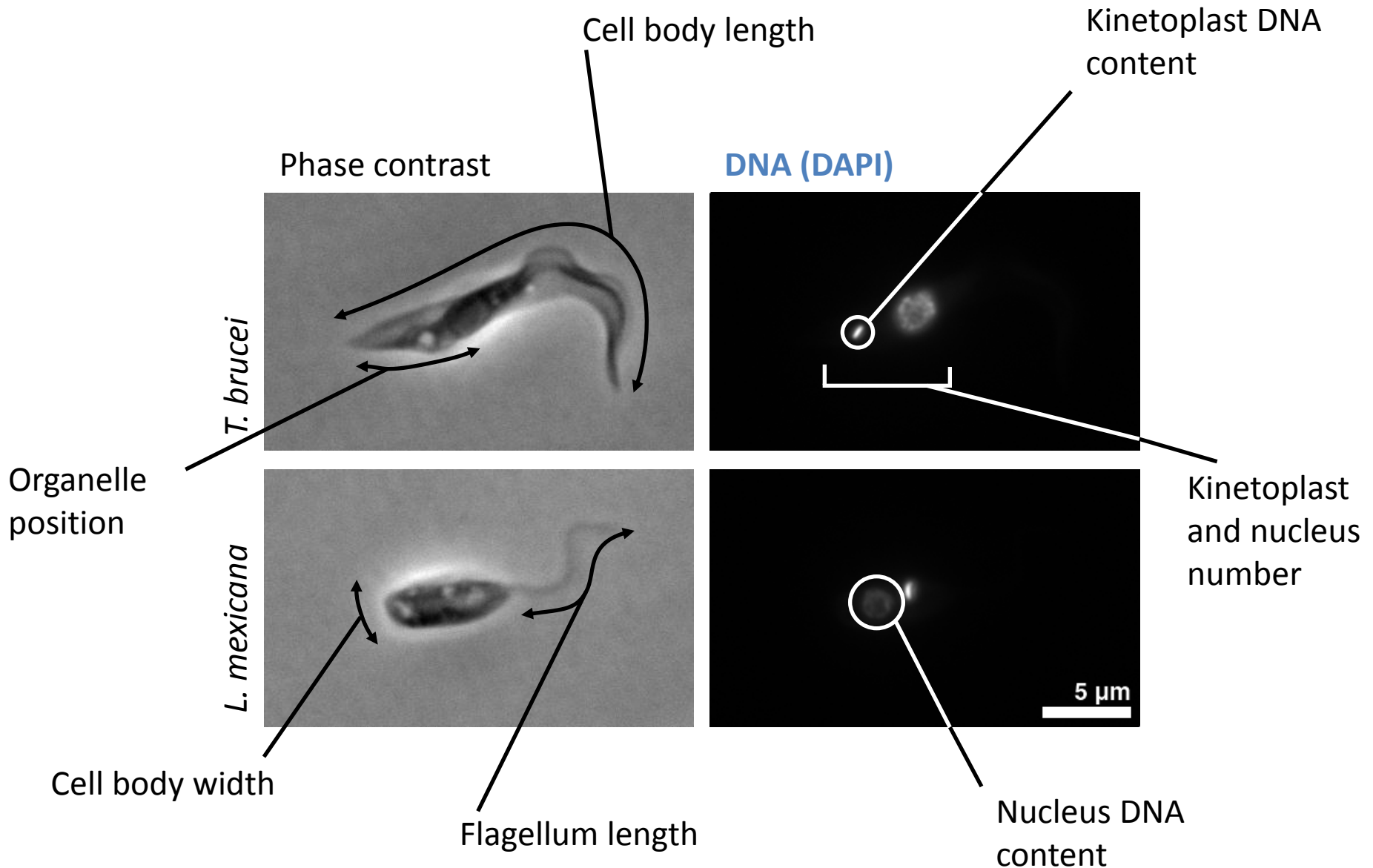




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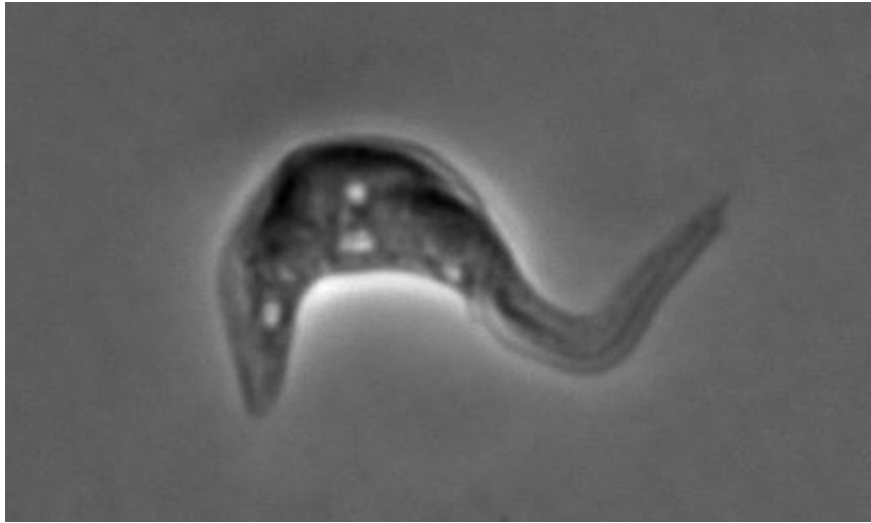
Beyond just counting Ks and Ns; high throughput image analysis of trypanosomatid cell organisation.

