CHILDREN'S MEDICAL RESEARCH INSTITUTE

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Quantification of Biological Systems Using MATLAB for Image Processing

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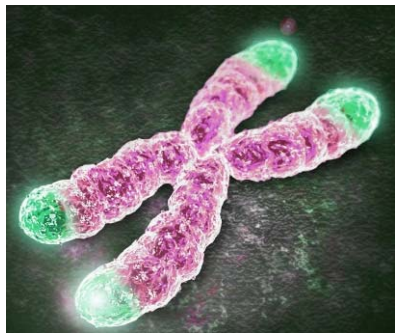
Image Processing

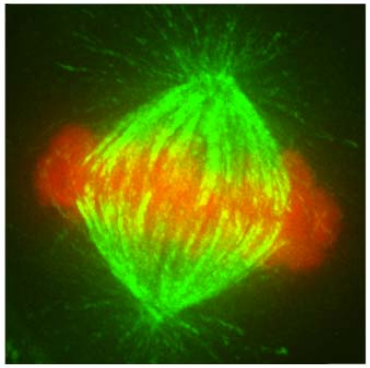
Project # 1:

- Proteins and Telomeres Colocalisation



Project # 2:

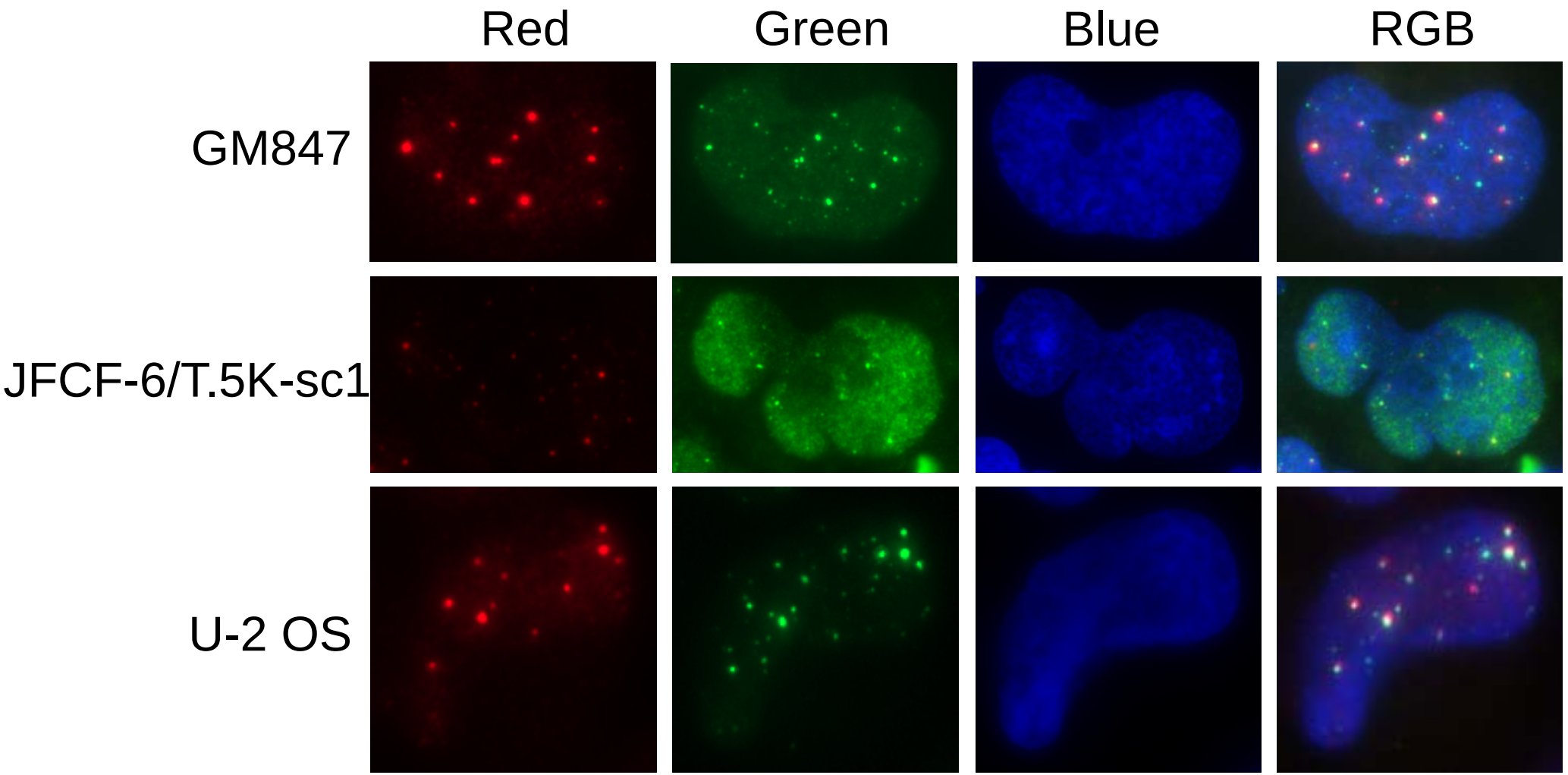
- DNA and Mitotic Spindle Features Quantification



Colocalisation Background

- Existing tools to study protein colocalisation use Pearson correlation or Mander's overlap coefficients to calculate the degree of colocalisation. These methods have a number of limitations.
- These methods have been shown to be greatly affected by the background noise.
- Most tools cannot automatically select a region of interest (ROI) and thus hinders analysis of a large number of images.
- Coefficient-based methods do not clearly report whether two signals are colocalised within a ROI, nor do they report the precise number of colocalisations in a specific region.

