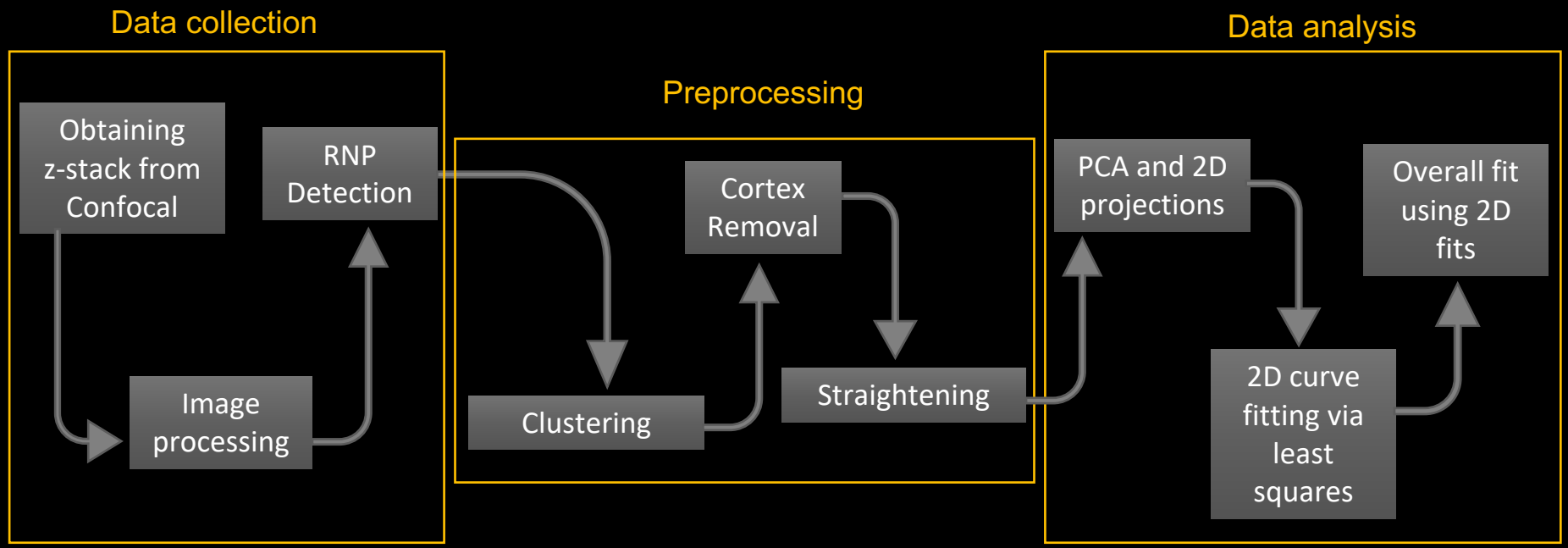
# Germplasm RNP Modeling

# Workflow


Data collection
Obtaining z-stack from Confocal
RNP Detection
Image processing
Preprocessing
Cortex Removal
Clustering
Straightening
Data analysis
PCA and 2D projections
Overall fit using 2D fits
2D curve fitting via least squares

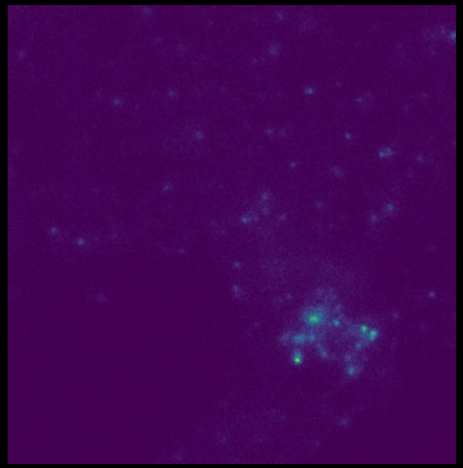
# Data collection

# Confocal microscopy

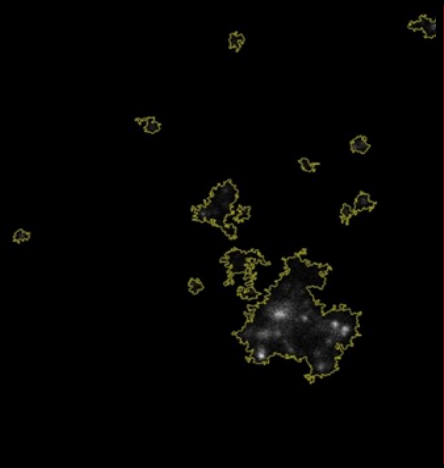
- Confocal microscopy was used to obtain the z-stack of germ plasm labeled for RNPs.
- Each z section was processed to obtain the location and radius of the RNPs present.

Composite image of 63 z sections (vasa-red, dazl-green)

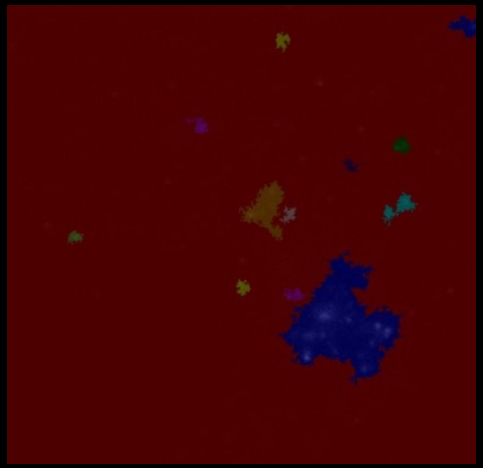
Original image



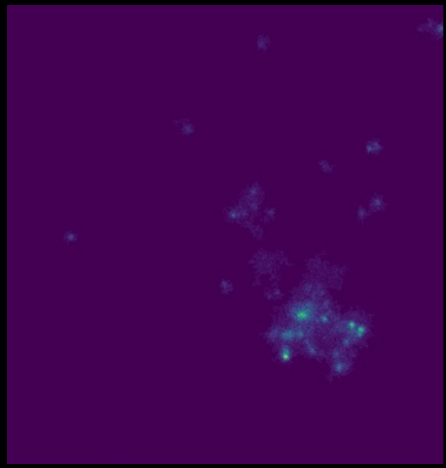
Watershed Segmentation



Labeled Segments



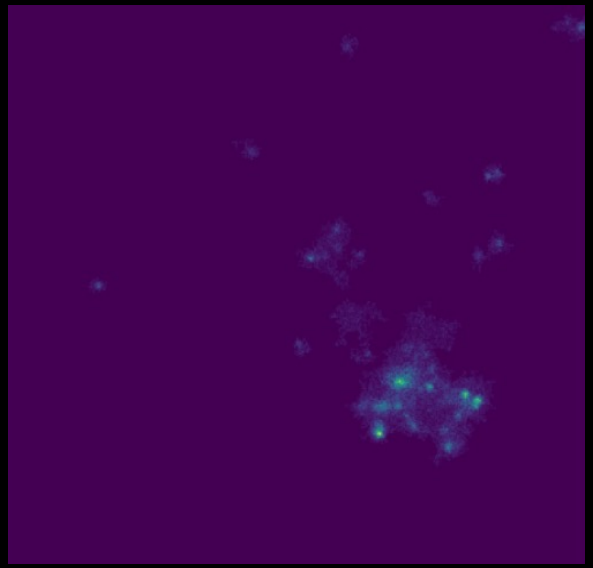
Cleaned image



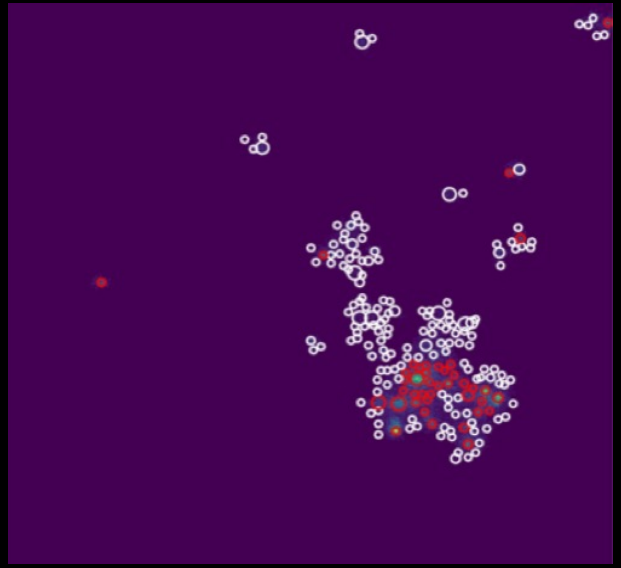
# Image processing

A watershed segmentation algorithm was used to isolate regions of fluorescence from the darker background.

