Leeds Institute of Molecular Medicine

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An algorithm to detect Copy Number Aberrations in cancer genomes of tumour specimens.

Arief Gusnanto, ***Stefano Berri***,
Henry M. Wood and Pamela Rabbitts

The cancer genome is often aneuploid

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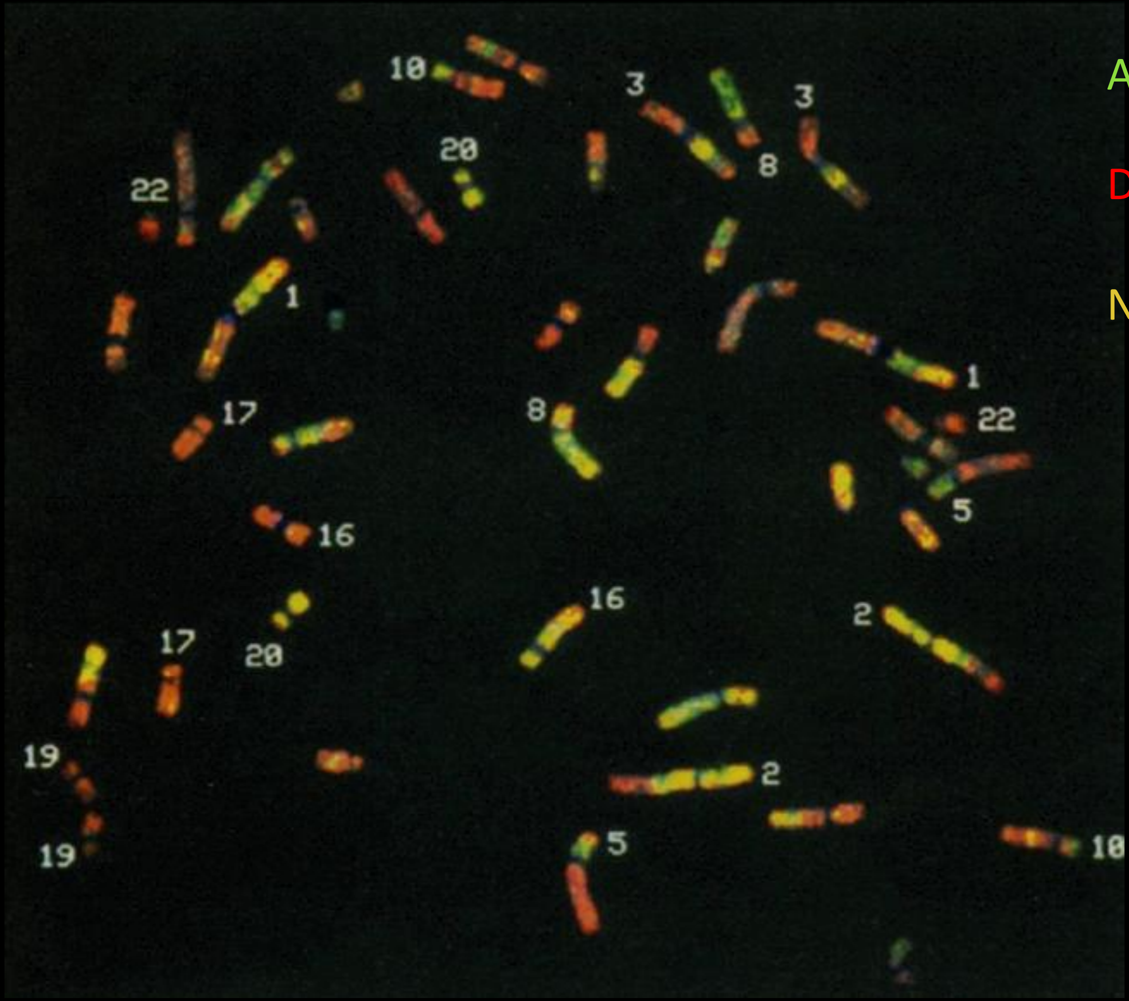

Amplifications

Deletions

Normal

Hartwell and Kastan. Science, 1994

Detecting abnormalities

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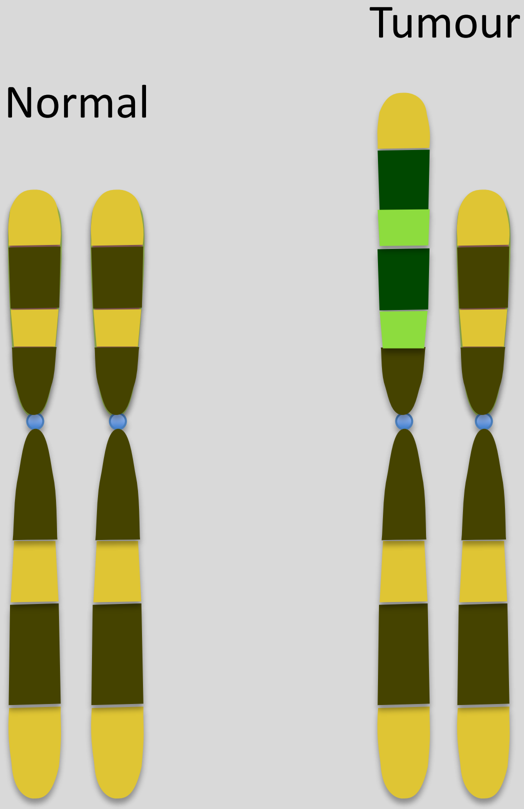
Why?