Micrographs hold a huge amount of data

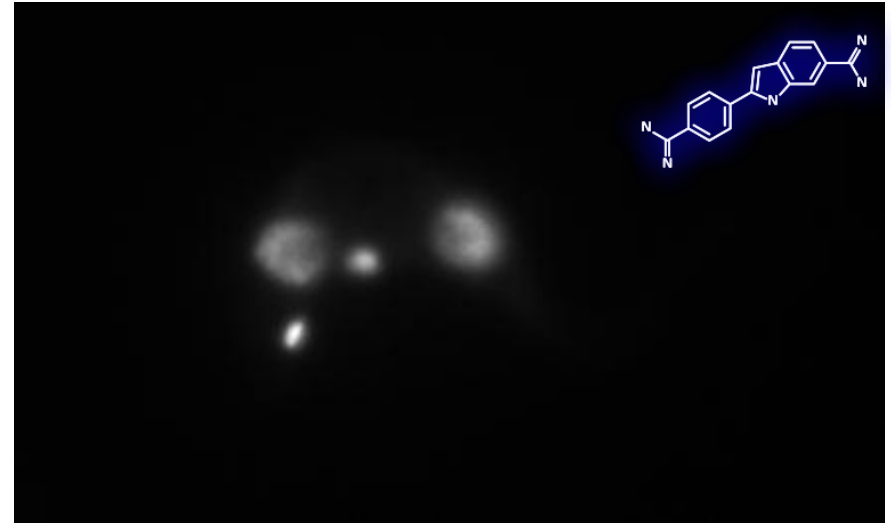


Identifying Ks & Ns by DNA sequence bias

Phase contrast



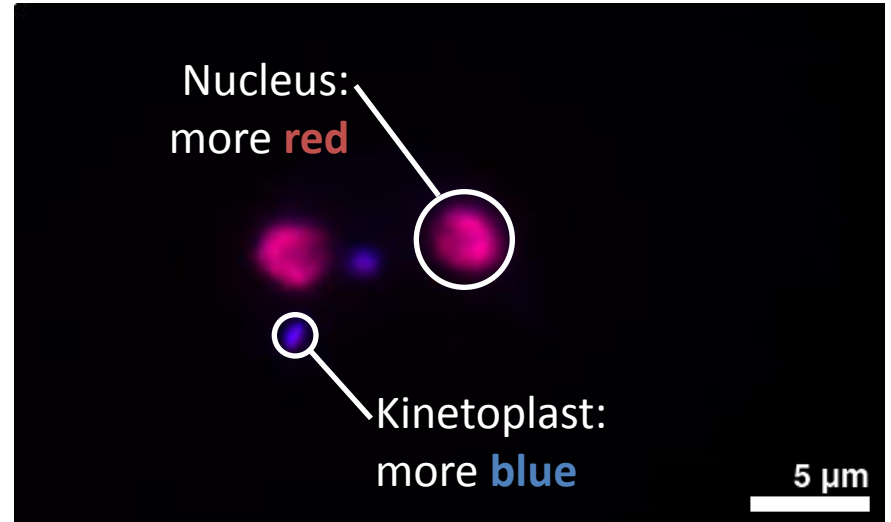
DNA (DAPI) – minor groove binding



DNA (PI) – base pair intercalating



DAPI/PI overlay



