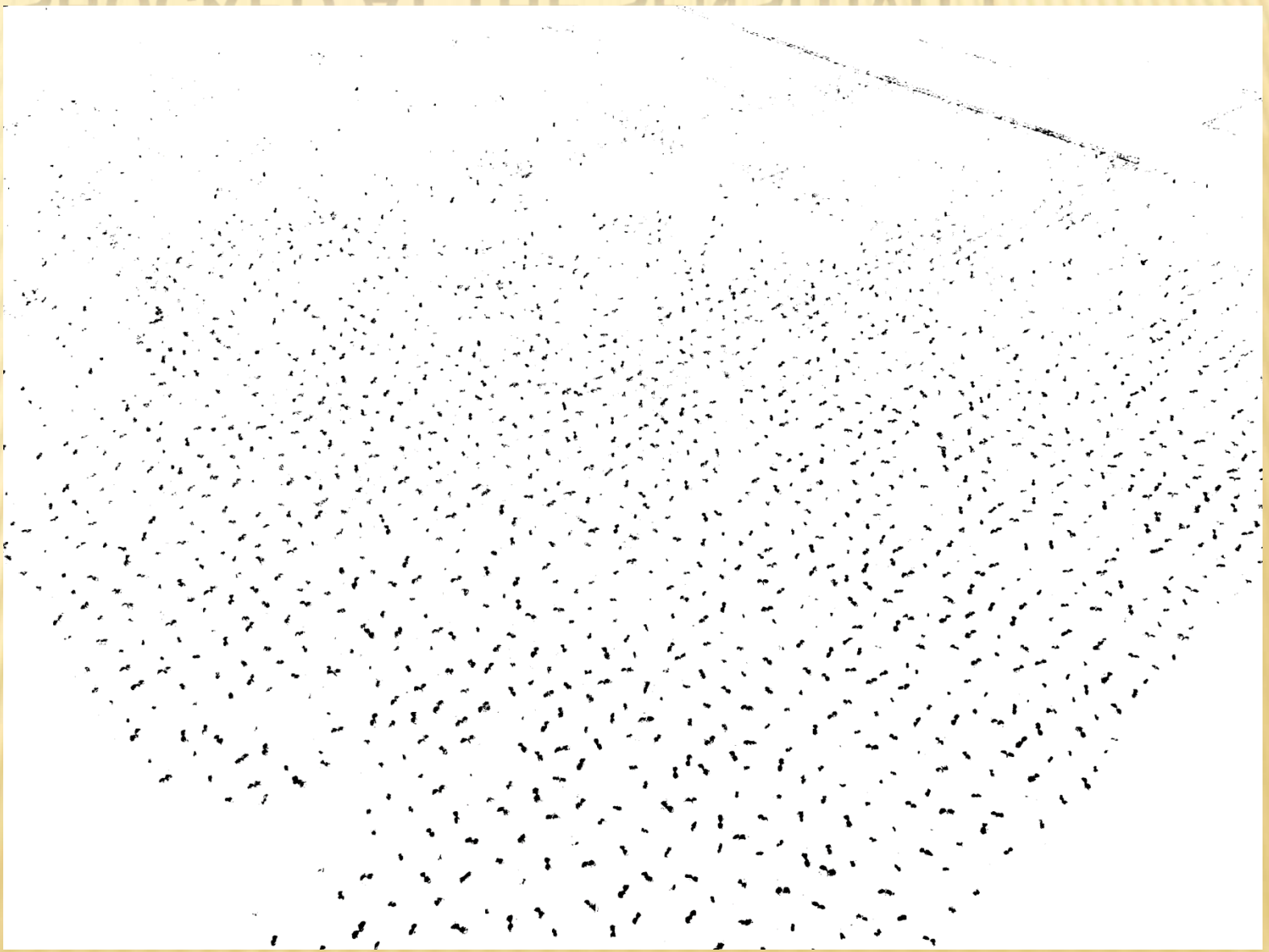
LOTS OF TRAYS



THIS TOOK ME A COUPLE MINUTES IS ALL



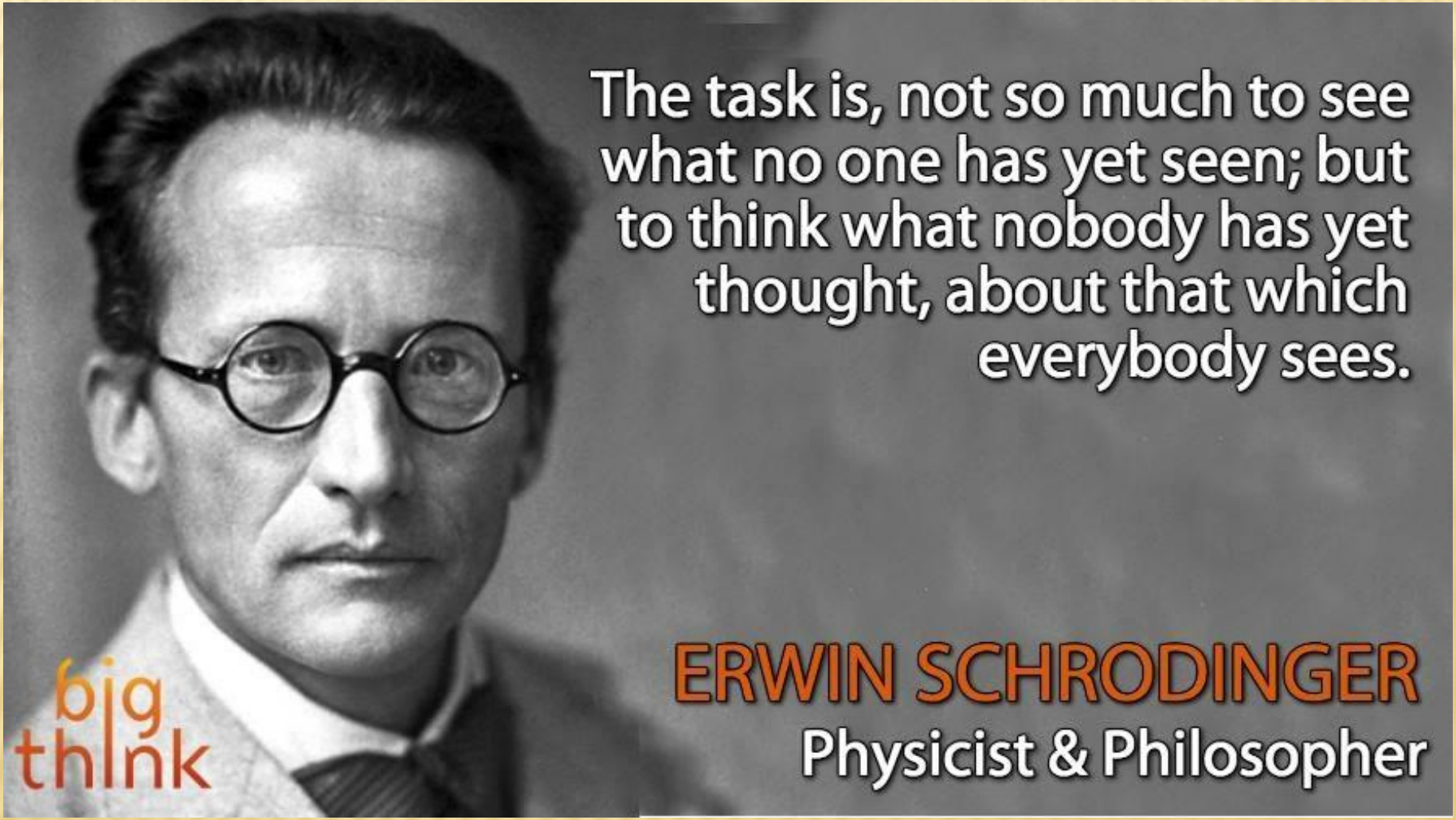
I WAS SHOCKED AT THE SENSITIVITY






One Thing To Be Sure….
This talk in 10 years will be
VASTLY different…
© Lee West Cultivated Images

QUESTIONS??


The task is, not so much to see what no one has yet seen; but to think what nobody has yet thought, about that which everybody sees.
ERWIN SCHRODINGER
Physicist & Philosopher
big think