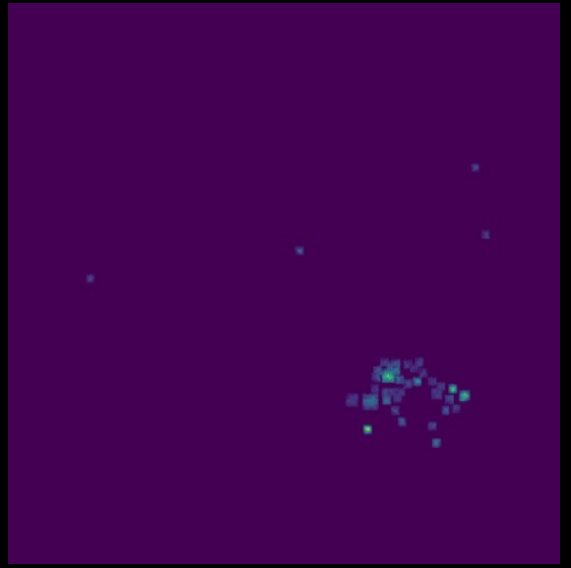
Cleaned Image



Laplacian of Gaussian



Highest Intensity Blobs
(Minimum Z-score kept = 0.5)

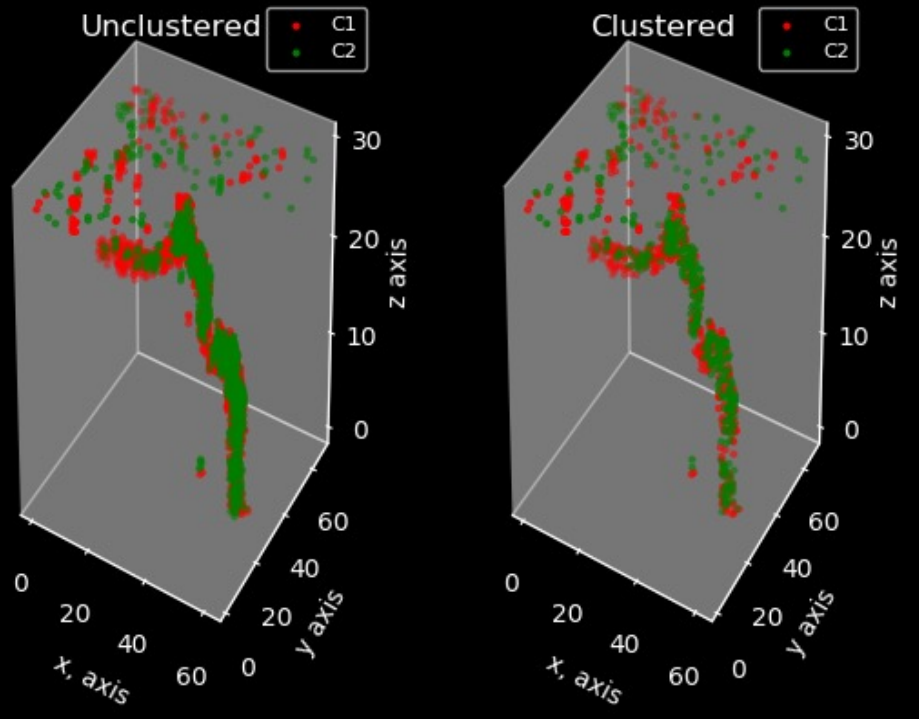


# RNP Detection

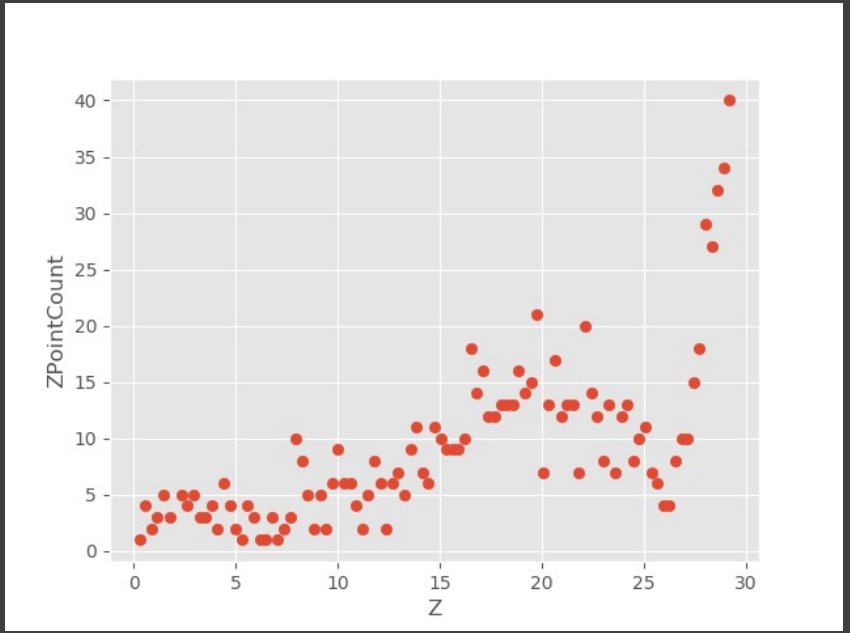
From the fluorescent regions, RNPs were detected using a Laplacian of Gaussian blob detection algorithm.