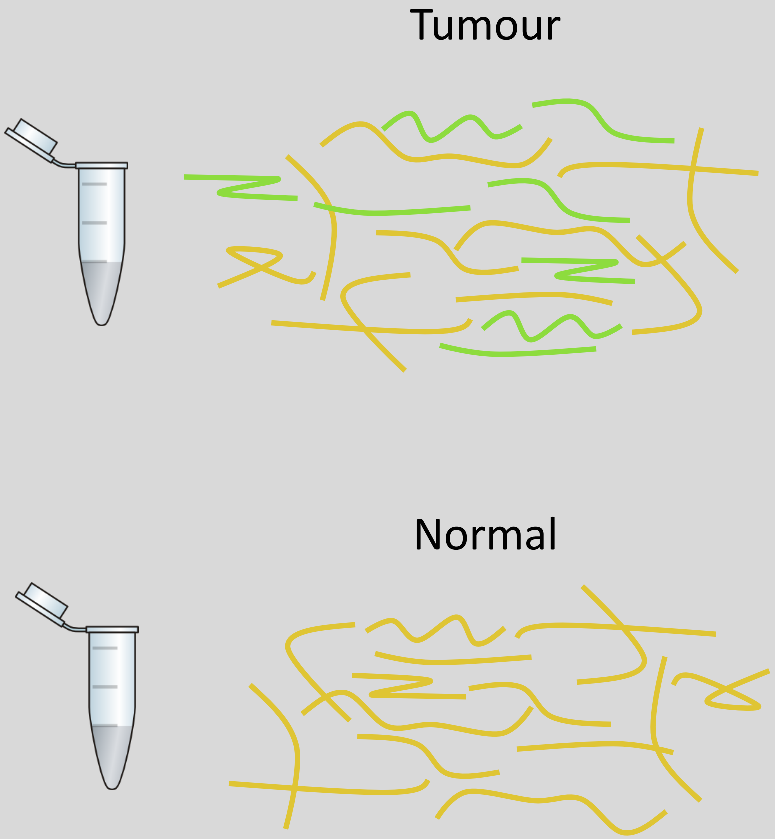
- Molecular characterisation and classification of tumours
- Diagnostic, prognostic and predictive tool
- Understand the biology of cancer

How?

- CGH
- aCGH (BAC or oligo)
- SNP microarray
- “NextGen” Sequencing
  - ✓ Tuneable resolution/cost
  - ✓ Re-use of data
  - ✓ Flexible platform
  - ✓ Technical independence Test – Control
  - ✓ Might become very cheap

Copy number by “NextGen” Sequencing





“NextGen” sequencing and reads mapping.

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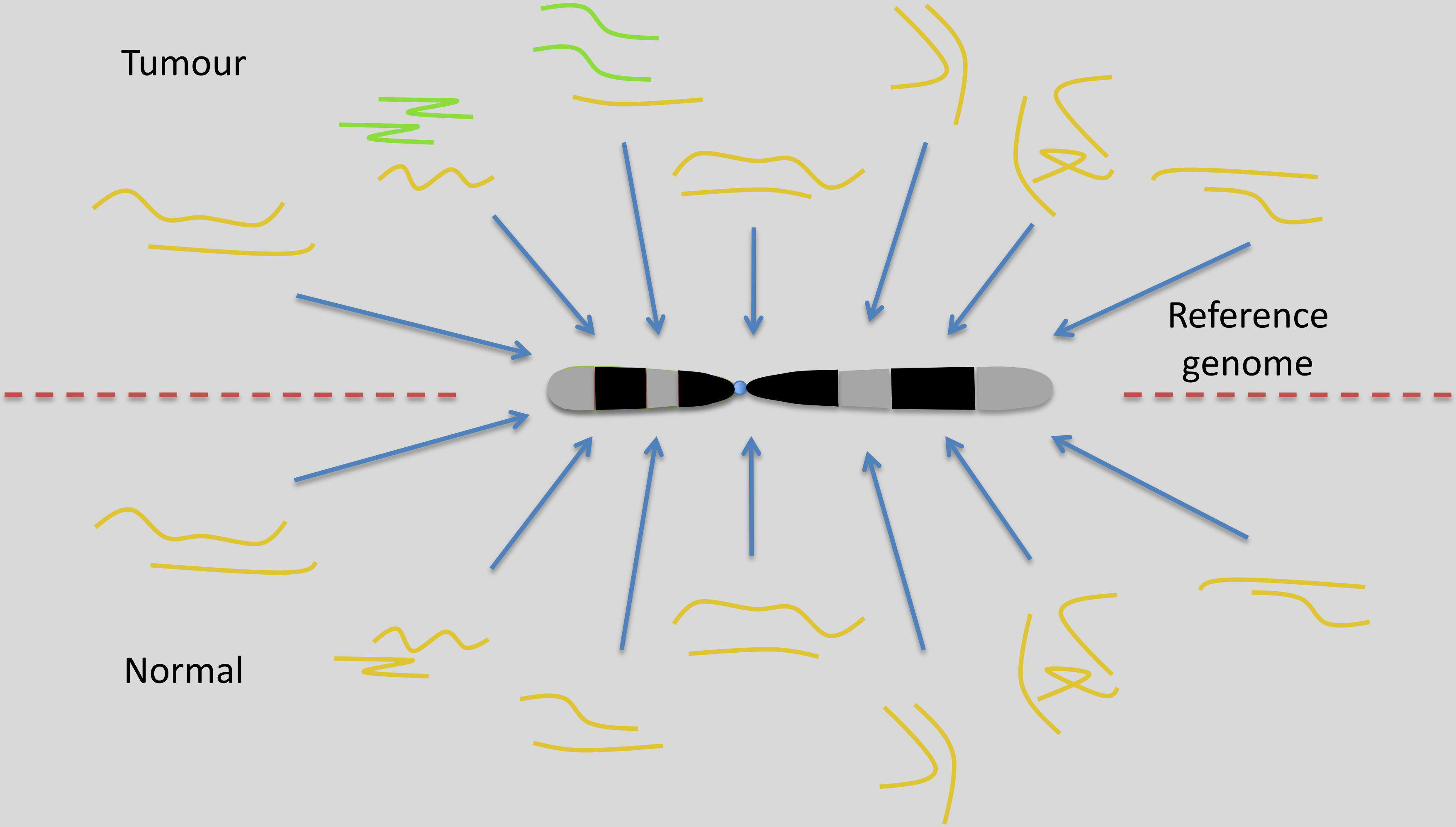