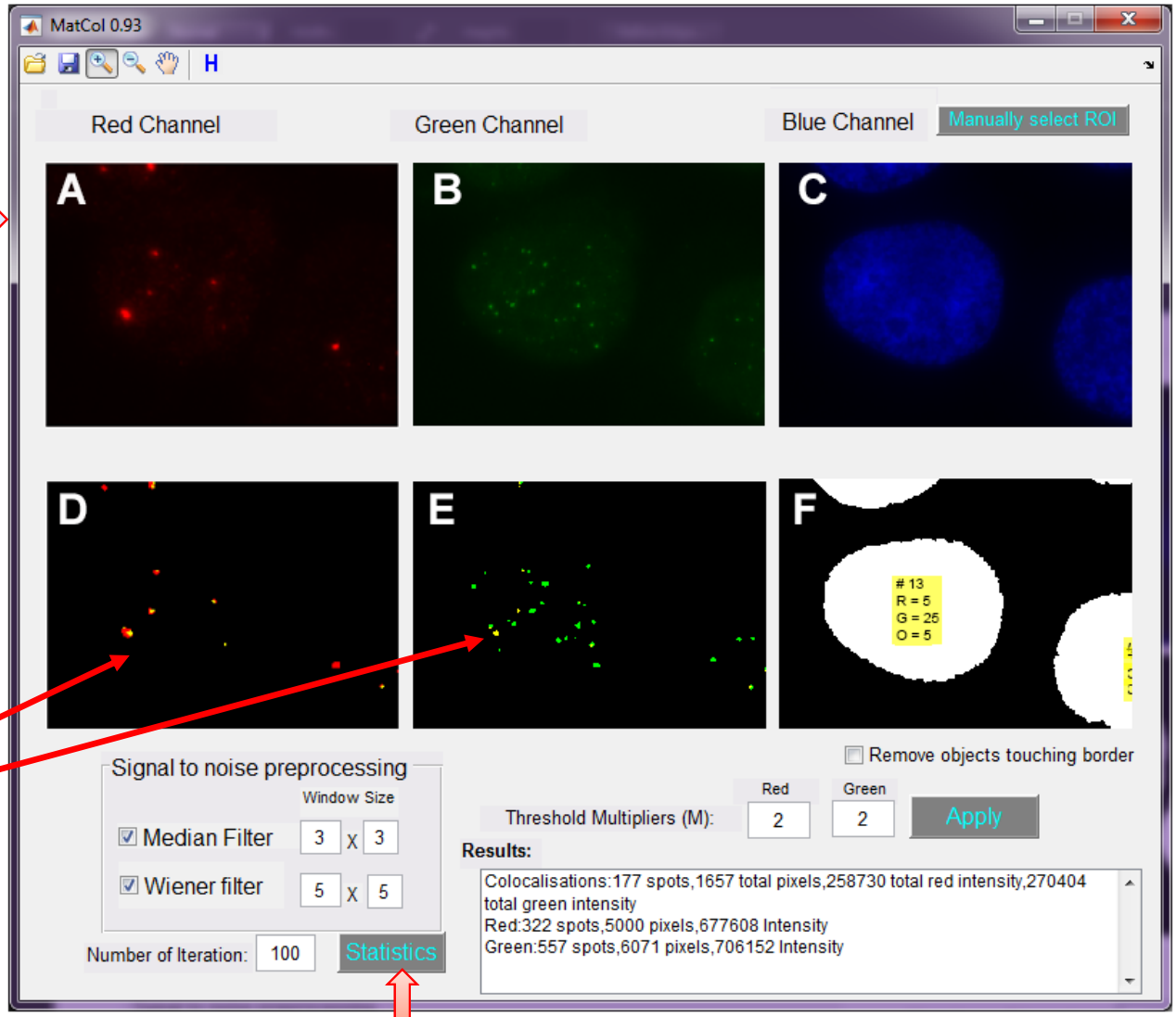
ROI-based background intensity selection



