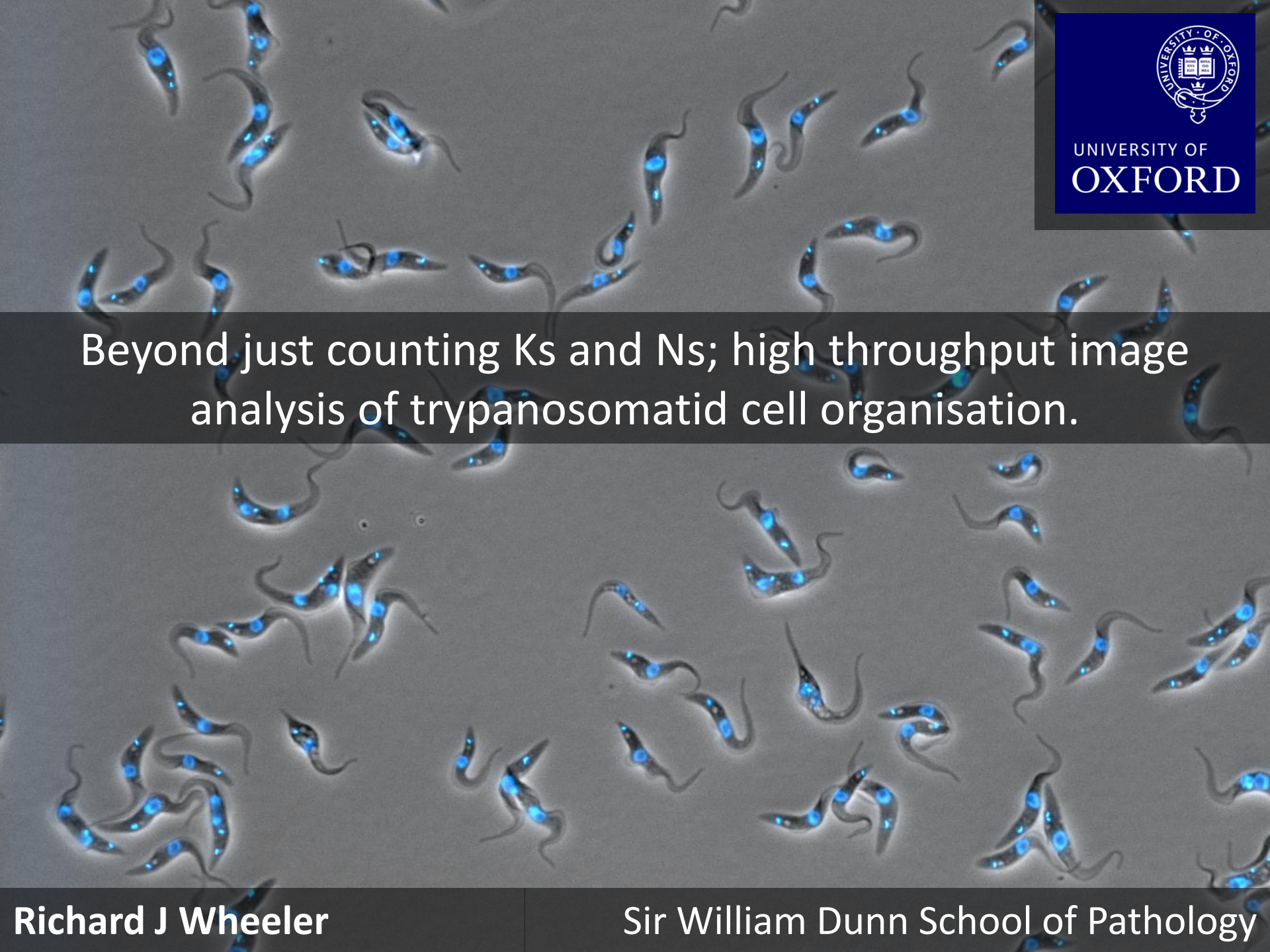
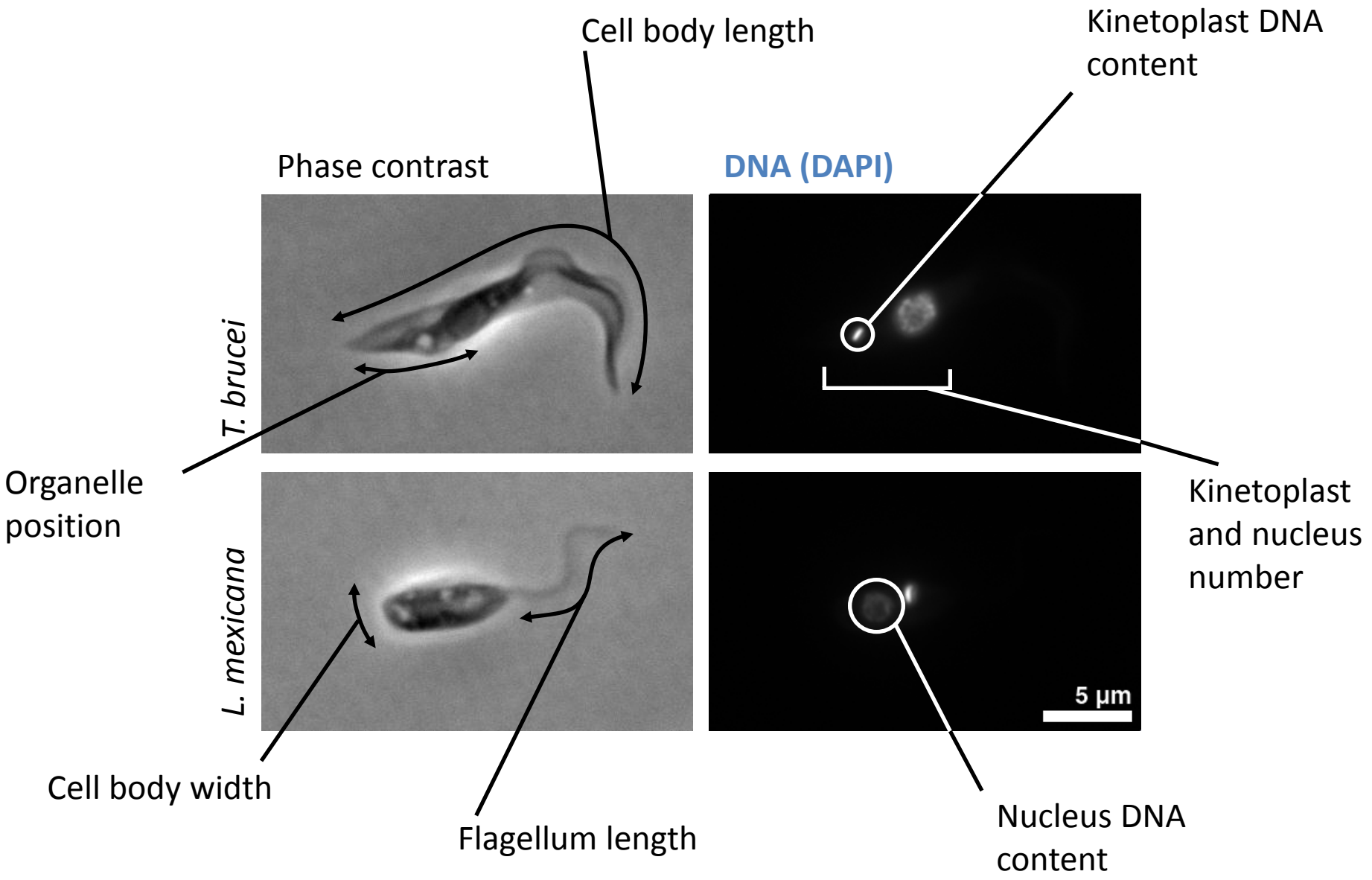