Counting number of sequences for each window

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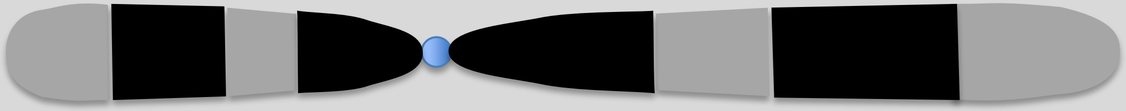
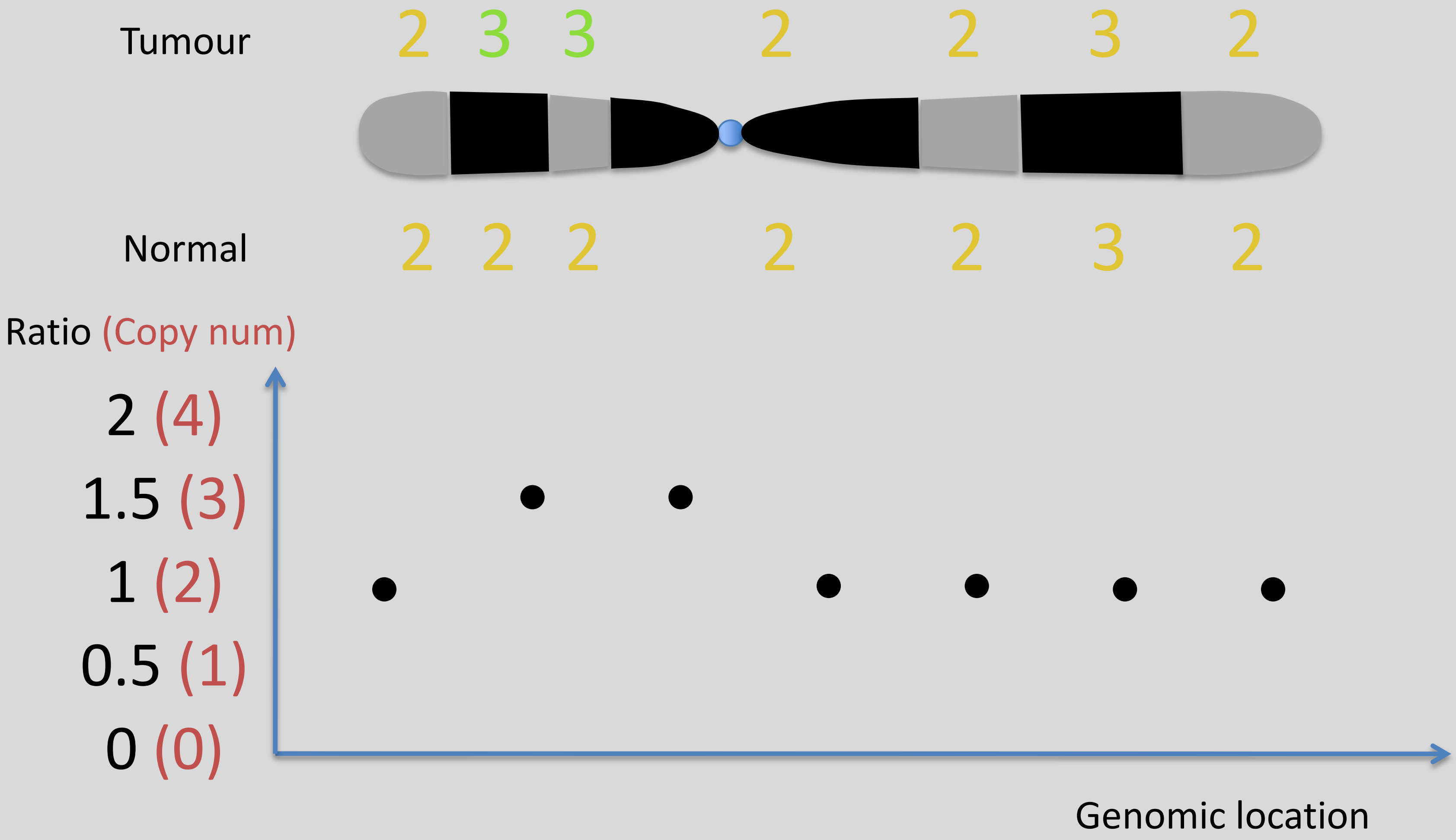
Tumour	2	3	3	2	2	3	2
Normal	2	2	2	2	2	3	2