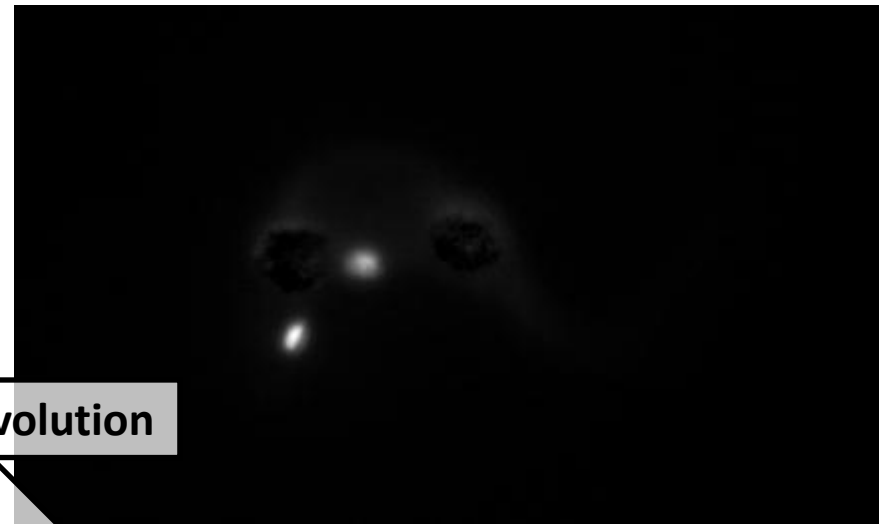
Splitting images by colour deconvolution

DNA (DAPI)

A-T rich DNA binding preference



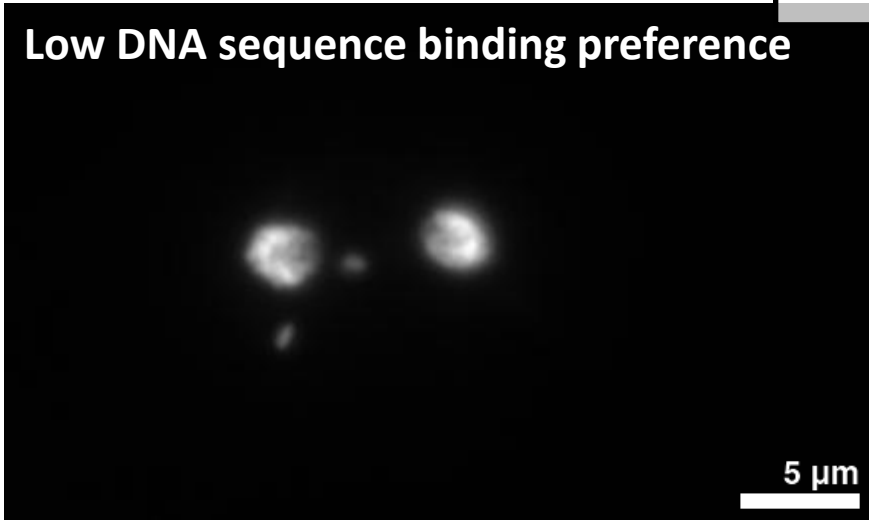
Kinetoplasts



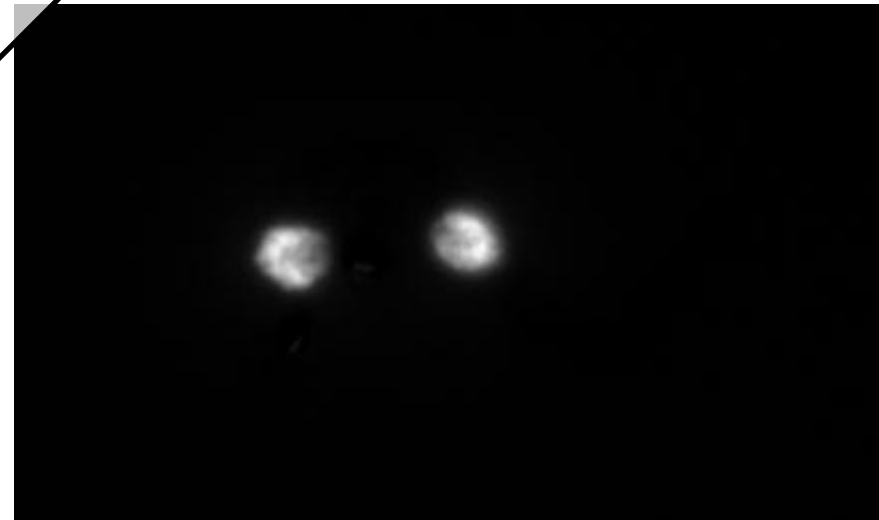
Colour Deconvolution

DNA (PI)