Beyond just counting Ks and Ns; high throughput image analysis of trypanosomatid cell organisation.

Richard J Wheeler

Sir William Dunn School of Pathology

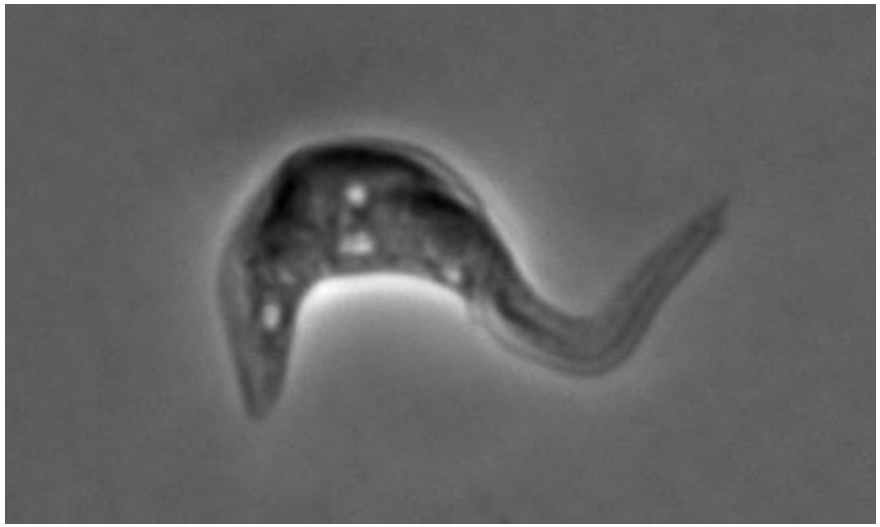
Micrographs hold a huge amount of data



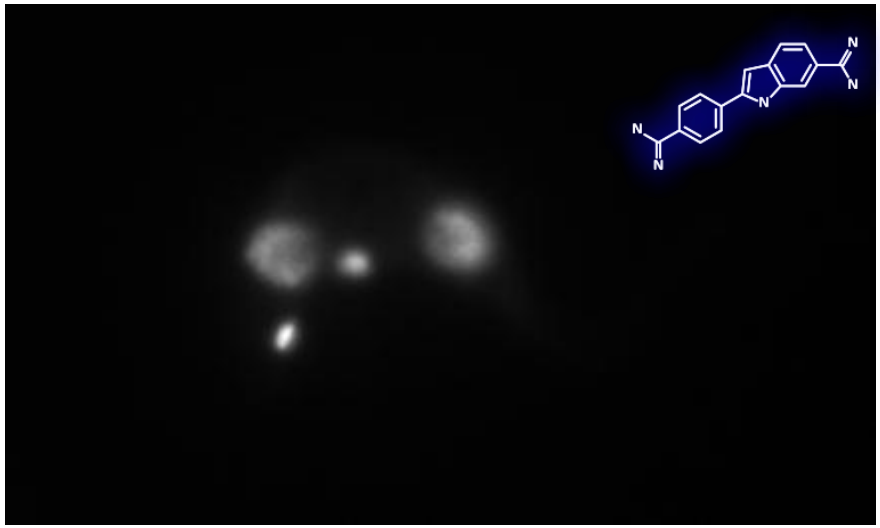
Procyclic 427 *Trypanosoma brucei* and promastigote WHO strain MNYC/BZ/62/M379 *Leishmania mexicana*