Counting number of sequences for each window



Toward the real data

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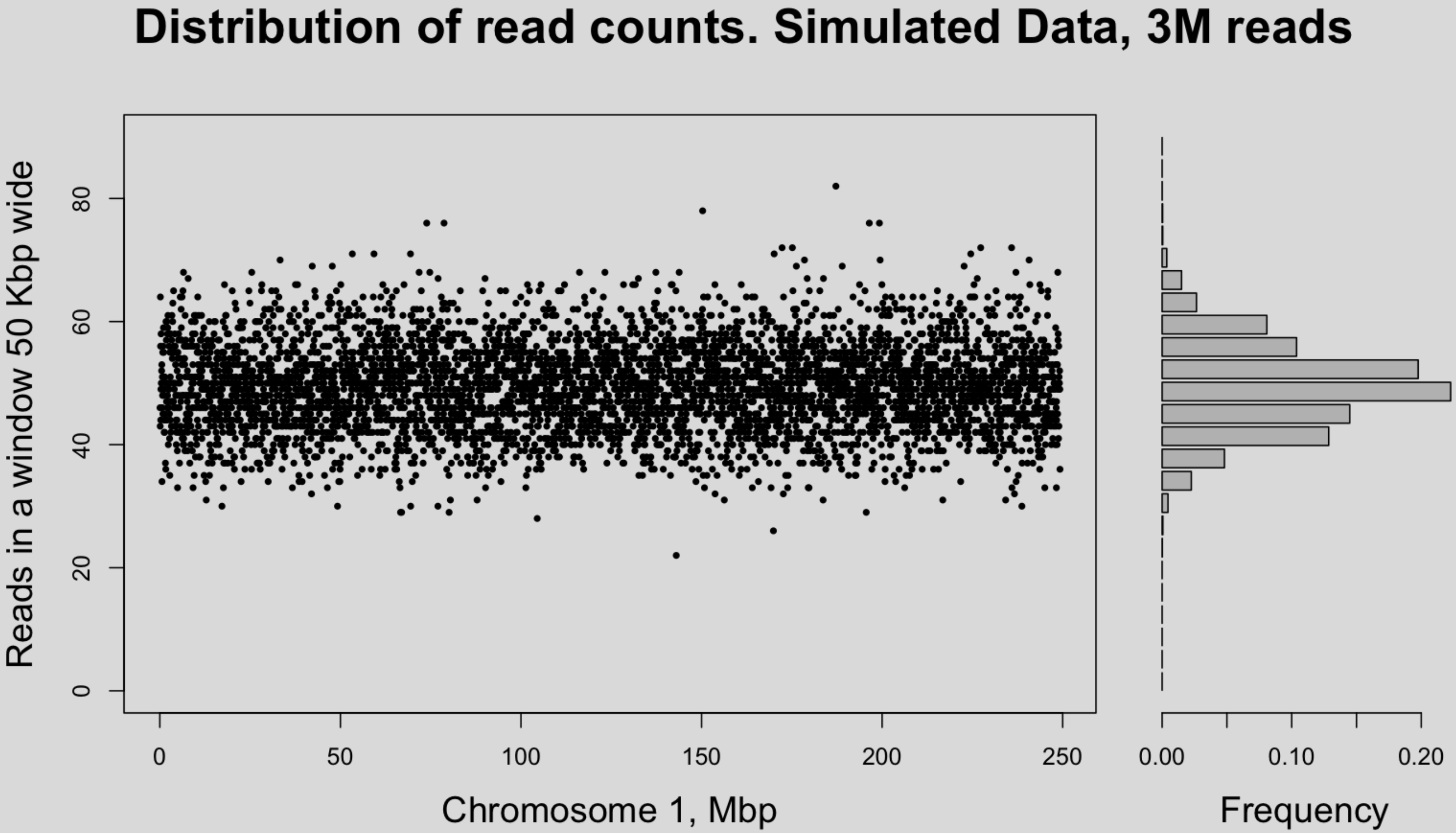
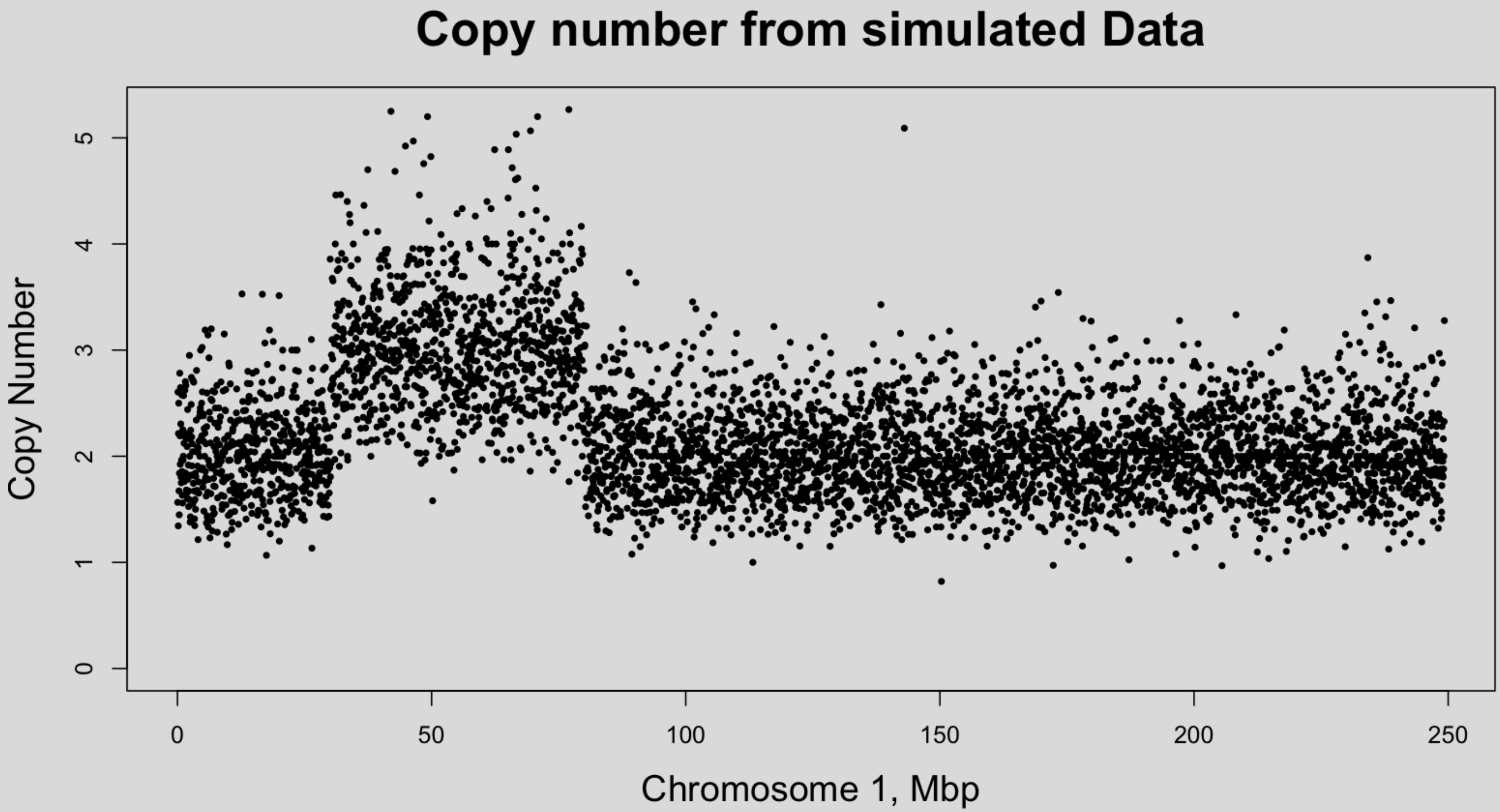