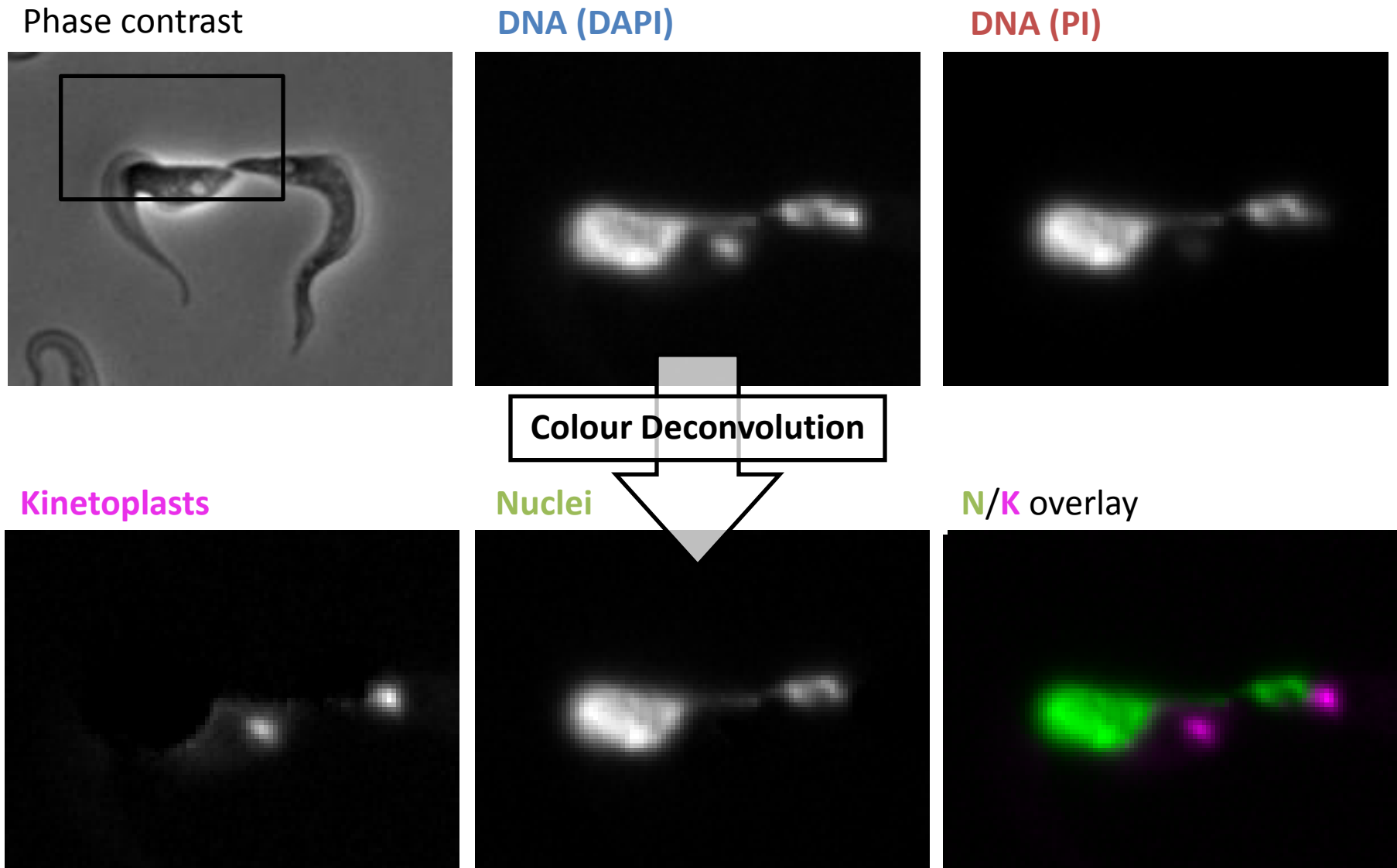
Low DNA sequence binding preference



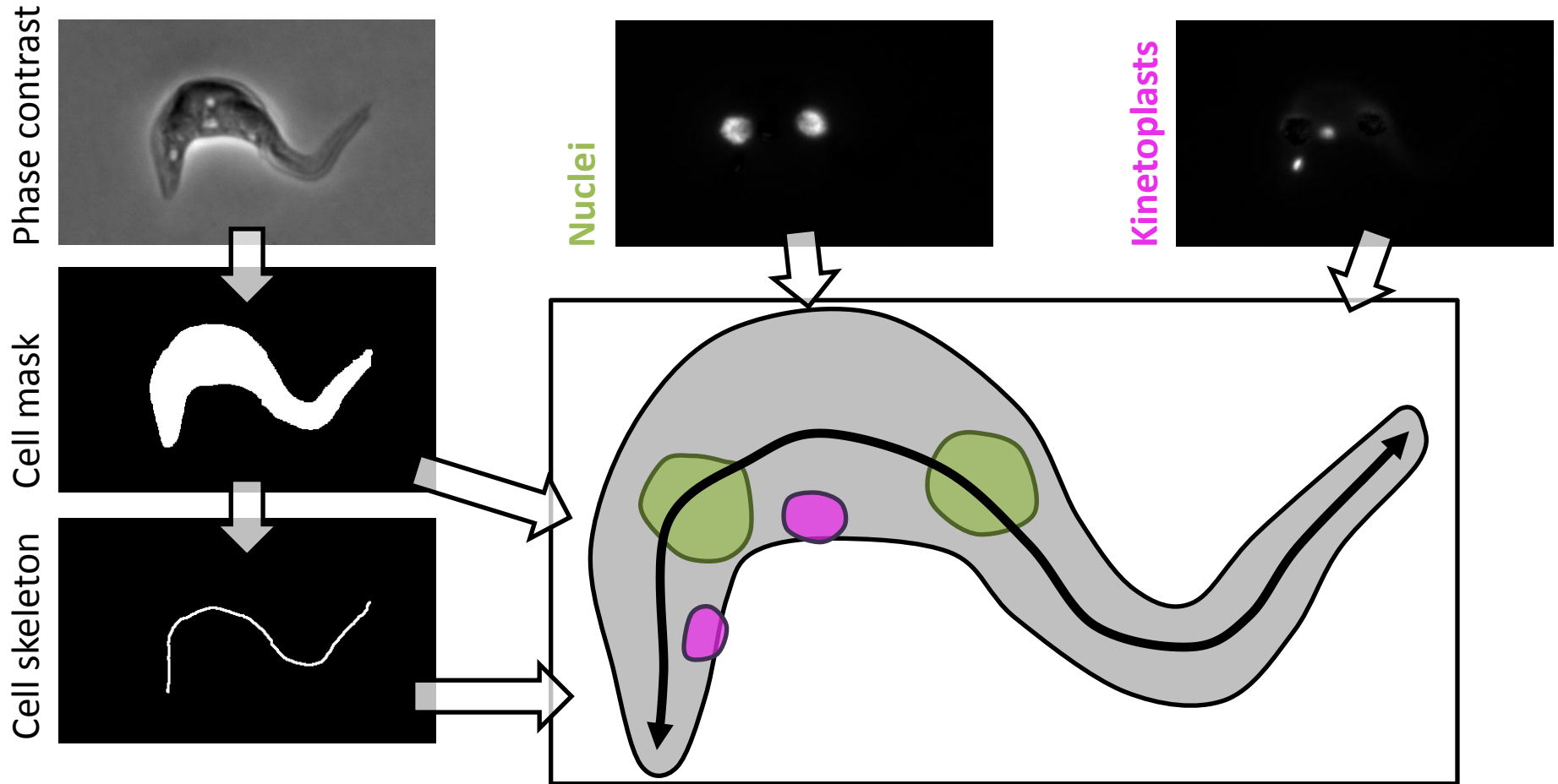
Nuclei



Analysing mutant morphologies



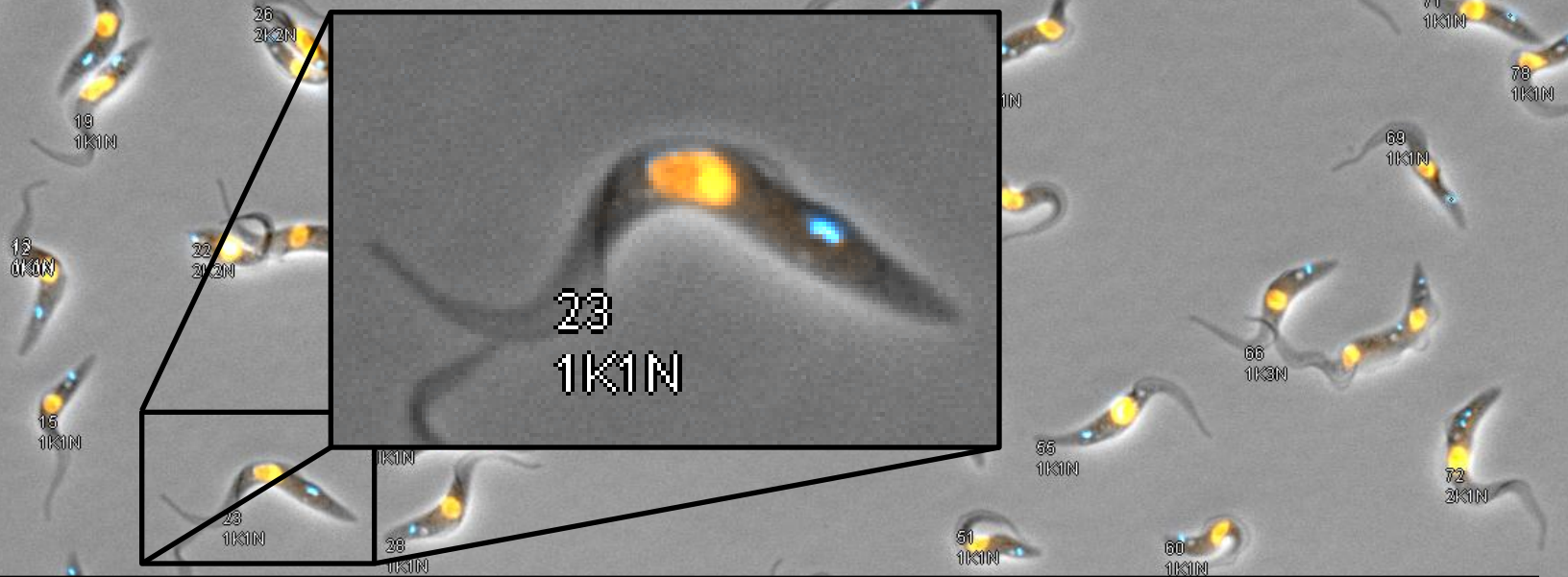
Automating analysis



Advantages of automation:

1. Far faster than manual analysis
2. Simpler to collect quantitative data
3. Less susceptible to experimentalist bias

Wild type *T. brucei* – automated analysis



	Cell Area	Total K DNA	Total N DNA	K #	N #	Cell Length	Cell Max Width	K1 Area	K1 DNA	N1 Area	N1 DNA	K1-Post Dist	K1 Off Axis Dist	N1-Post Dist	N1 Off Axis Dist
23	31.68	52271	392936	1	1	15.71	2.24	0.77	36496	5.52	252335	4.65	0.26	9.78	0.17