Identifying Ks & Ns by DNA sequence bias

Phase contrast

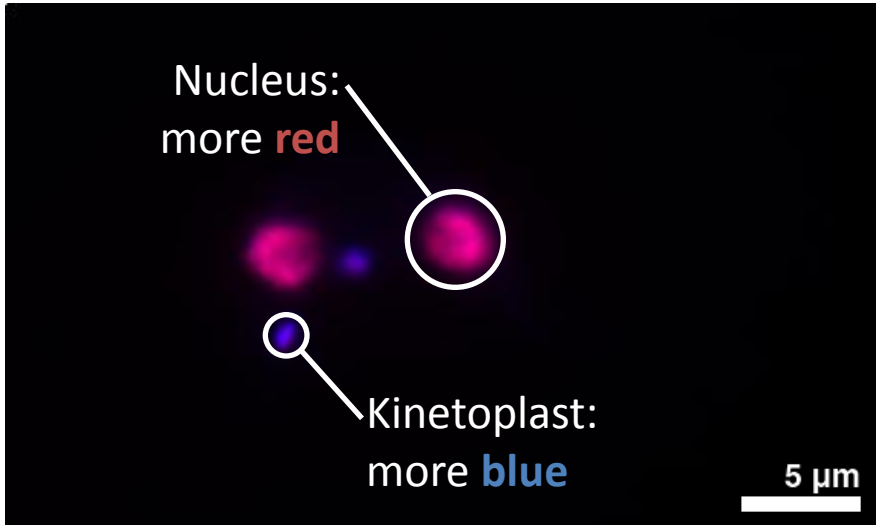


DNA (DAPI) – minor groove binding



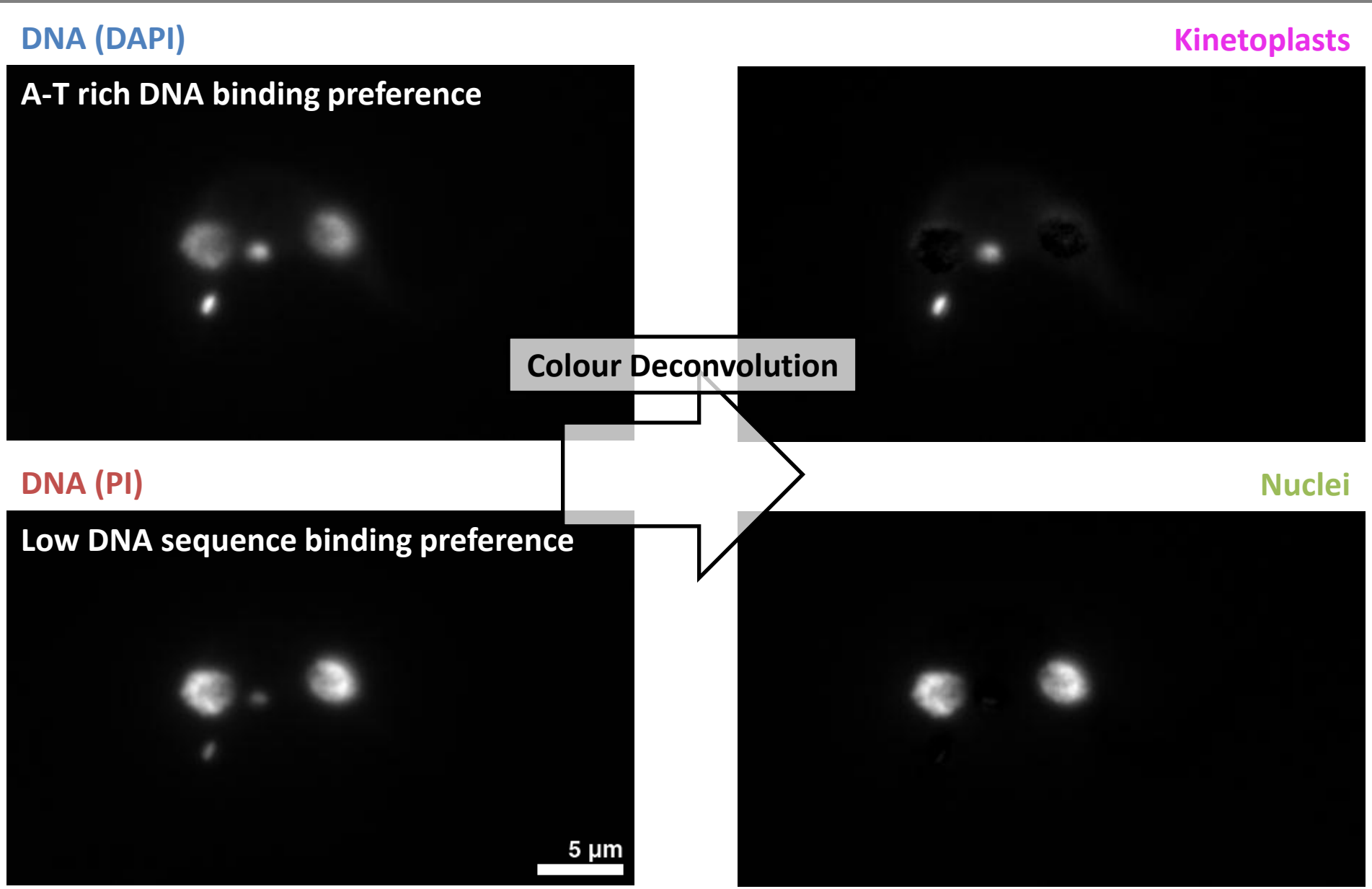
DNA (PI) – base pair intercalating

DAPI/**PI** overlay



Procyclic 427 *T. brucei*

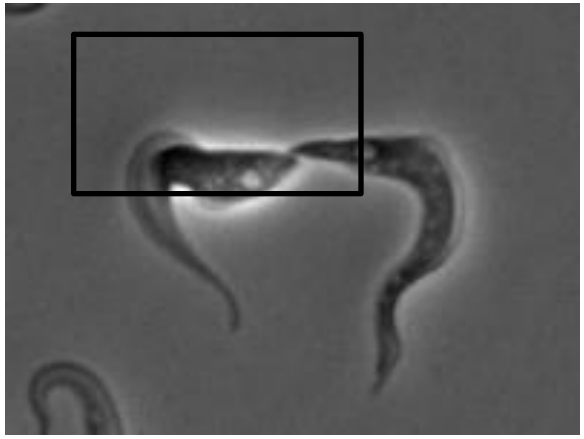