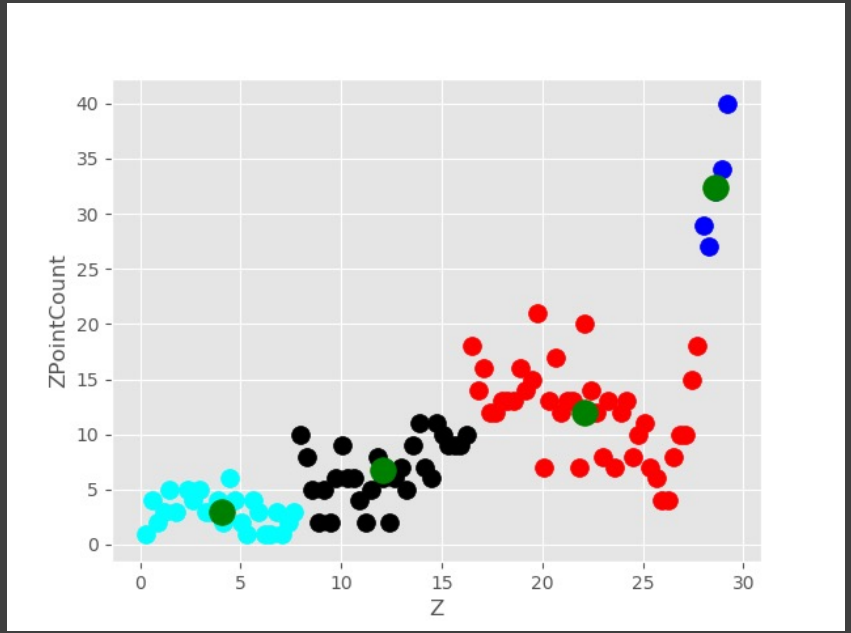
# Preprocessing

# Visualization & Clustering

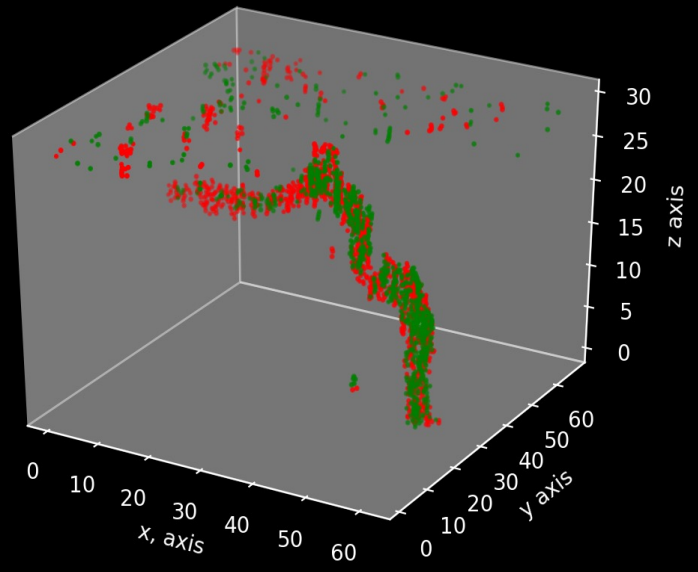



WT dnd-dazl

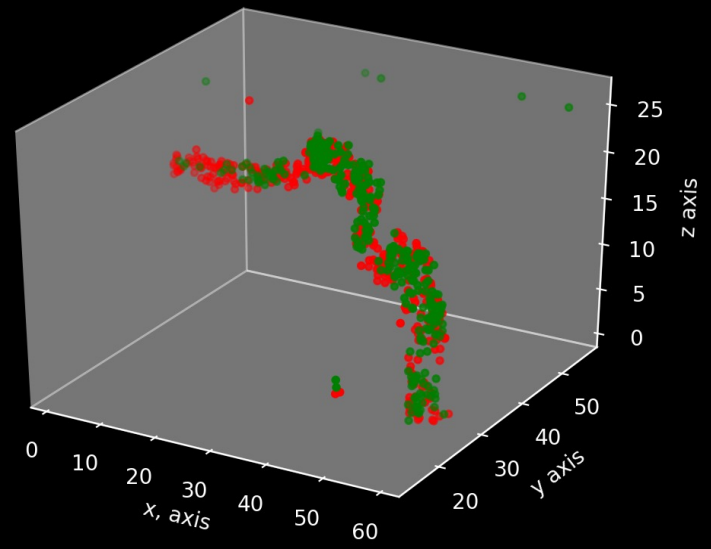



# Cortex Removal

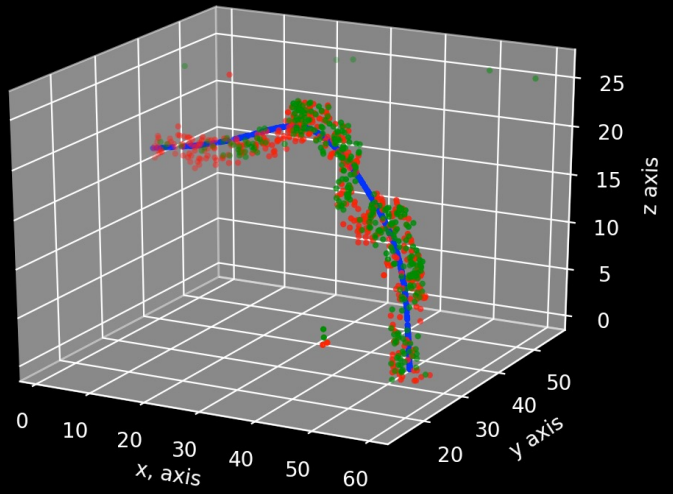



With cortex

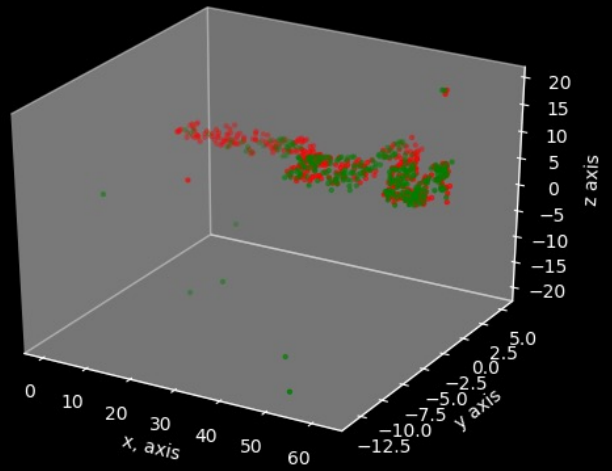



Without cortex

# Straightening



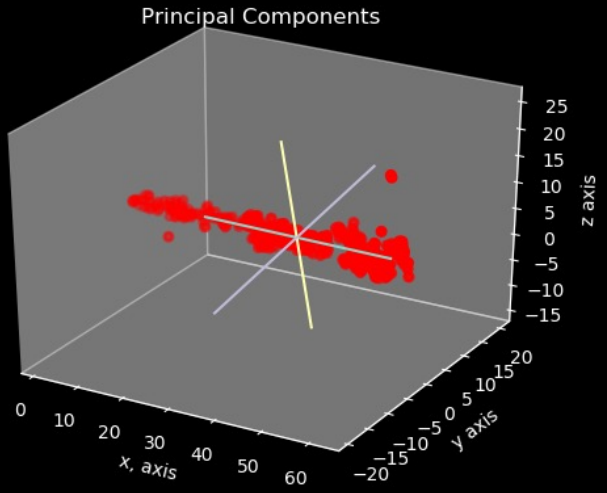

First principal curve

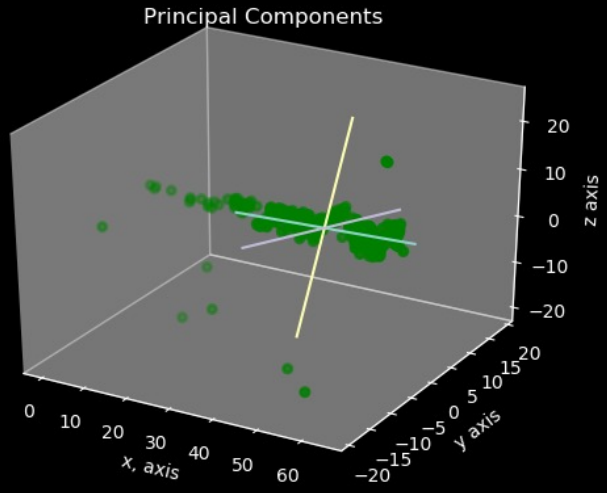


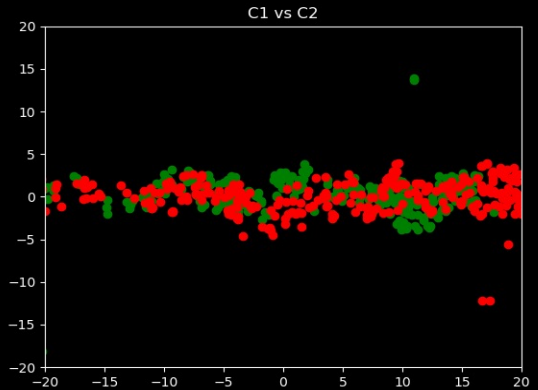

Straightened aggregate

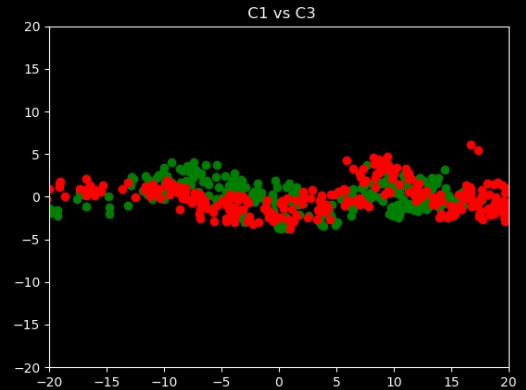
# Data analysis

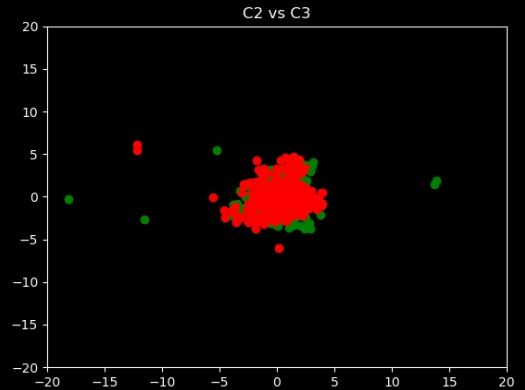
# Principal components



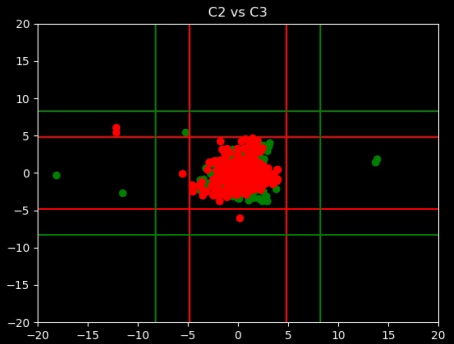

C1 vs C2
20
15
10
5
0
−5