1. Totally repeatable and leaves a full “audit trail”

2. Manual cross-analysis is possible

Wild type *T. brucei* – automated DNA analysis

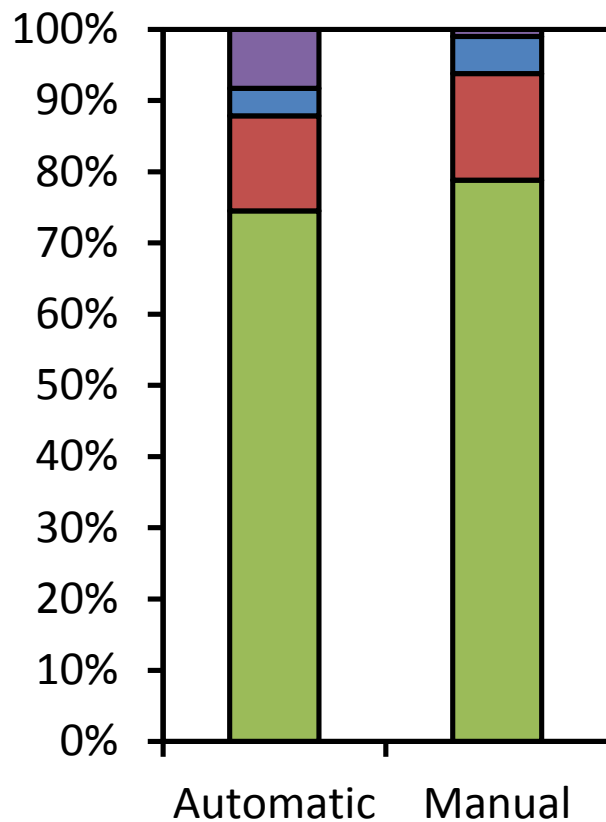


1K1N

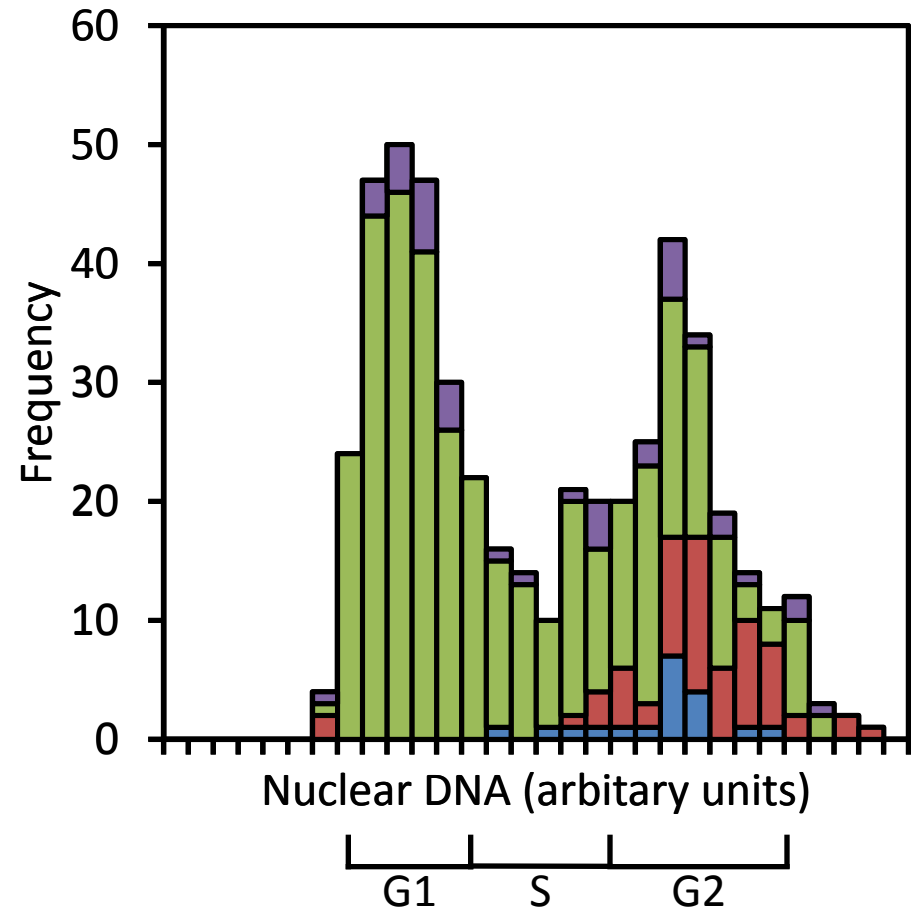
2K1N

2K2N

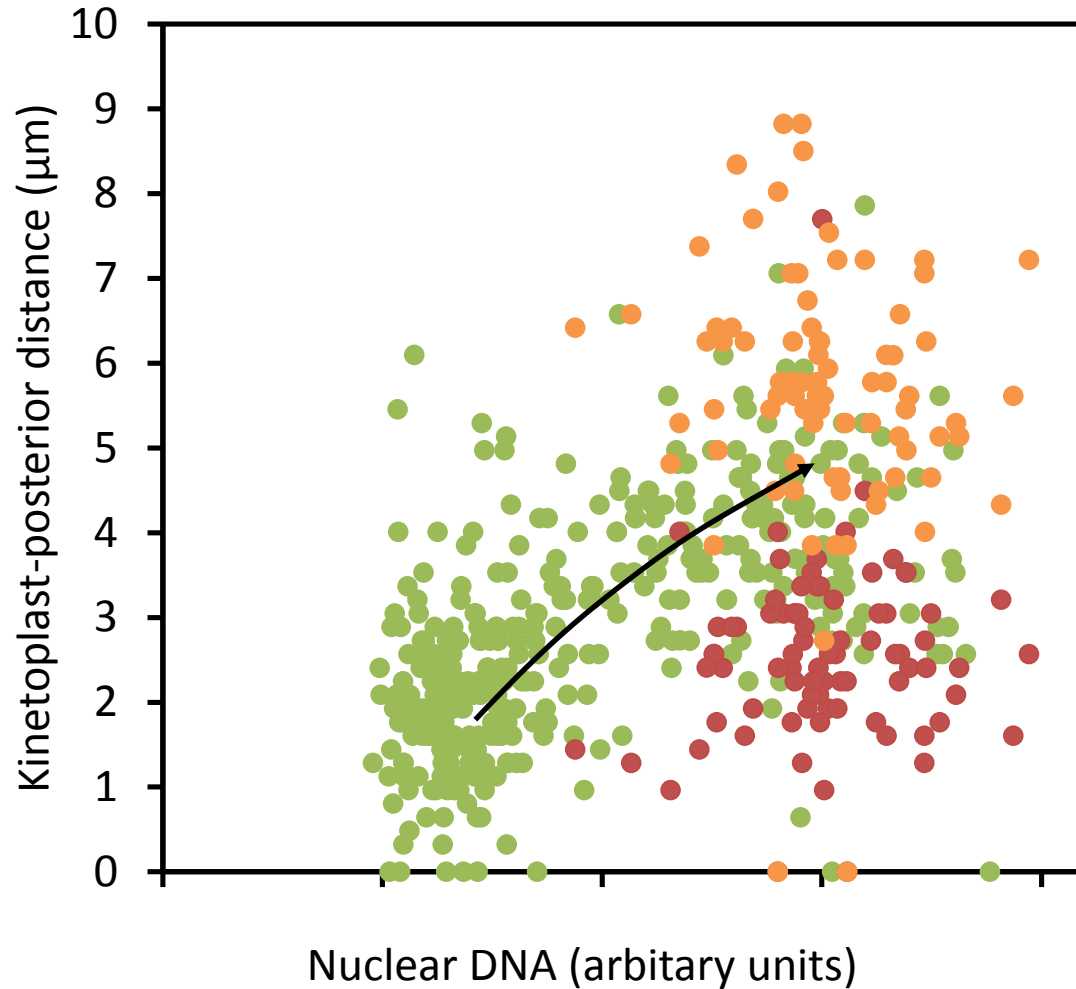
Other



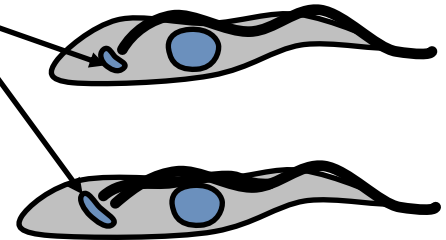
n ≈ 500



Kinetoplast movement through the cell cycle



1K1N



2K1N



Anterior kinetoplast



Posterior kinetoplast



Conclusions

- Kinetoplasts and nuclei can be identified **unambiguously** by microscopy with the **simple** double DNA staining technique
- Using colour deconvolution signal from kinetoplast and nuclear DNA can be **quantitatively** split to **two separate images**
- This approach allows **unbiased** and **more accurate** analysis of nuclei and kinetoplasts
- I have developed automated **quantitative morphometric analysis tools** based on these colour deconvolved images
- These automated analysis tools allow:
 - Extraction of **quantitative** data from micrographs **far faster** than possible manually
 - Entirely **unbiased** and **repeatable** data collection
 - Complete **record of all data** collected and options for manual cross checking

Using this approach yourselves

The screenshot displays the ImageJ software interface with several windows open:

- ImageJ**: The main application window with a menu bar (File, Edit, Image, Process, Analyze, Plugins, Window, Help) and a toolbar. The title bar reads "15.09.2010_TBru_DAPISYBR_40xFields.tif - Pos001_S001 (33.3...". The image area shows a field of cells with yellow nuclei and blue kinetoplasts.
- Colour Deconvolution**: A dialog box with the option ☒ "Modify all images currently open".
- K/N Count**: A dialog box with the following settings:
 - ☒ "Process all images currently open"
 - ☒ "Give a visual display of results"
 - Cell thresholding options:
 - Rolling ball radius: 15 px
 - Minimum cell area: 500.00 px²
 - Skeleton branches: 10.00 px
 - Kinetoplast thresholding options:
 - Thresholding type: Manual
 - Minimum threshold value: 5000
 - Maximum threshold value: 10000
 - Automatic threshold type: MaxEntropy
- Results**: A table window showing the analysis results for 8 cells.

File	Edit	Font	
	Cell Area (um ²)	Total Kinetoplast DNA	Total Nu
2	26.949	39707	414138
3	29.315	57081.000	297611
4	21.704	3178	92879
5	40.938	46644	131886
6	26.435	20970	390145
7	31.372	85572.000	308555
8	13.526	1046	58155

Acknowledgements

- Eva Gluenz
- Keith Gull
- ... and all the other members of the Gull lab.



Sir William Dunn School of Pathology

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