MatCol GUI

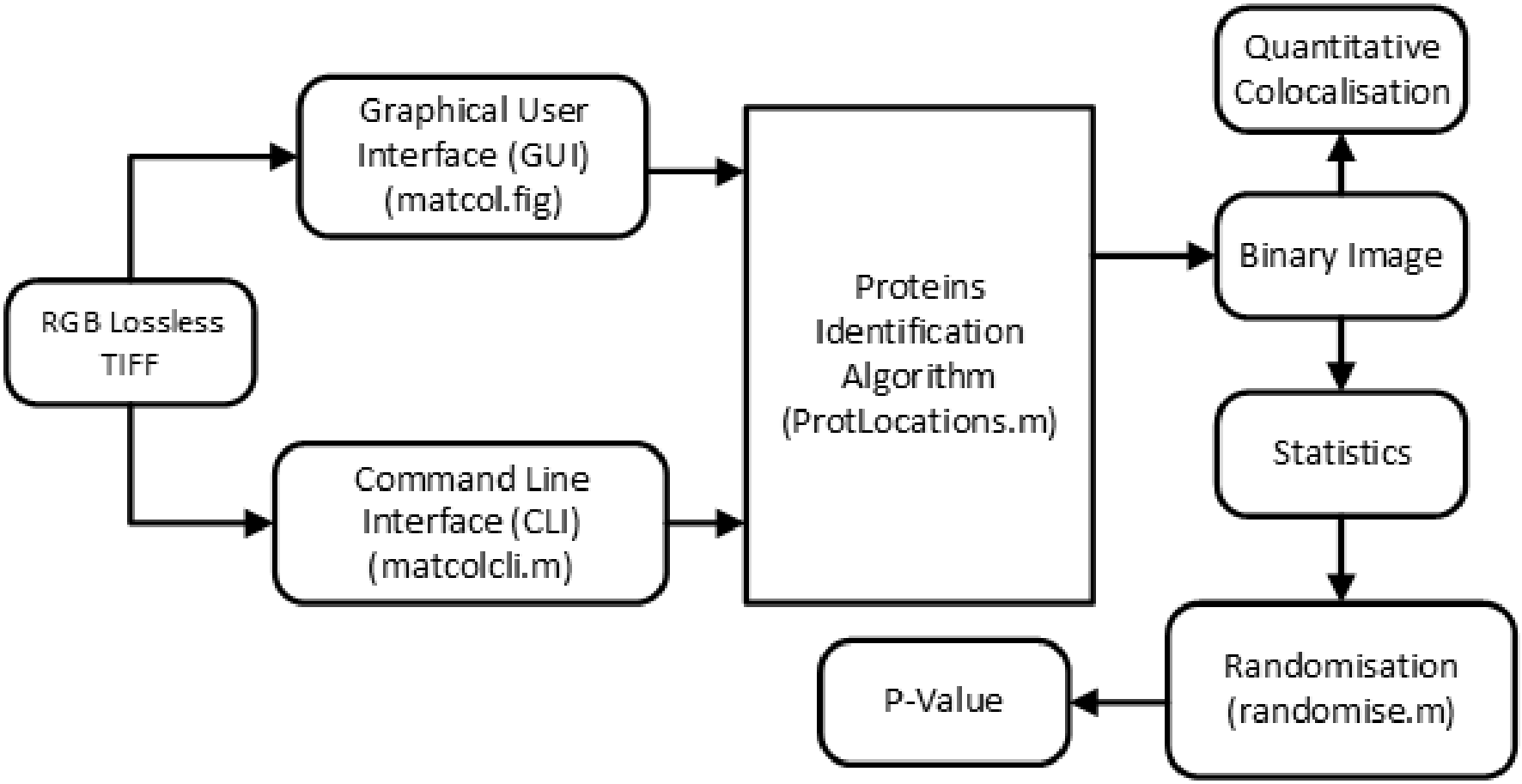
Windows of the red, green and blue channels, and their respective binary versions are provided.

Colocalisation are shown in yellow

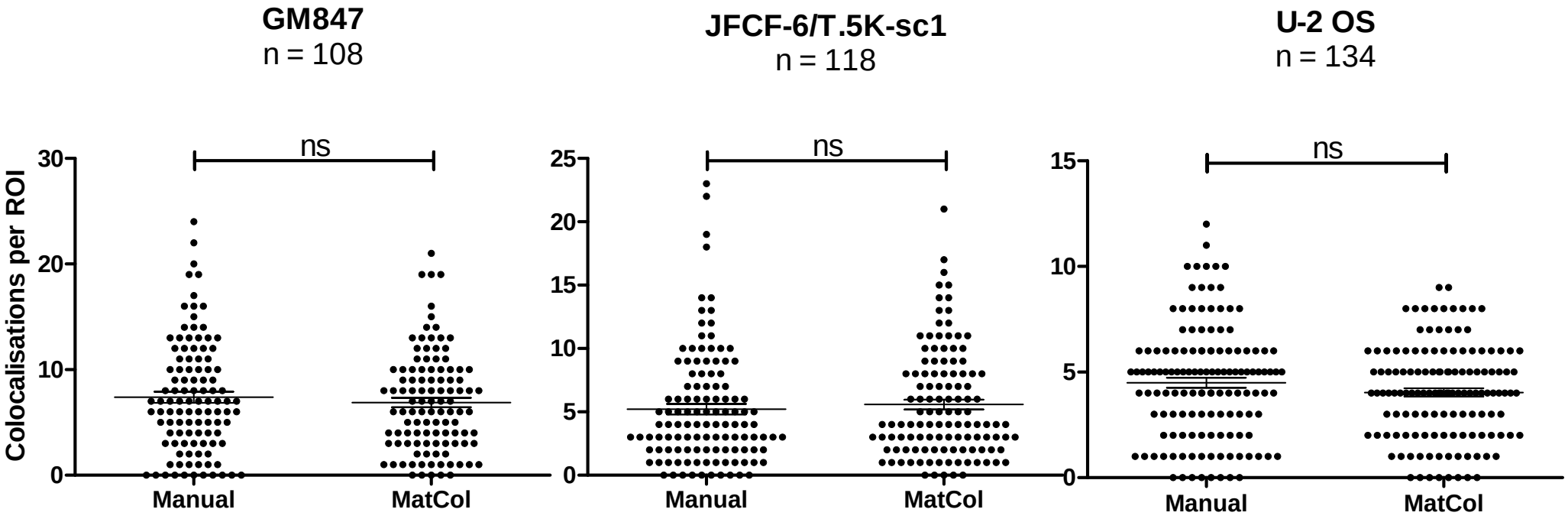


P-Value is calculated by the Student's t-test.