Ratio Test/Control



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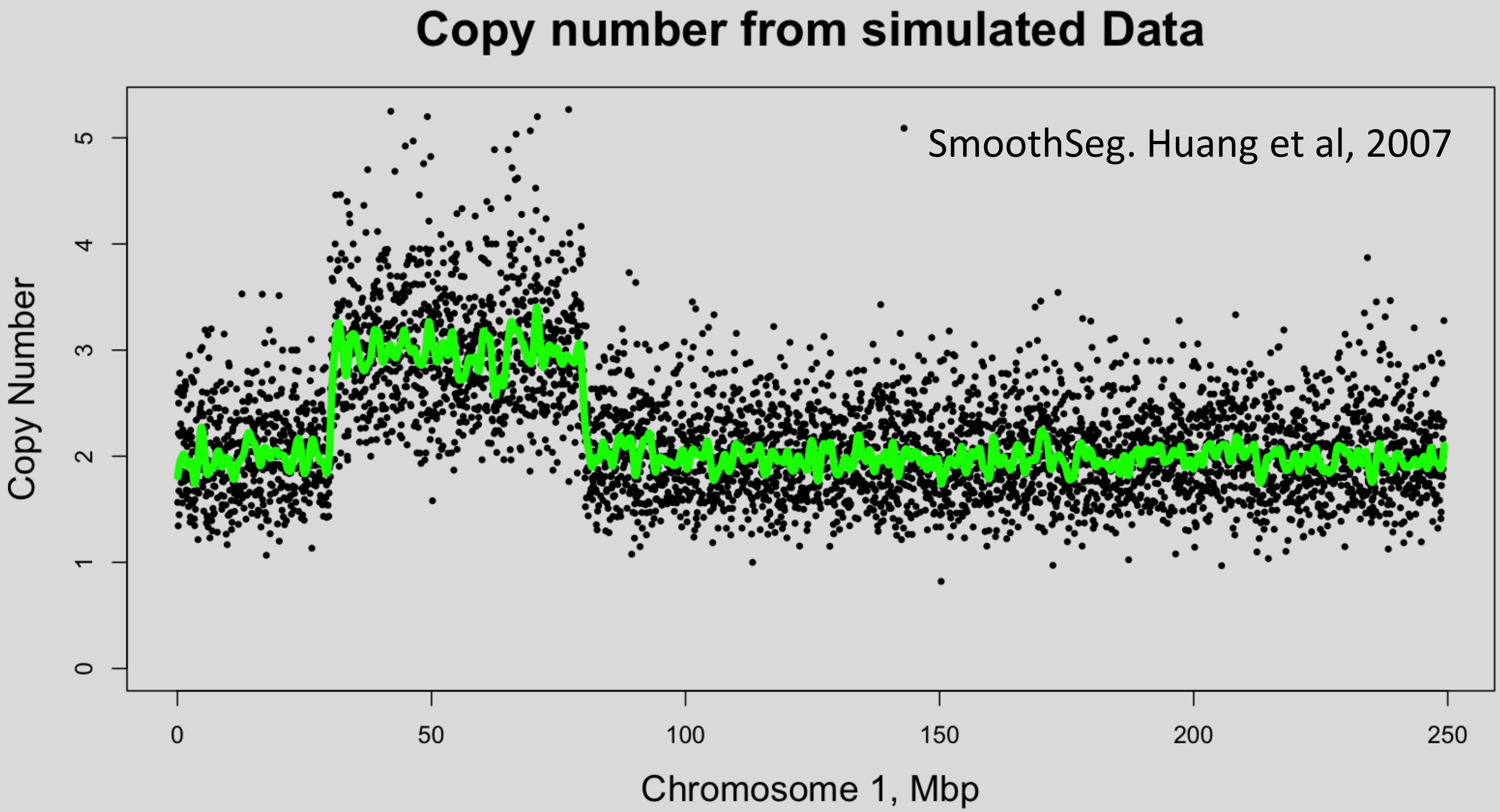