Splitting images by colour deconvolution



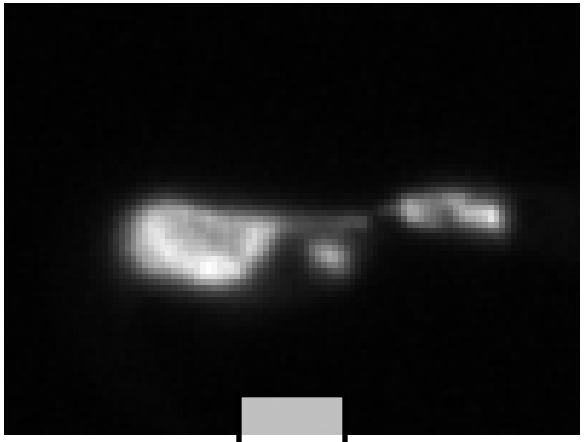
Procyclic 427 *T. brucei*

Analysing mutant morphologies

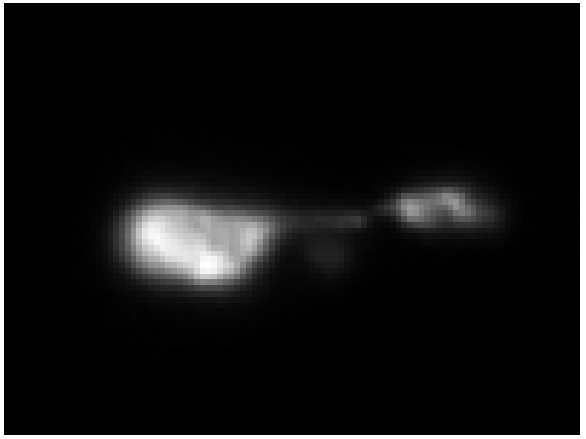
Phase contrast



DNA (DAPI)

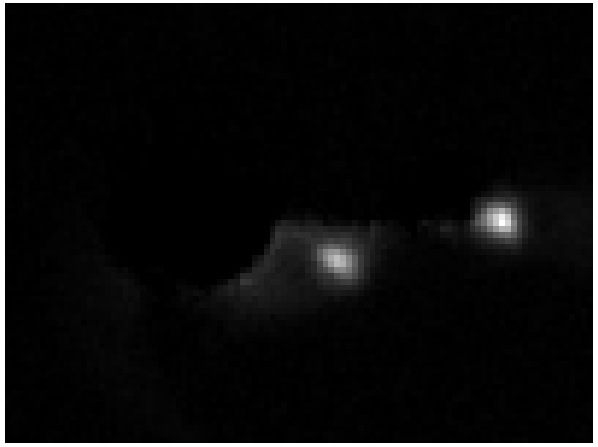


DNA (PI)