Modular software design

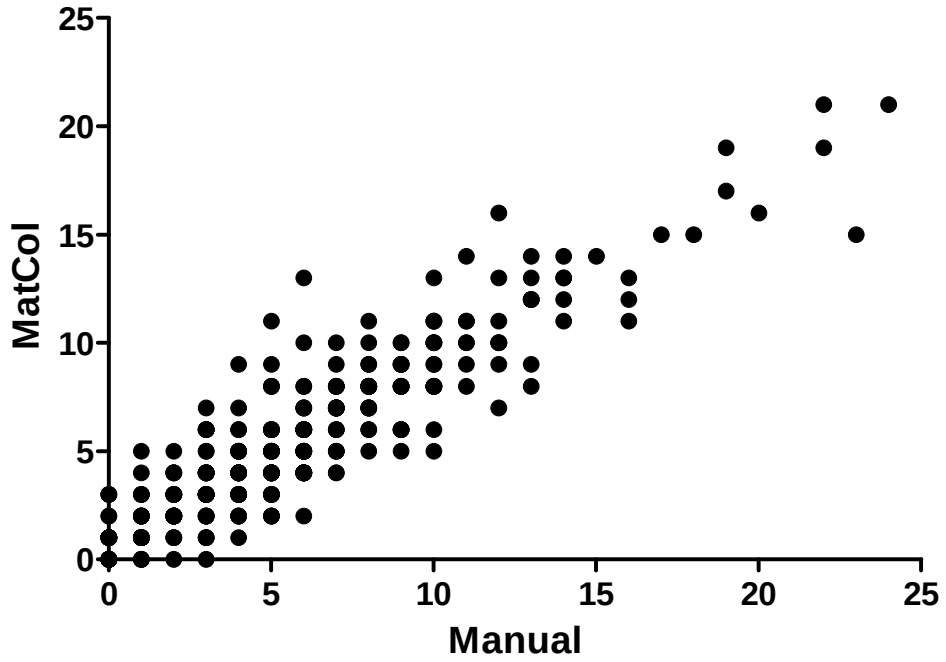


Non-significant difference between manual and MatCol quantification



Column graphs of manual versus MatCol colocalisations per ROI for each cell line. ns = not significant using unpaired Student's t-test.

Significant correlation between the manual and automated quantification



XY plot of the combined colocalisation data of three cell lines. A significant correlation between the manual and automated MatCol colocalisation was found (Pearson=0.91, P<0.0001).

MatCol Colocalisation Summary

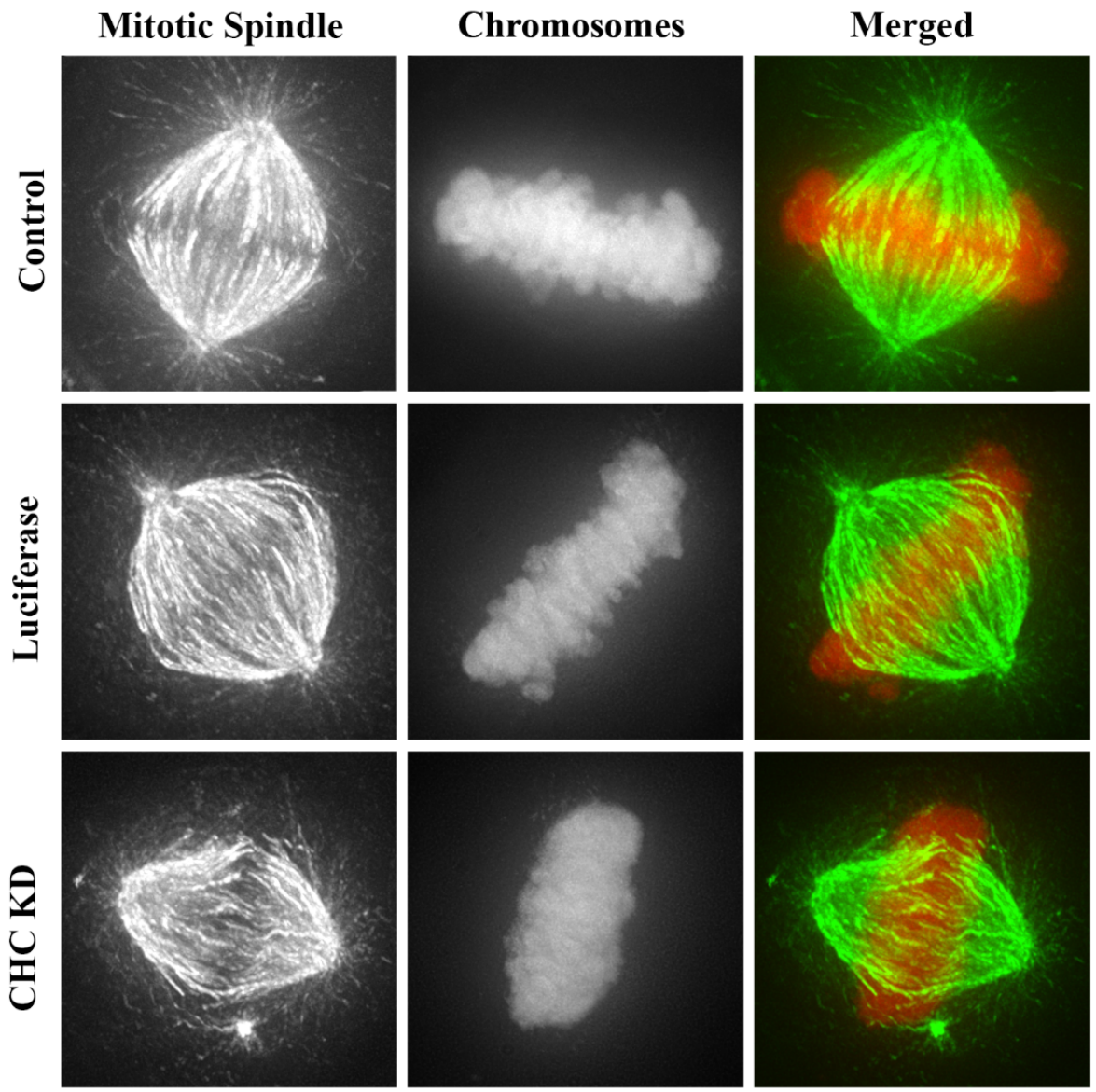
- MatCol is a novel and user-friendly tool that addresses the need to study the colocalisation of two biological features.
- MatCol has enabled us to efficiently, automatically, and without bias quantify colocalisations.
- MatCol reports colocalisation as a quantity independent of intensity.
- MatCol enables the measurement of statistical significance of the observed colocalisation of two fluorescence signals against overlap by random chance.