−10
−15
−20
−20
−15
−10
−5
0
5
10
15
20

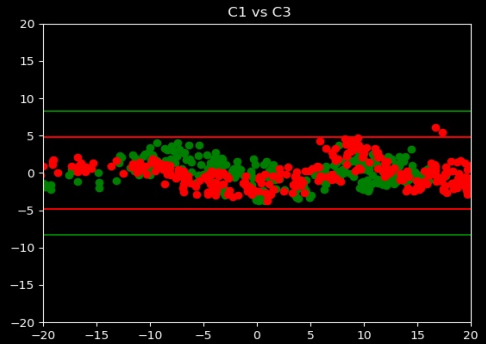

C1 vs C3
20
15
10
5
0
−5
−10
−15
−20
−20
−15
−10
−5
0
5
10
15
20

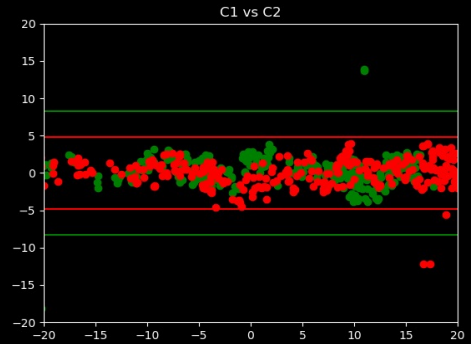

C2 vs C3
20
15
10
5
0
−5
−10
−15
−20
−20
−15
−10
−5
0
5
10
15
20

# 2D Plots

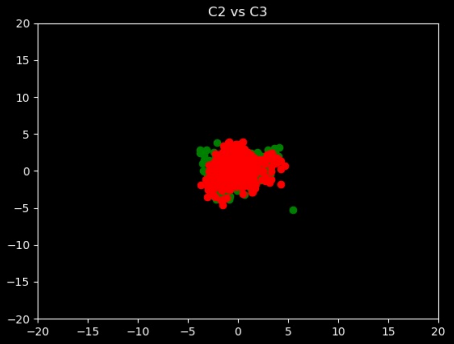
# Removing Outliers

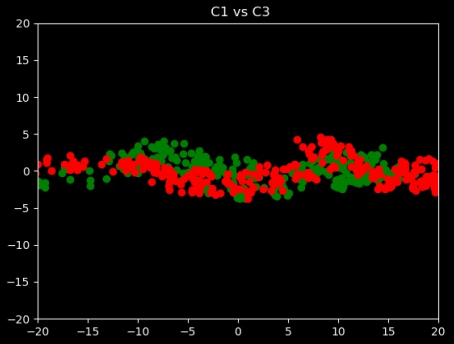


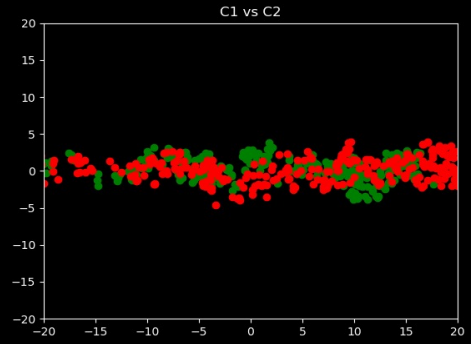



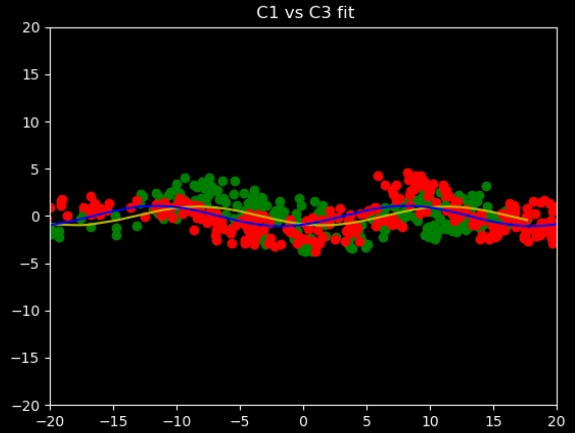

Outlier boundaries

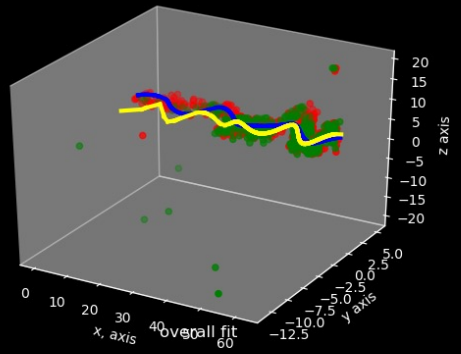


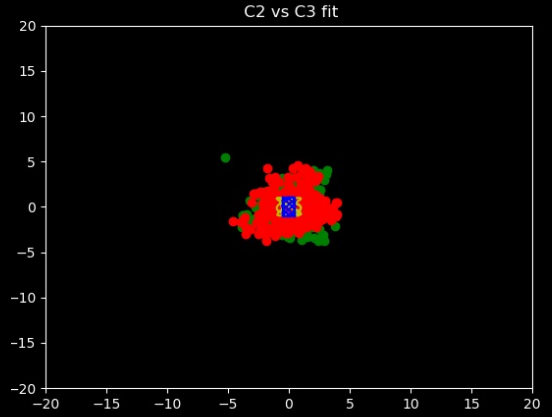



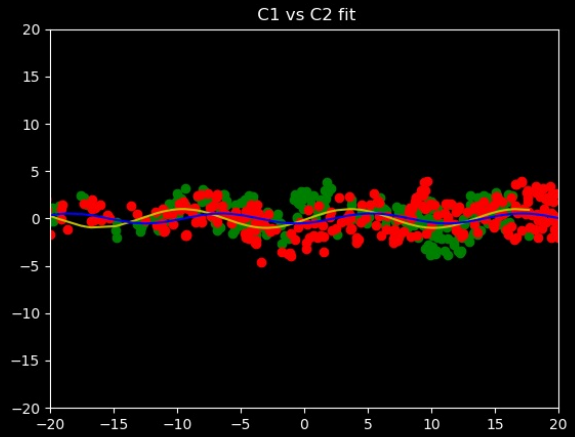




Cleaned 2D plots



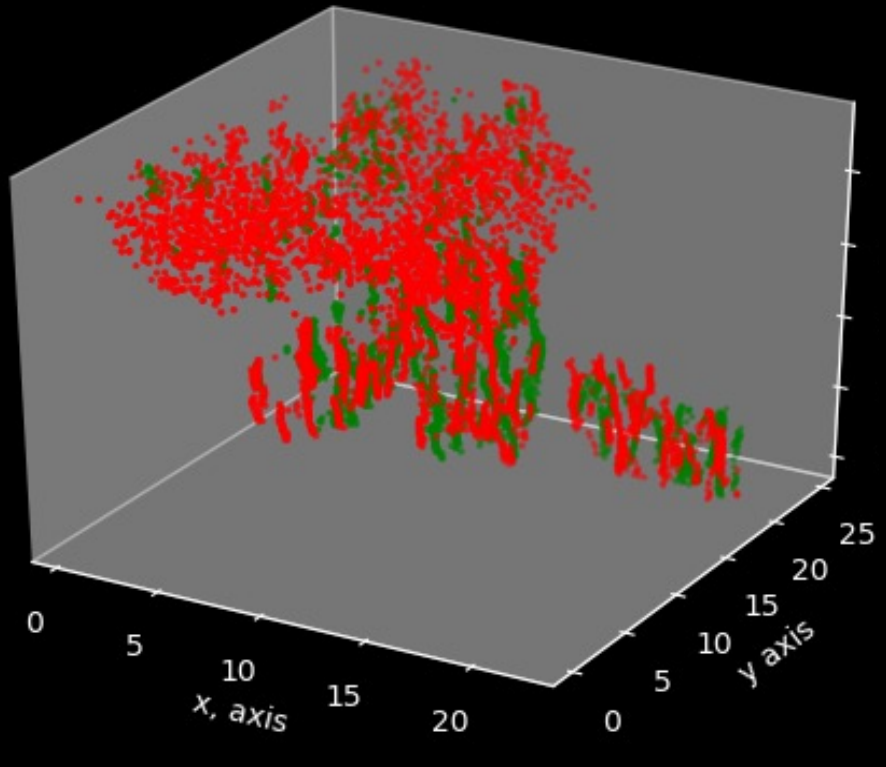



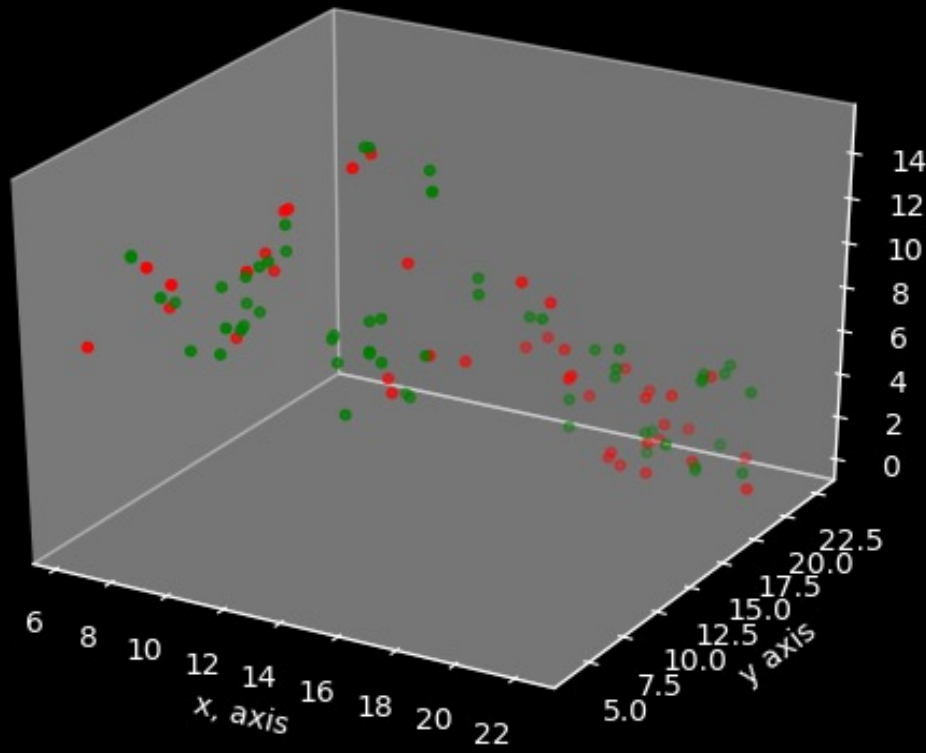