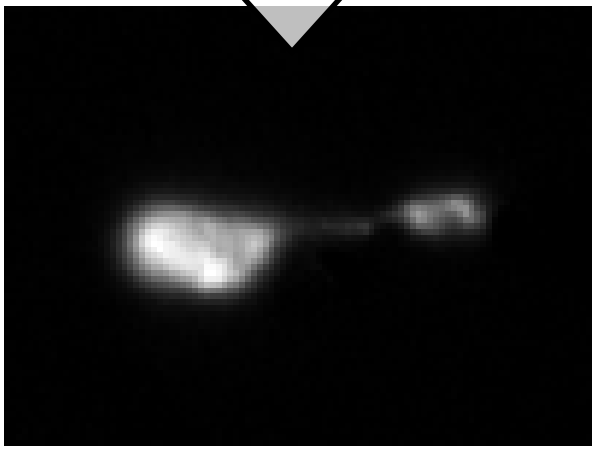


Colour Deconvolution

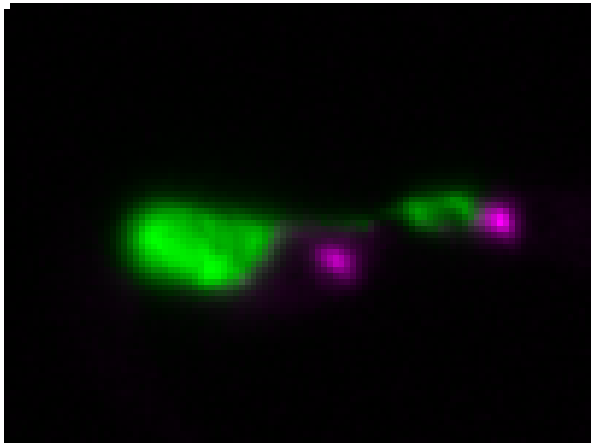
Kinetoplasts



Nuclei

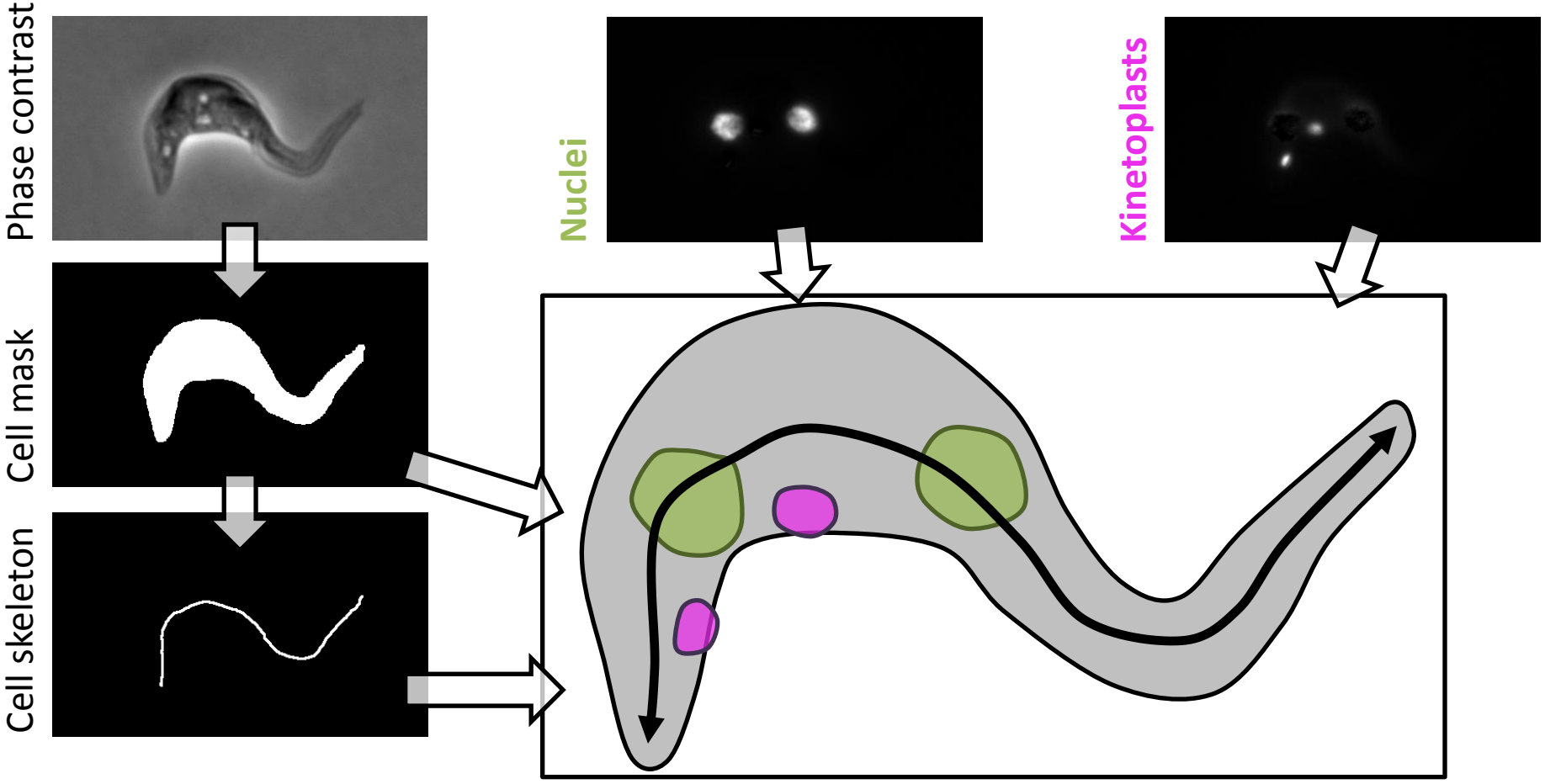


N/K overlay



Inducible procyclic *T. brucei* SCC1-mutAB cell line. Gluenz *et al.* 2008