Project # 2:

Quantification of DNA and Mitotic Spindles

- Untreated sample (control)
- Luciferase (+ve control)
- Clathrin heavy chain Knockdown (CHC KD)

Sample Images



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Properties Measured

Length & area

- Area
- Convex area
- Compactness
- Eccentricity
- Perimeter
- Solidity
- Extent
- Major axis Length
- Minor axis length

Intensity

- Mean intensity
- Median intensity
- Total intensity

Texture based Analysis

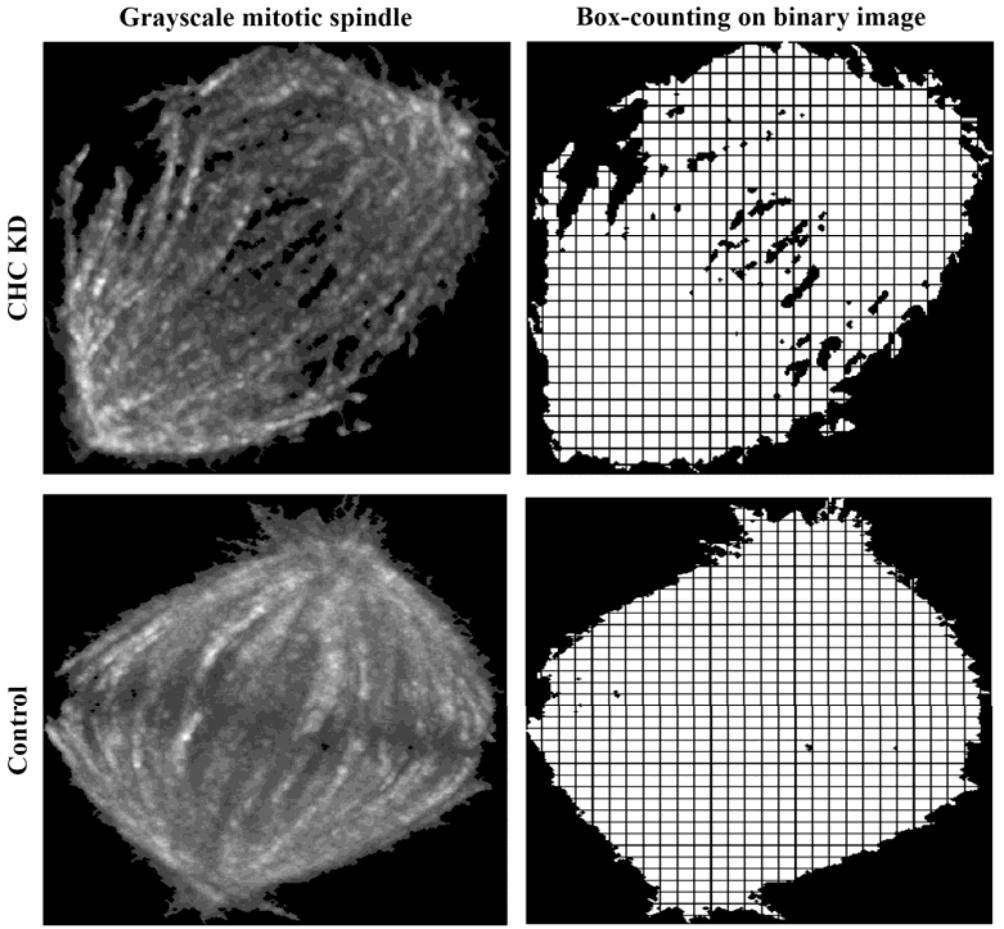
- Entropy
- Standard deviation

Other properties

- Orientation
- Percent Density
- Fractal Dimension
- Euler No.

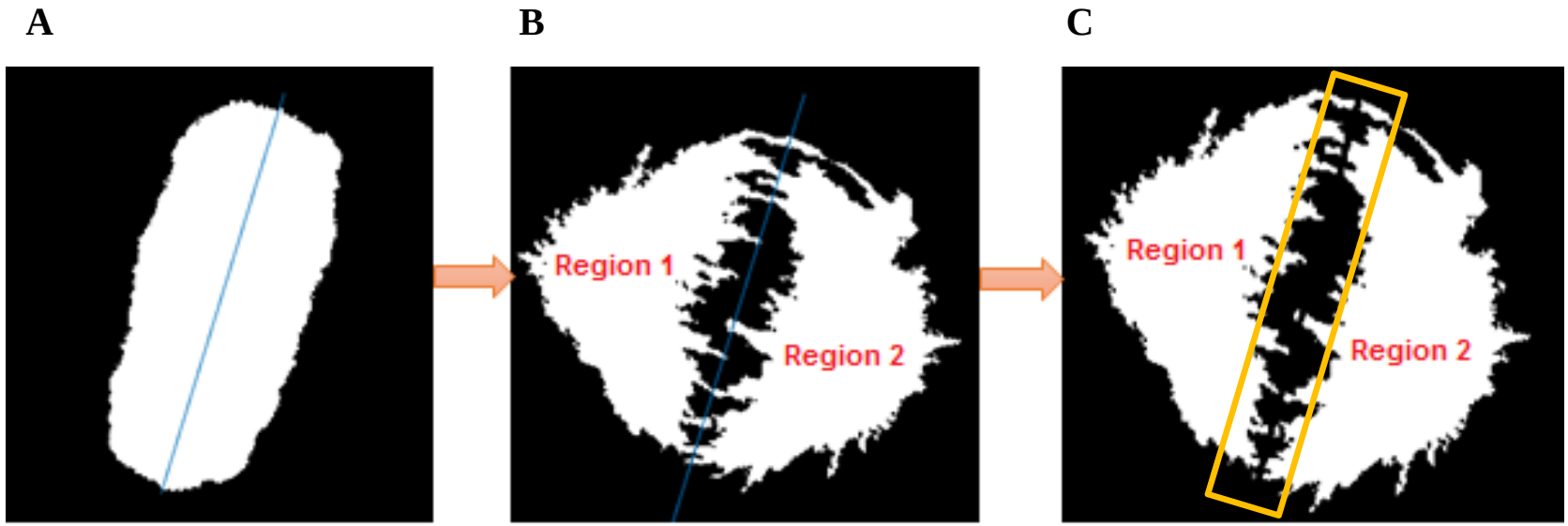
Computing the fractal dimension

- Fractals are infinitely complex patterns that are self-similar across different scales
- Describes irregularity of an object