# Curve Fitting

# Results

# Aura dnd nanos

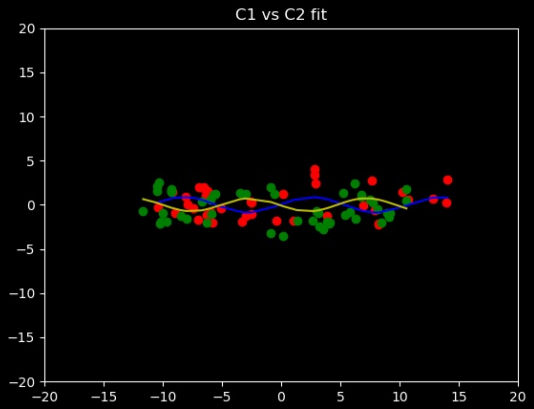



Original




Clustered & Cortex removed

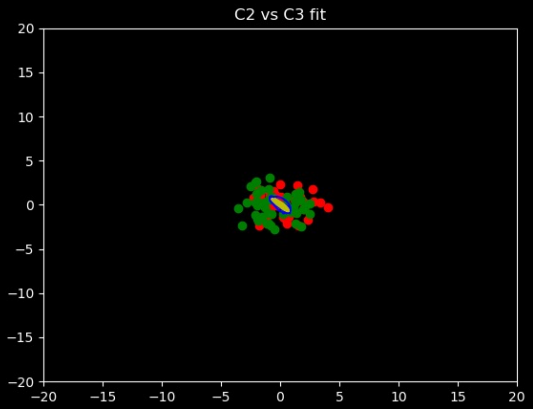
Fit:
r = 0.87
pi = 10.83
ph = 4.76

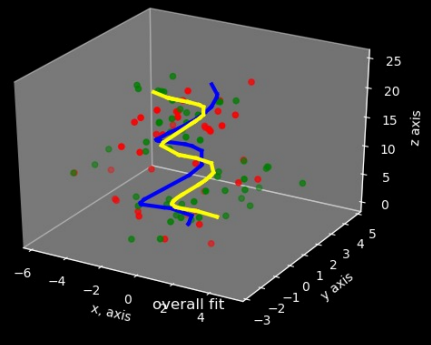



SE:
r = 0.33
pi = 1.3
ph = 0.49

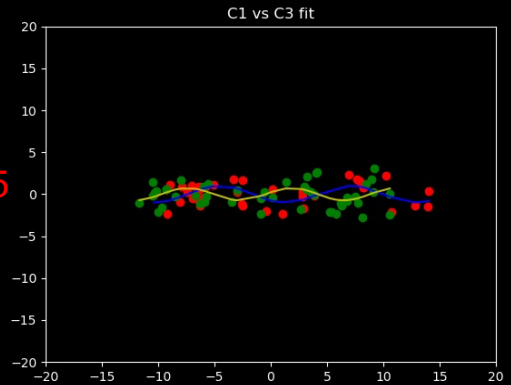
Fit:
r = 0.73
pi = 9.83
ph = 3.256

SE:
r = 0.369
pi = 0.85
ph = 0.42




Fit:
r = 0.98
pi = 11.6
ph = 4.005

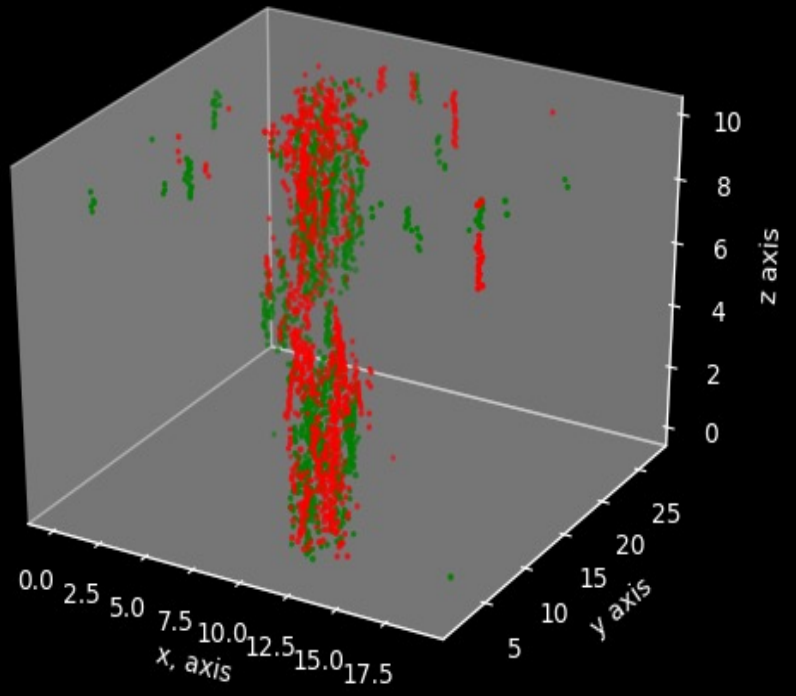



SE:
r = 0.28
pi = 0.88
ph = 0.27

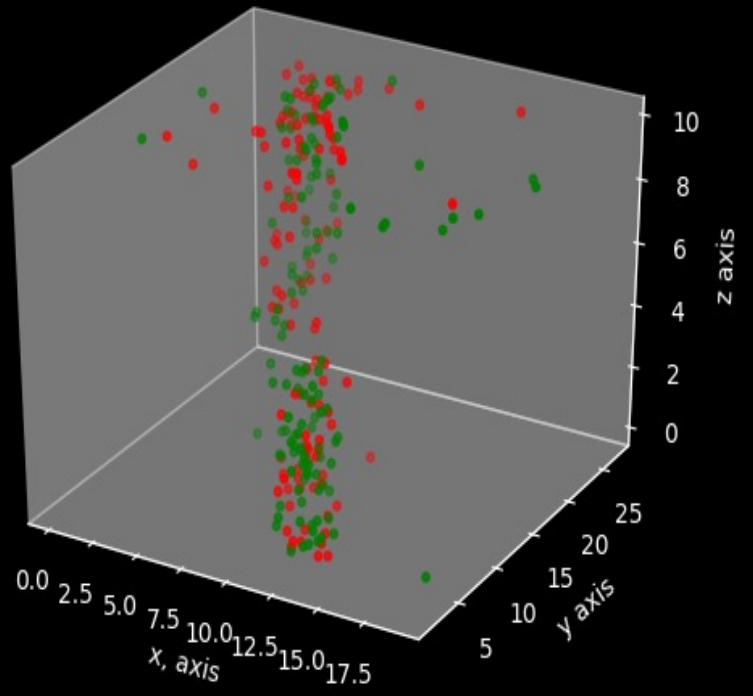
Fit:
r = 0.73
pi = 9.26
ph = 5.02

SE:
r = 0.3114
pi = 0.643
ph = 0.346

# WT vasa dazl



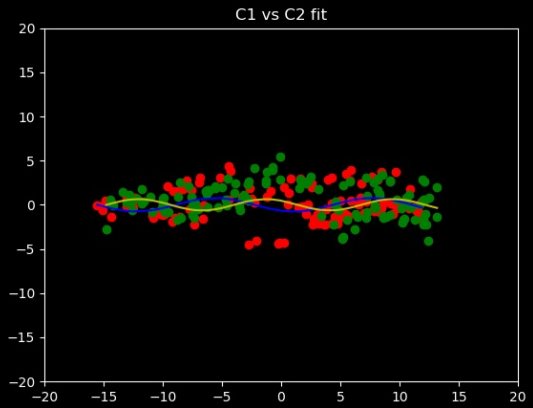

Original