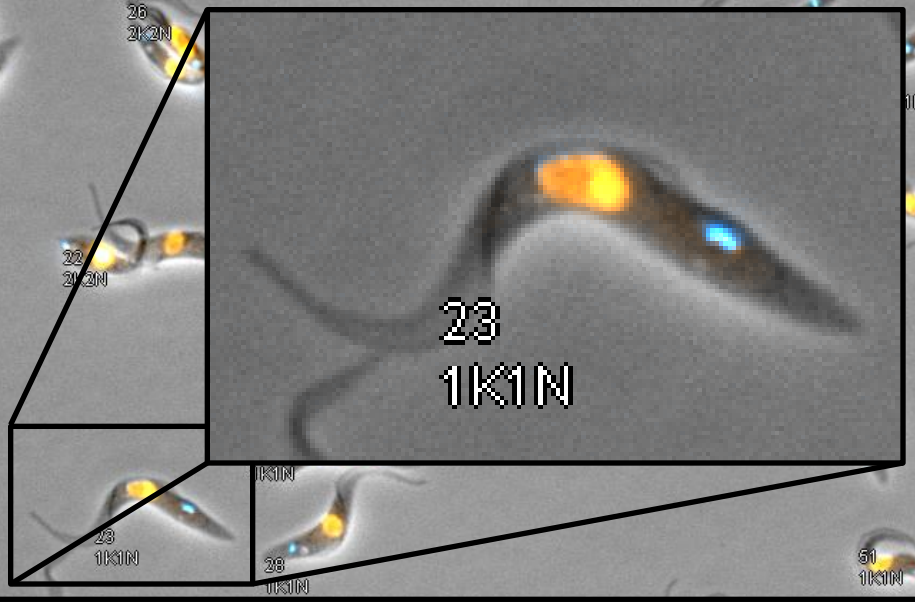
Automating analysis



Advantages of automation:

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

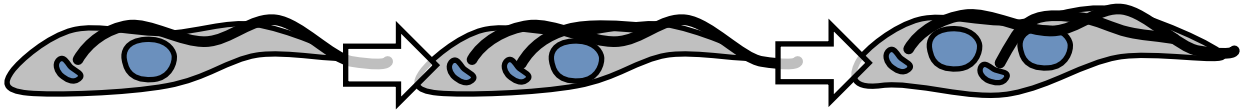
Wild type *T. brucei* – automated analysis