$$fractal\ dimenison = \frac{\log(no.of\ self similar\ pieces)}{\log(magnification\ factor)}$$

Comparing the area between two regions



Samples	df	P-value
Control & Luciferase	99	0.691
Control & CHC KD	95	0.004
Luciferase & CHC KD	92	0.003

Two sample t-test

Good

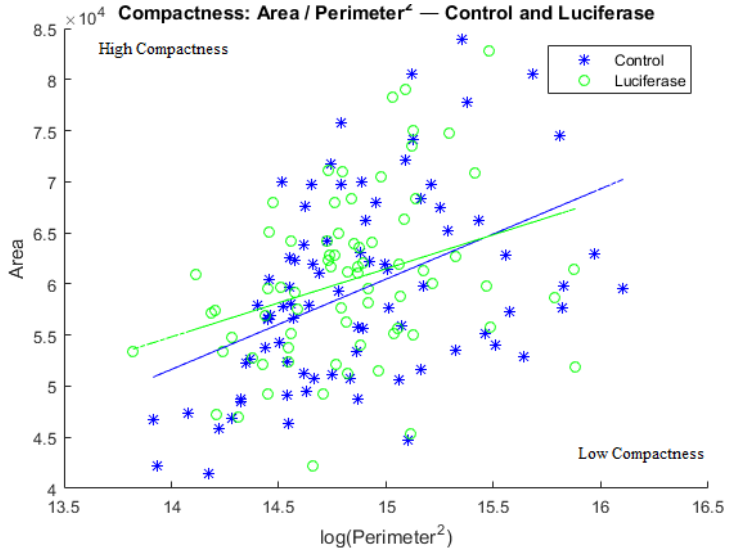
Neutral

Bad

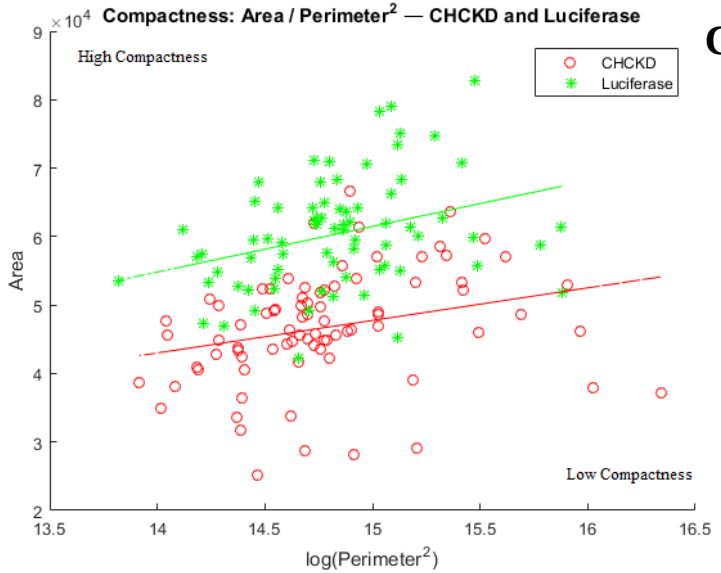
No.	Image Property	Control and Luciferase		Control & CHC KD		Luciferase and CHC KD	
		T/F*	P-value	T/F*	P-value	T/F*	P-value
1	Area	0	0.5572	1	7.00E-16	1	2.15E-18
2	ConvexArea	0	0.5118	1	1.70E-09	1	9.48E-13
3	Compactness	0	0.3248	1	0.0319	1	0.0019
4	Eccentricity	0	0.0754	1	0.013	1	5.14E-05
5	Entropy	1	0.048	0	0.1115	0	0.5209
6	EulerNumber	1	0.0039	0	0.9733	1	6.79E-04
7	Fractal_Dimension	0	0.4241	1	3.34E-05	1	7.30E-07
8	Intensity(mean)	0	0.0649	0	0.2	0	0.4216
9	Intensity(median)	0	0.5671	0	0.7591	0	0.7391
10	Intensity(total)	1	0.0291	1	1.21E-04	1	1.78E-08
11	Major Axis Length	0	0.8679	1	0.0017	1	4.84E-04
12	Minor Axis Length	1	0.0062	1	1.68E-12	1	3.59E-17
13	Orientation	0	0.3414	0	0.5828	0	0.1131
14	Percent Density	0	0.9067	0	0.2974	0	0.2471
15	Perimeter	0	0.2137	0	0.1464	0	0.7524
16	Solidity	0	0.6201	1	1.88E-06	1	1.72E-06
17	Standard Deviation	1	0.0108	1	0.022	0	0.5278
18	Extent	0	0.5817	1	0.0028	1	4.00E-04
19	Satellites	0	0.4358	0	0.3811	0	0.1258