Clustered & Cortex removed

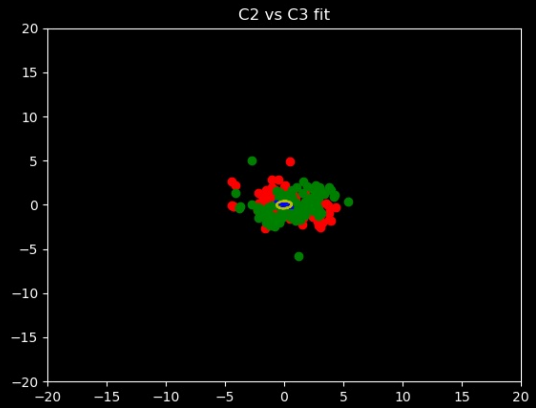
Fit:
r = 0.75
pi = 13.28
ph = 4.20

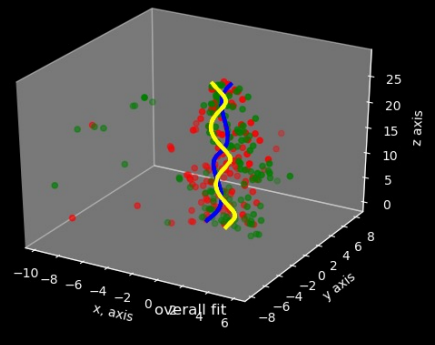
SE:
r = 0.26
pi = 1.01
ph = 0.29



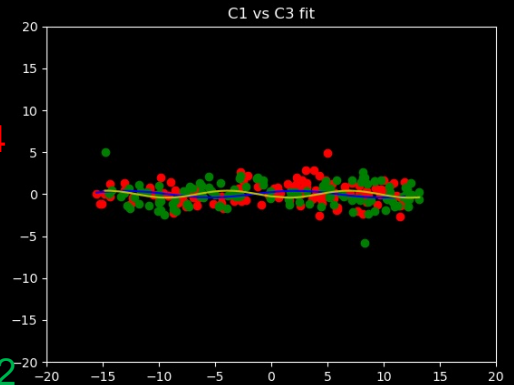

Fit:
r = 0.63
pi = 10.72
ph = 0.75

SE:
r = 0.246
pi = 0.74
ph = 0.35




Fit:
r = 0.39
pi = 15.14
ph = 0.67




SE:
r = 0.15
pi = 1.92
ph = 0.4

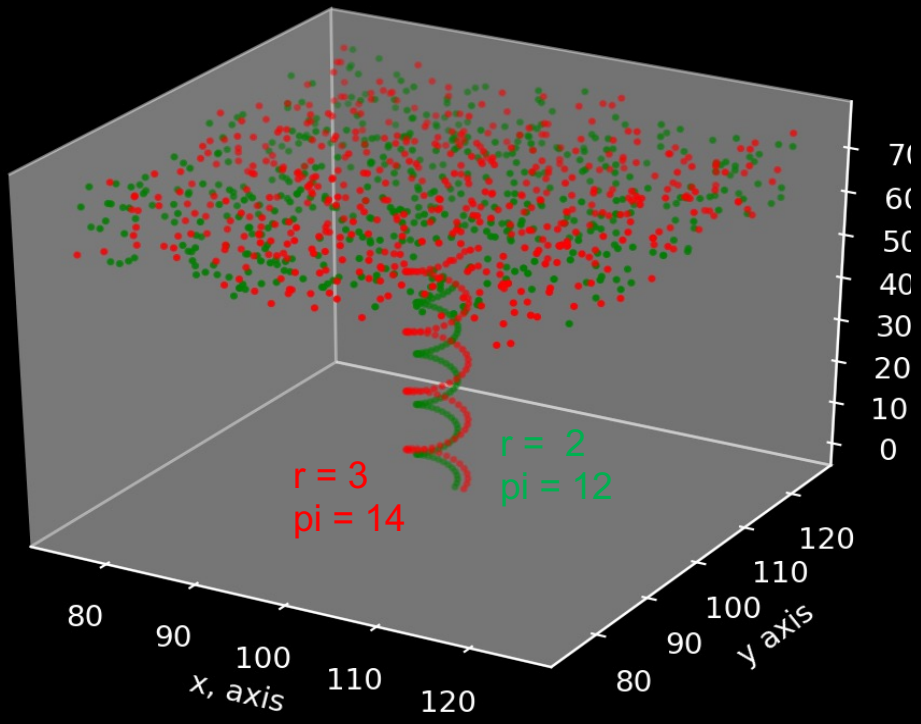
Fit:
r = 0.416
pi = 10.82
ph = 2.24

SE:
r = 0.158
pi = 0.945
ph = 0.411

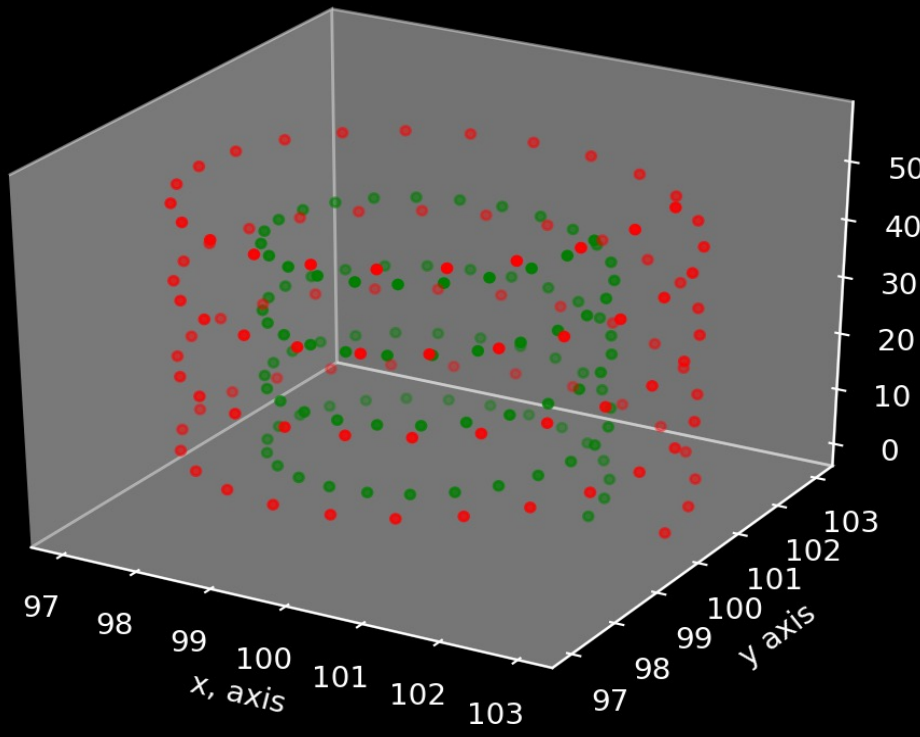
# We can use computer generated models with known patterns to understand what we see biologically

Synthetic models:
1. Perfect Helix
2. Helix with random error
3. Not a helix (stacks)