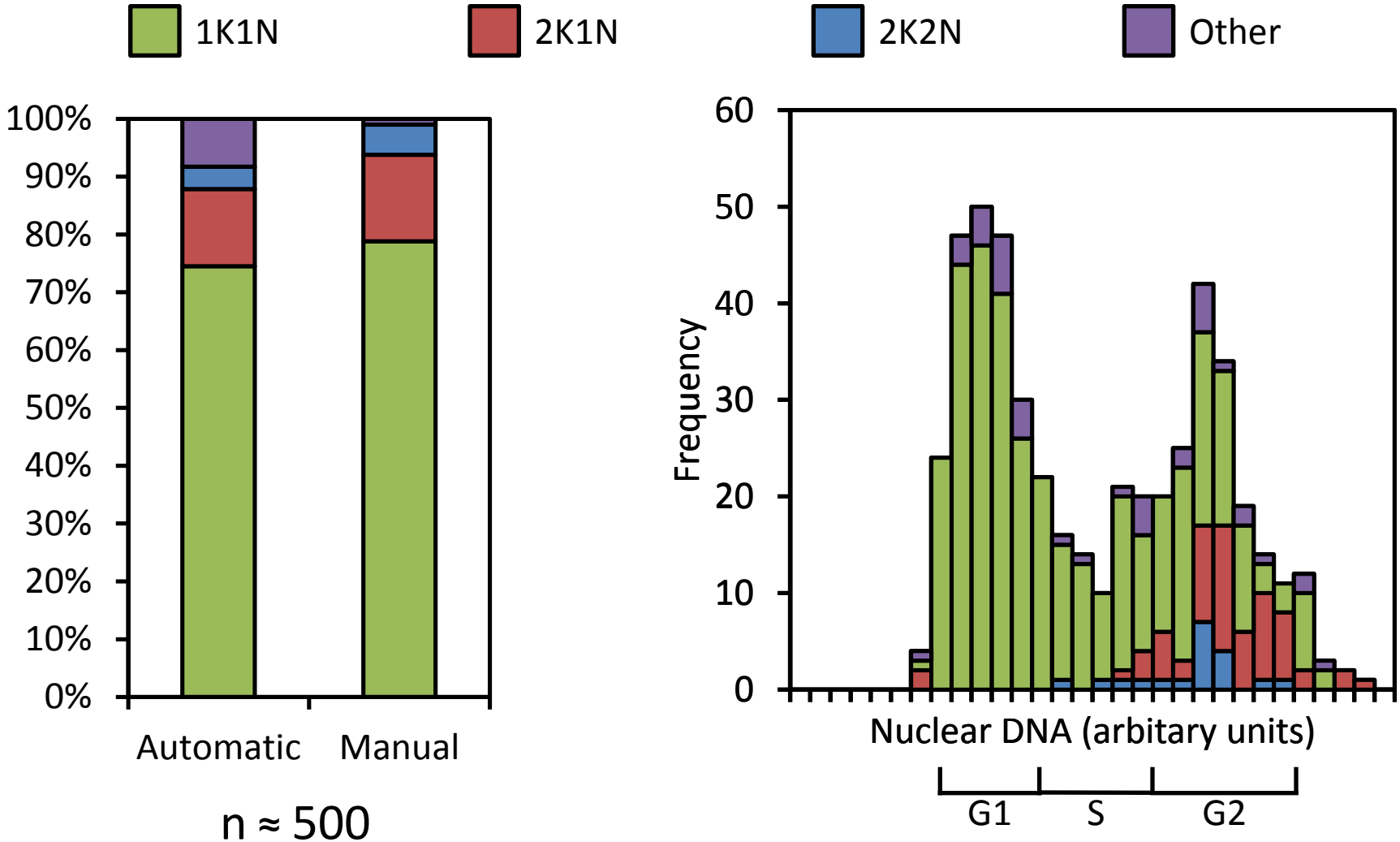


	Cell Area	Total K DNA	Total N DNA	K #	N #	Cell Length	Cell Max Width	K1 Area	K1 DNA	N1 Area	N1 DNA	K1-Post Dist	K1 Off Axis Dist	N1-Post Dist	N1 Off Axis Dist
23	31.68	52271	392936	1	1	15.71	2.24	0.77	36496	5.52	252335	4.65	0.26	9.78	0.17

1. Totally repeatable and leaves a full “audit trail”
2. Manual cross-analysis is possible

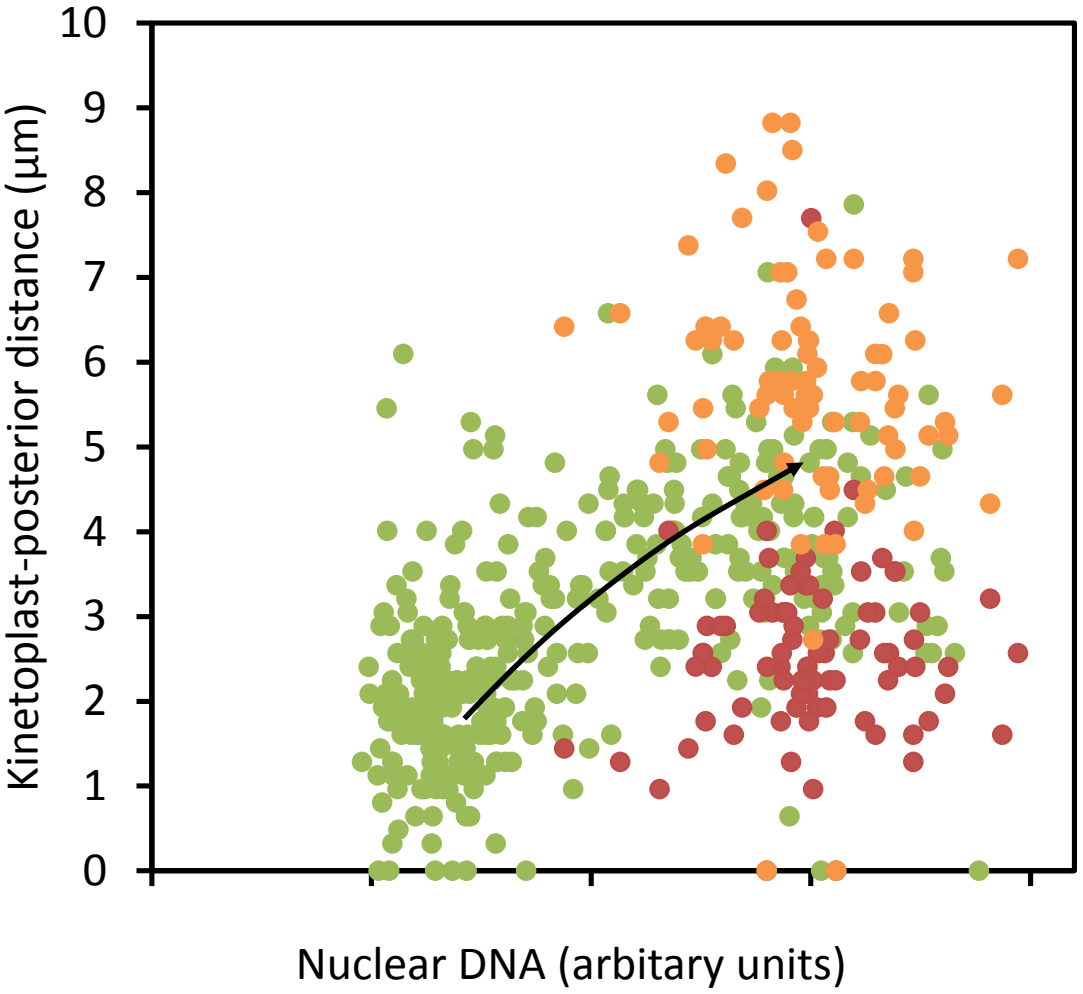
Wild type *T. brucei* – automated DNA analysis