SmoothSeg. Huang et al, 2007

Resolution and noise go together!

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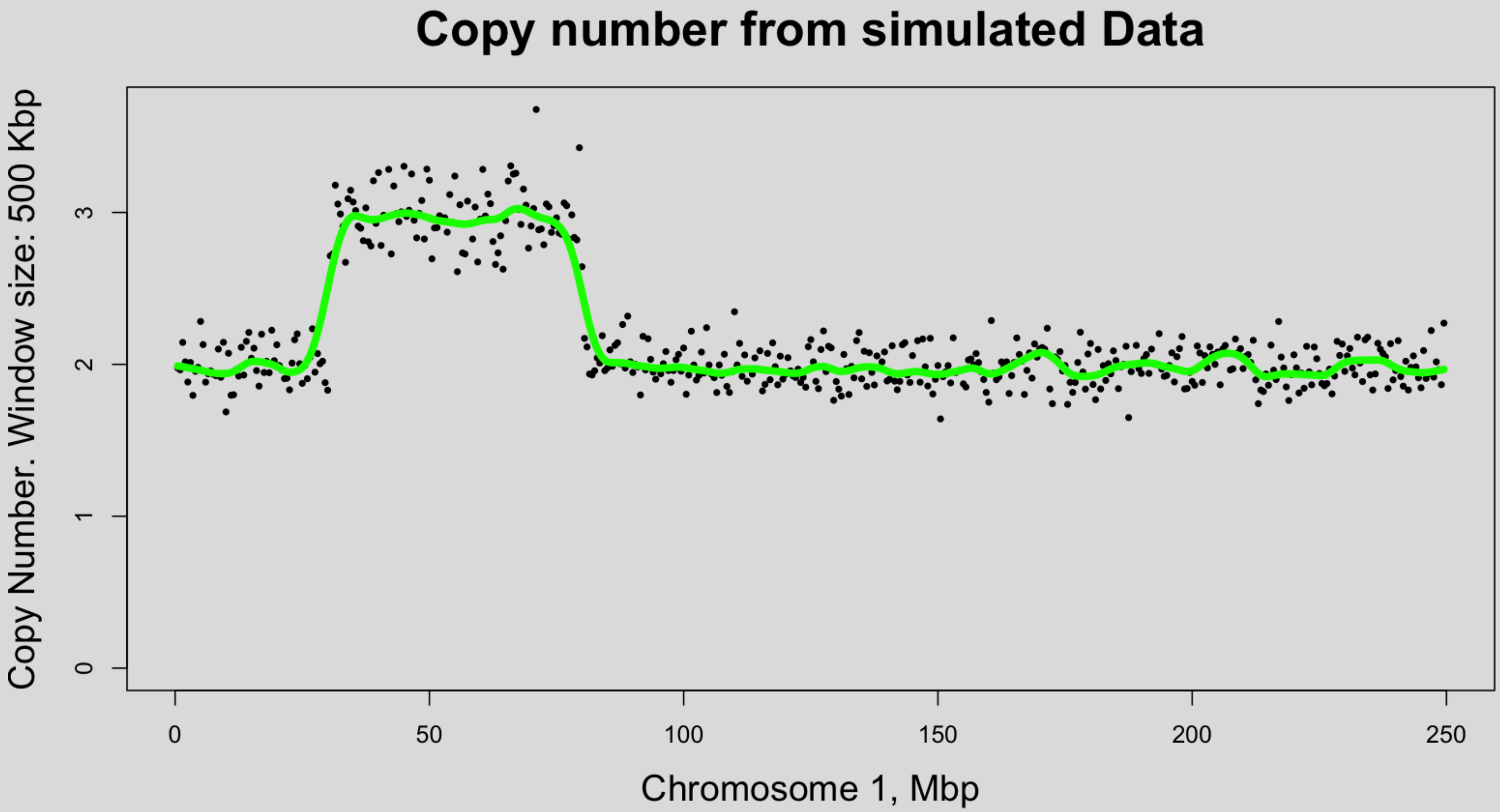
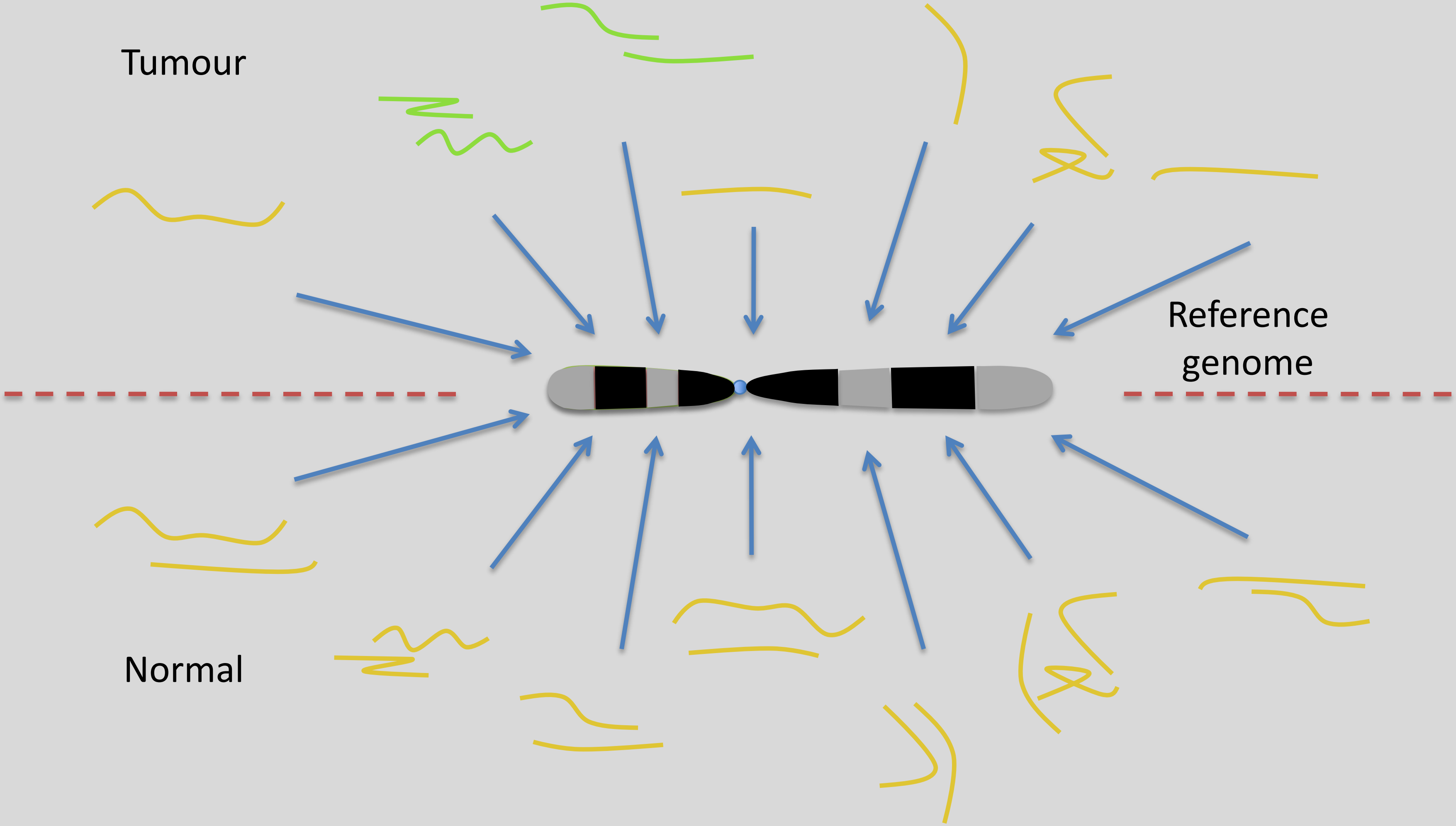


Different number of total reads



Total number of reads varies.