# Perfect helices



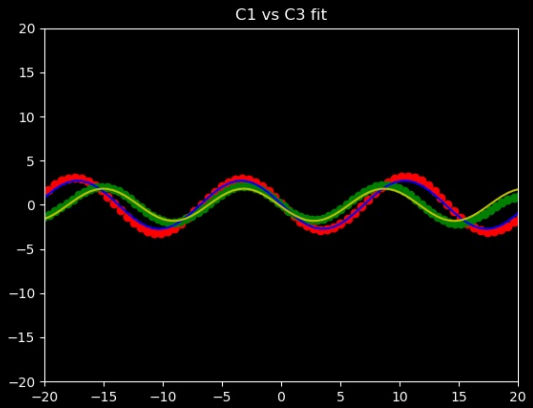

Original model




Cortex removed

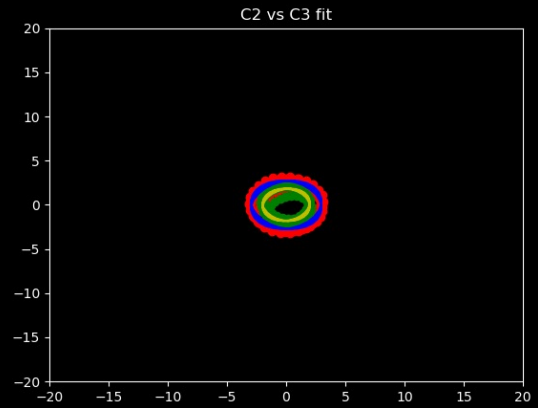
Fit:
r = 2.73
pi = 13.82
ph = 3.11

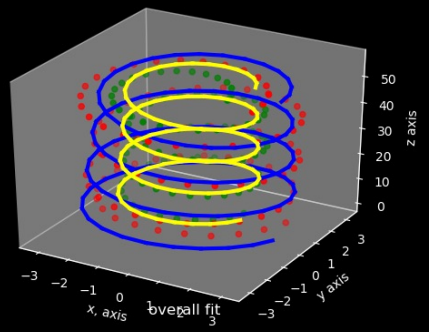
SE:
r = 0.07
pi = 0.05
ph = 0.03



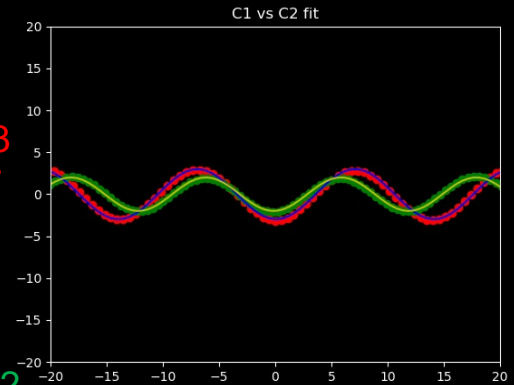

Fit:
r = 1.82
pi = 11.85
ph = 1.67

SE:
r = 0.058
pi = 0.052
ph = 0.03




Fit:
r = 2.98
pi = 14.03
ph = 4.67

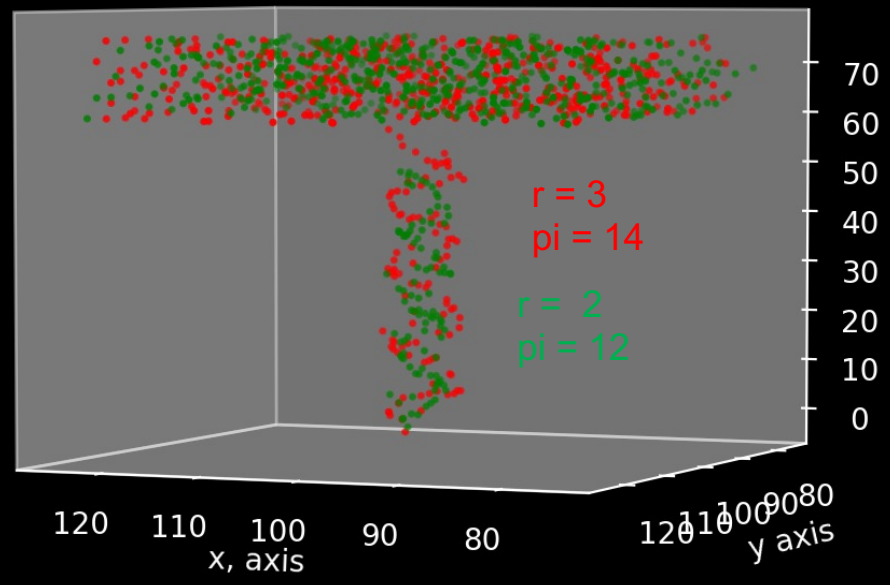



SE:
r = 0.024
pi = 0.015
ph = 0.008

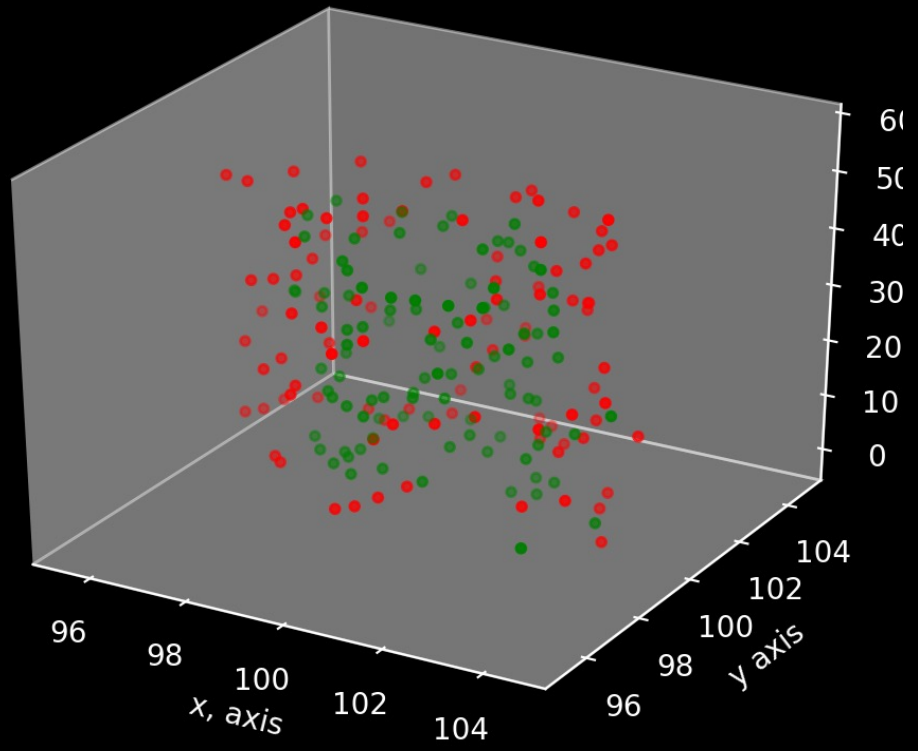
Fit:
r = 1.99
pi = 12.02
ph = 3.22

SE:
r = 0.017
pi = 0.014
ph = 0.008

# Helices with random error



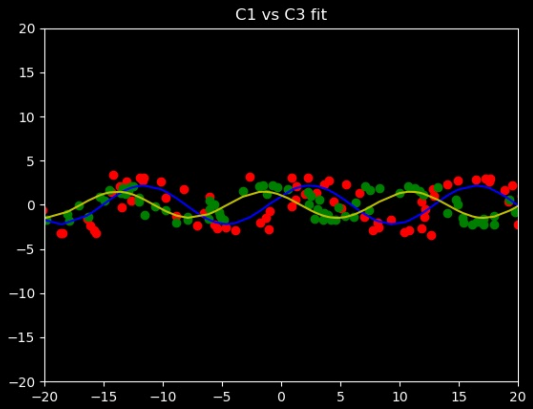

Original model




Cortex removed

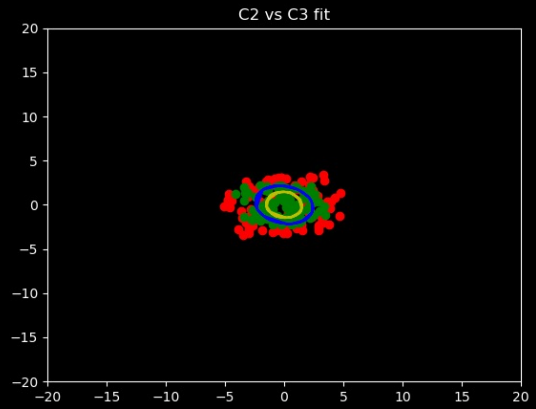
Fit:
r = 2.18
pi = 13.99
ph = 5.19

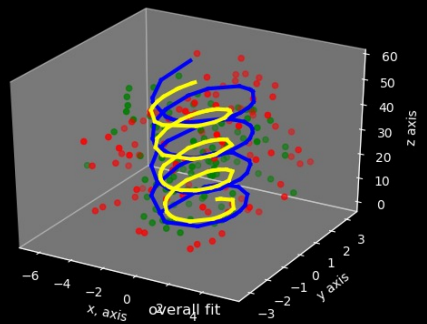
SE:
r = 0.21
pi = 0.18
ph = 0.09



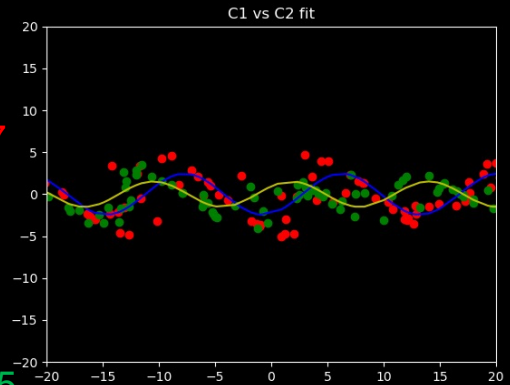

Fit:
r = 1.47
pi = 12.38
ph = 2.31

SE:
r = 0.13
pi = 0.16
ph = 0.09




Fit:
r = 2.45
pi = 13.97
ph = 3.47

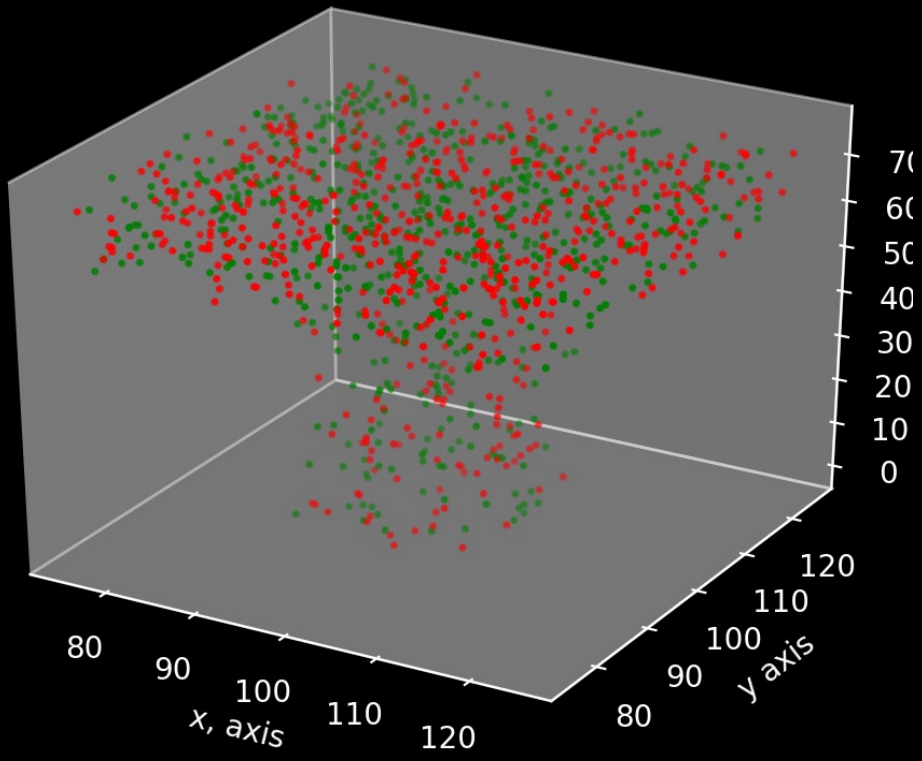



SE:
r = 0.25
pi = 0.21
ph = 0.11

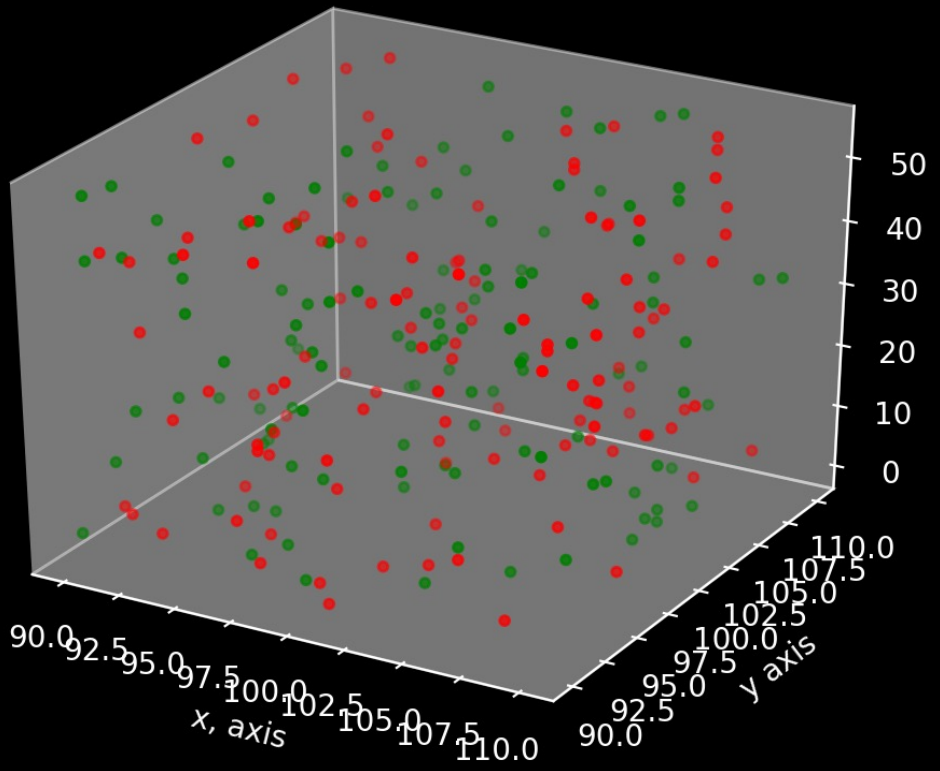
Fit:
r = 1.51
pi = 12.25
ph = 5.37

SE:
r = 0.22
pi = 0.236