Clustering Image Properties

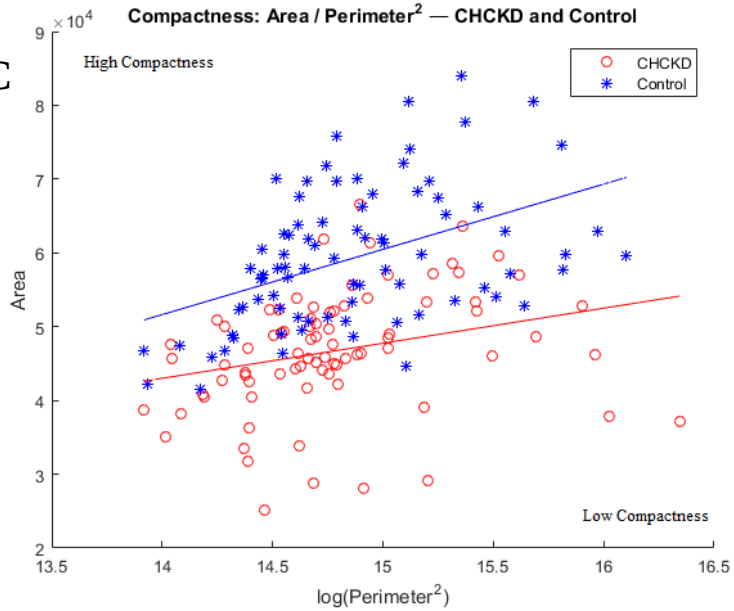
A



B

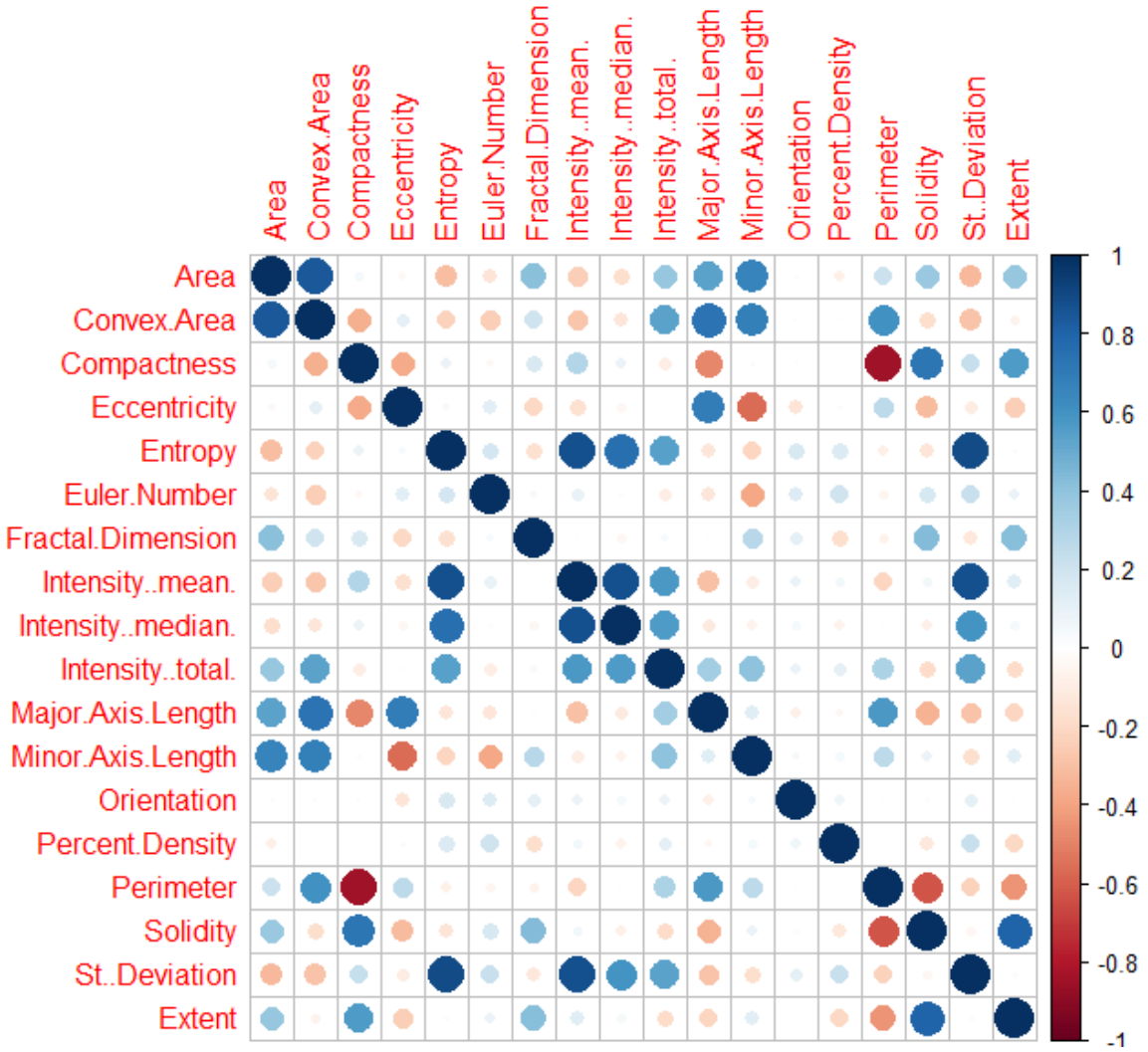


C



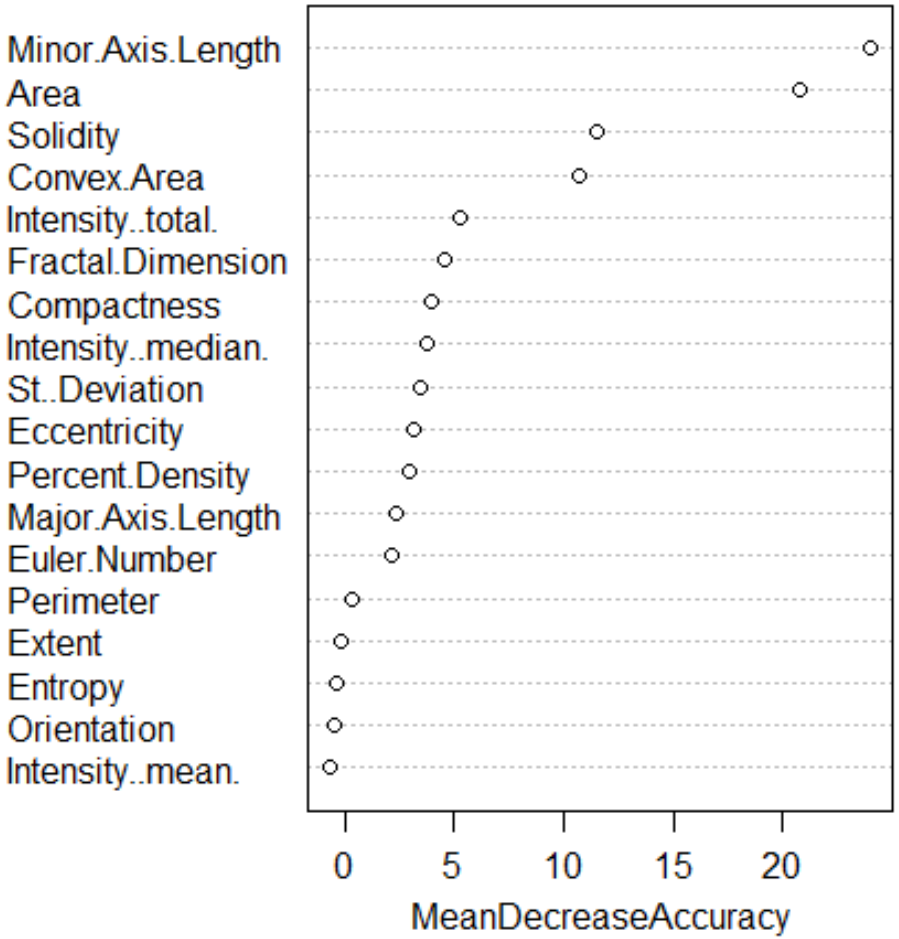
Pearson correlation coefficient heatmap

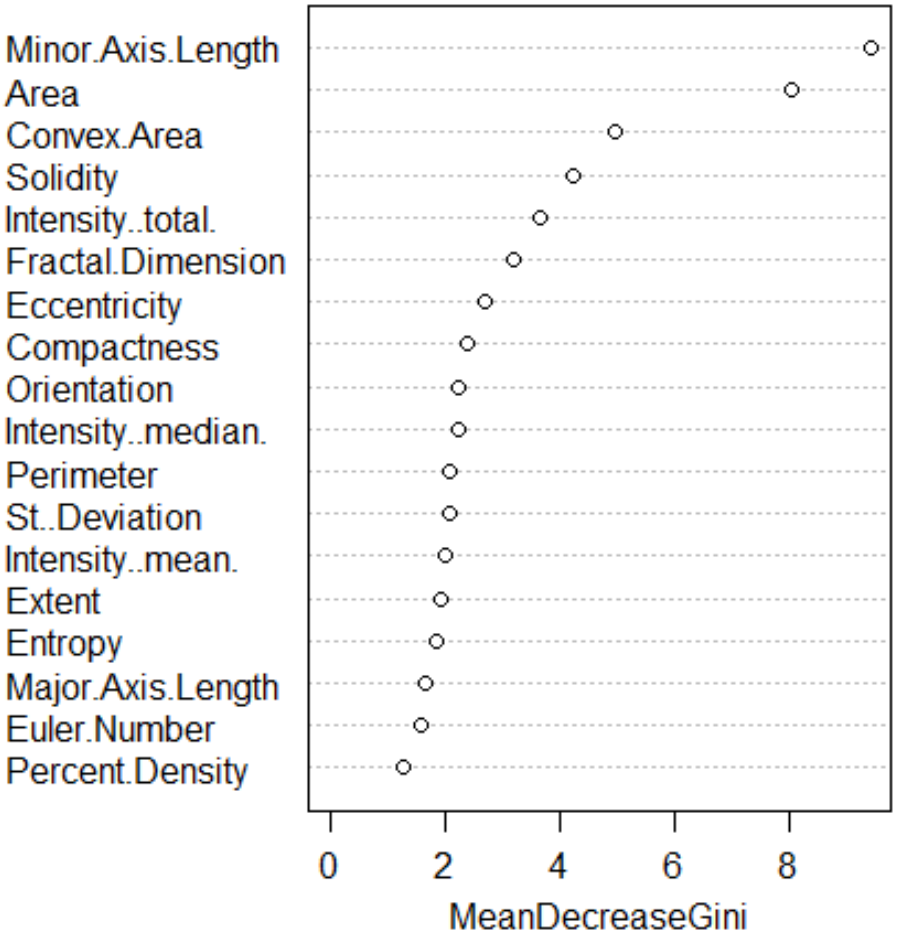
CHC data



Highly correlated fields could be excluded from the prediction model

Random Forest





Accuracy	Sensitivity	Specificity
0.80	0.714	0.862

Summary

- The programs provide a means of quantifying image characteristics rapidly

- High throughput image analysis reduces labour, sampling error and subjectivity

- Automatic image processing can detect changes not discernible to the human eye

Acknowledgement

- A/Prof. Jonathan Arthur
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شكرا

Díky

תודה

धन्यवाद

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Ευχαριστώ

Merci

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