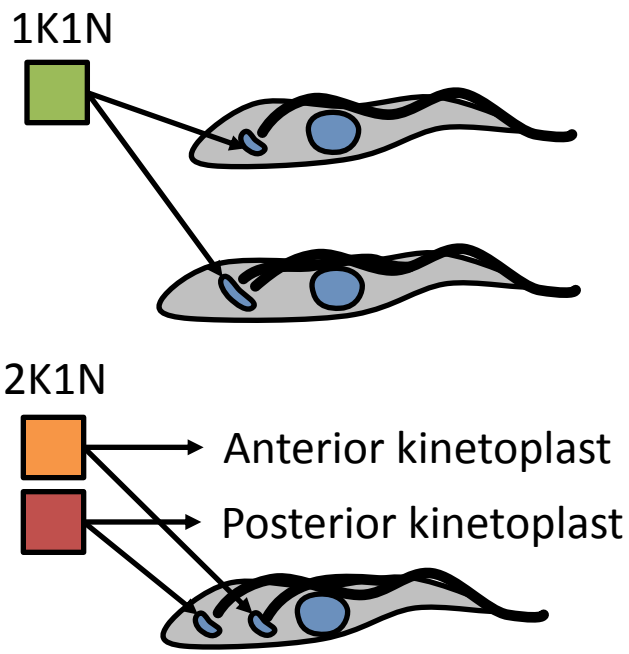




Procyclic 427 *T. brucei*

Kinetoplast movement through the cell cycle



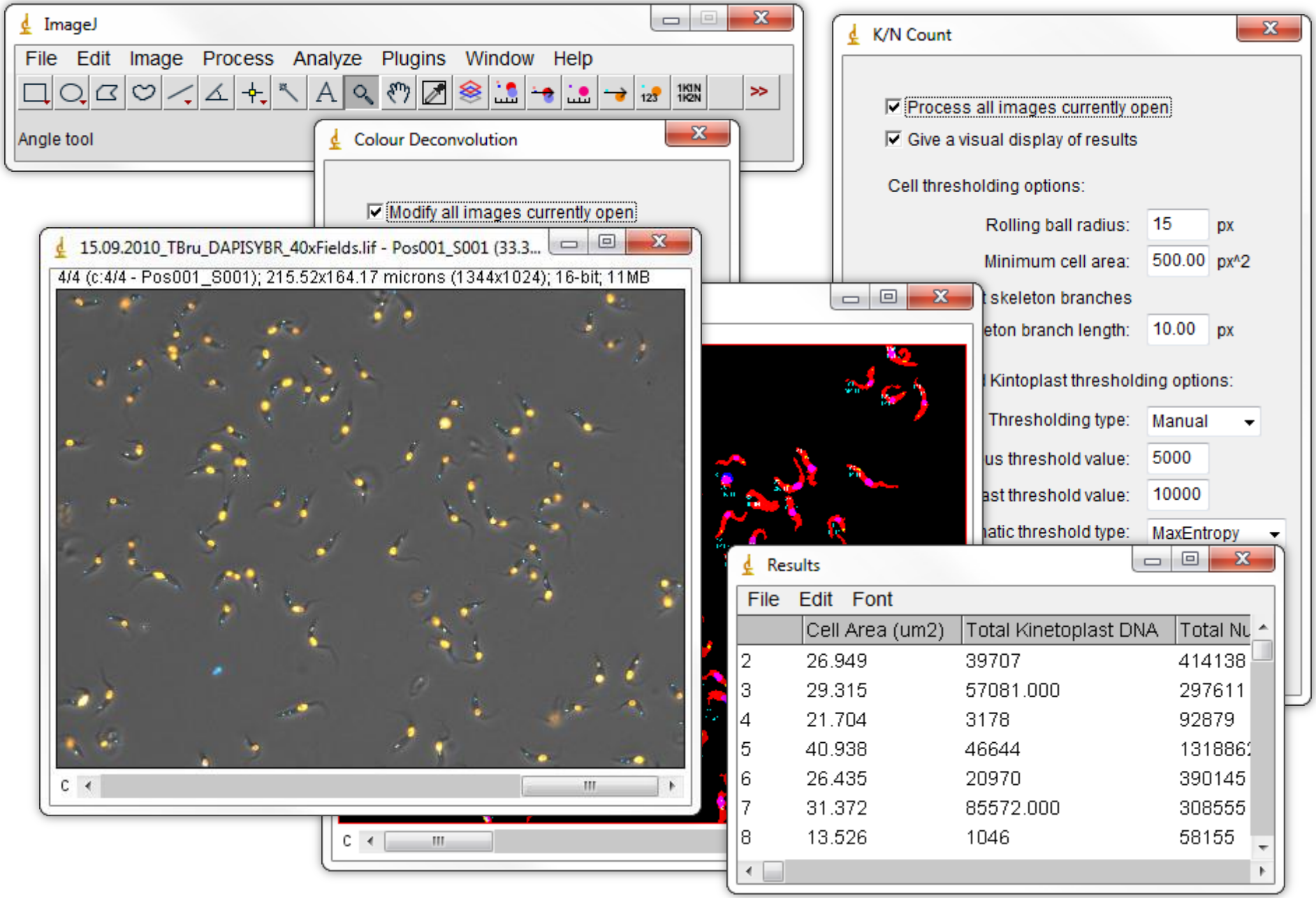


Procyclic 427 *T. brucei*

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



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- Keith Gull
- … and all the other members of the Gull lab.

Sir William Dunn School of Pathology

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