ph = 0.134

# Not a helix

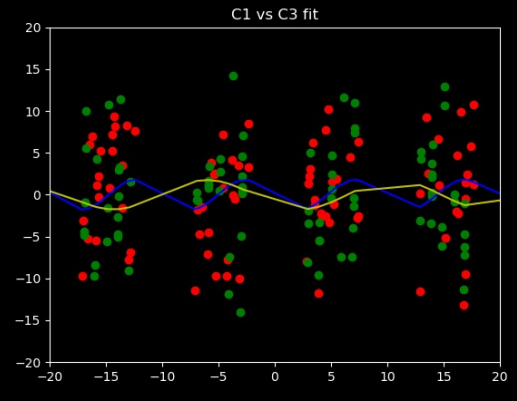



Original model




Cortex removed

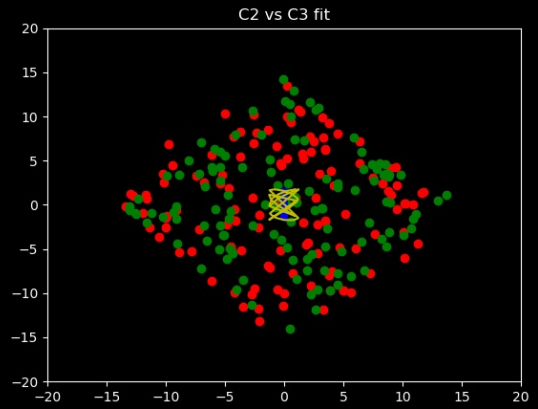
Fit:
r = 1.78
pi = 9.71
ph = 3.26

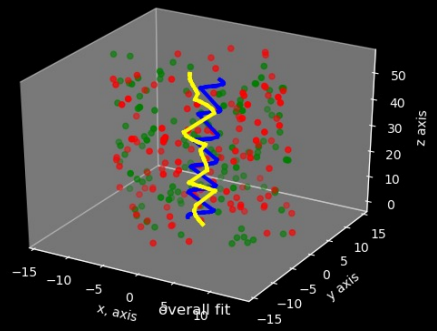



SE:
r = 0.76
pi = 0.41
ph = 0.45

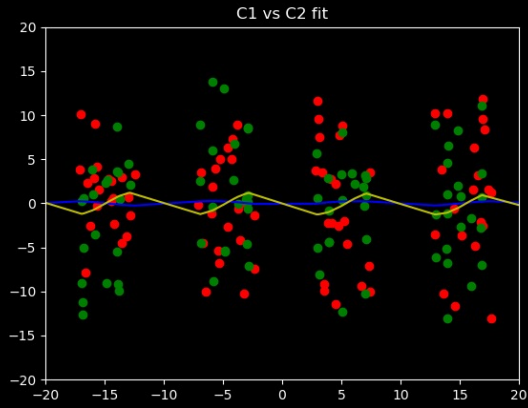
Fit:
r = 1.75
pi = 16.63
ph = 2.27

SE:
r = 0.76
pi = 0.97
ph = 0.39




Fit:
r = 0.258
pi = 12.008
ph = 3.07




SE:
r = 0.831
pi = 4.119
ph = 3.08

Fit:
r = 1.258
pi = 10.15
ph = 2.93

SE:
r = 0.8
pi = 0.624
ph = 0.667

# Directions

- **Ideas for synthetic models**
- Keeping radius and pitch constant, introduce 3D spatial error between 1 -50% of the radius for a regular helix and calculate standard error for each case.
- Keeping pitch constant, introduce changes in the radius and calculate the standard error of fits.
- Keeping radius constant, introduce changes in the pitch and calculate the standard error of fits.
- Combine changes in pitch and radius and calculate standard error of fits.
- Combine changes in pitch and radius with different percentages of 3D spatial error and quantify standard error.
- **We can use these models to elucidate what we see in zebrafish germ plasm.**