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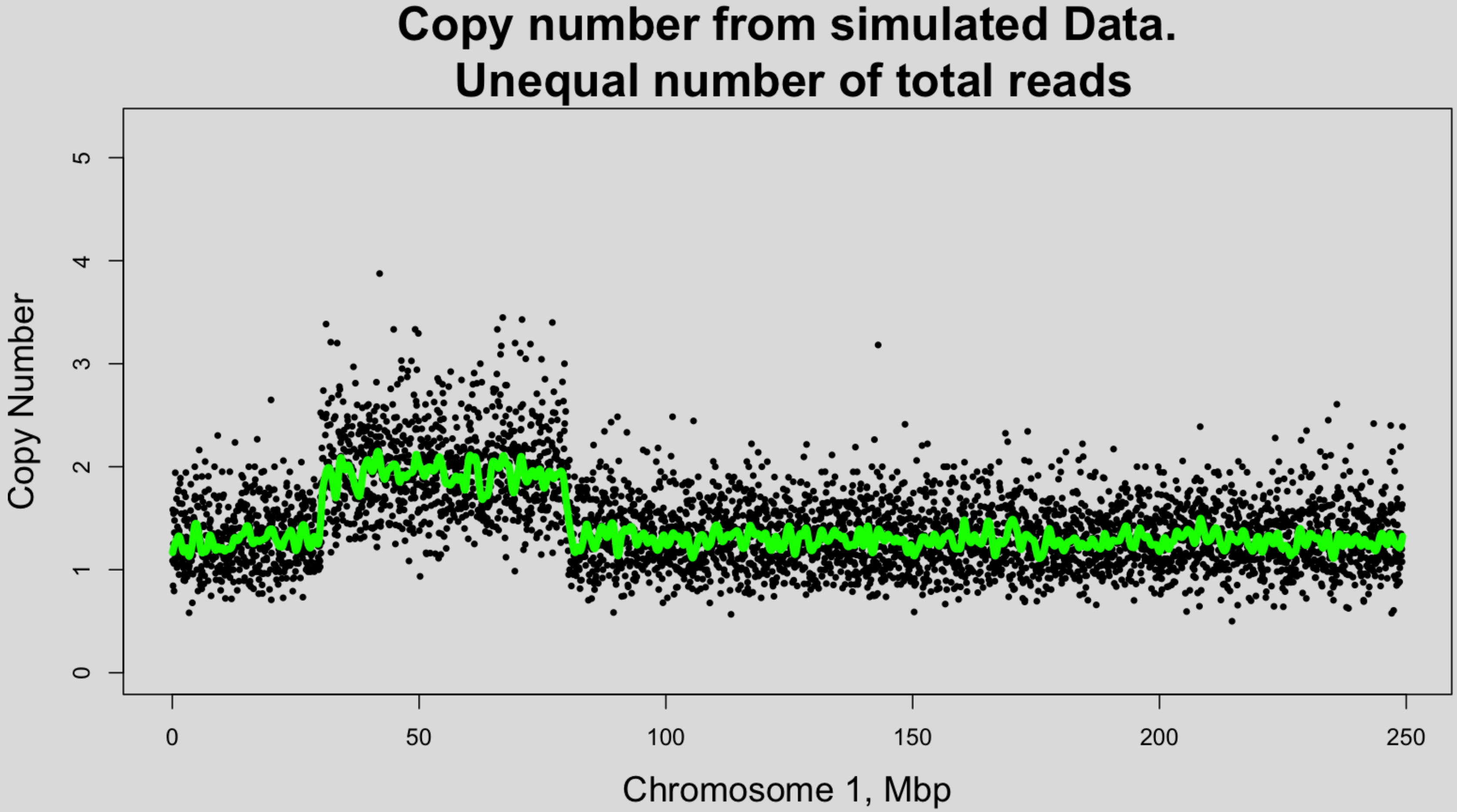

Normalization. A crucial step

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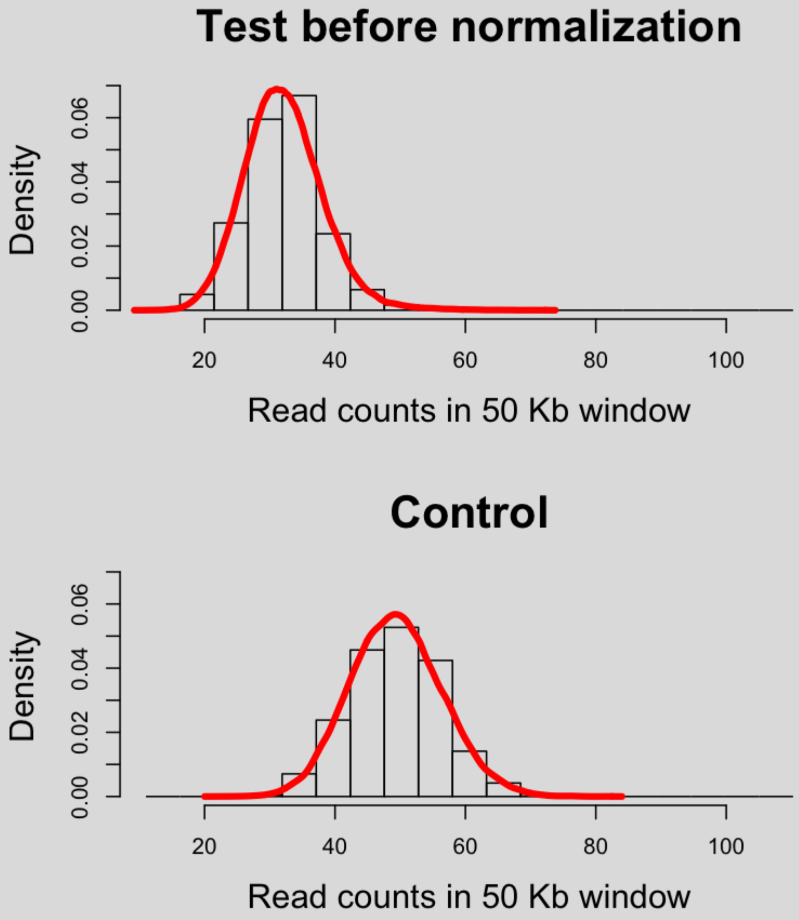

Normalization. A crucial step

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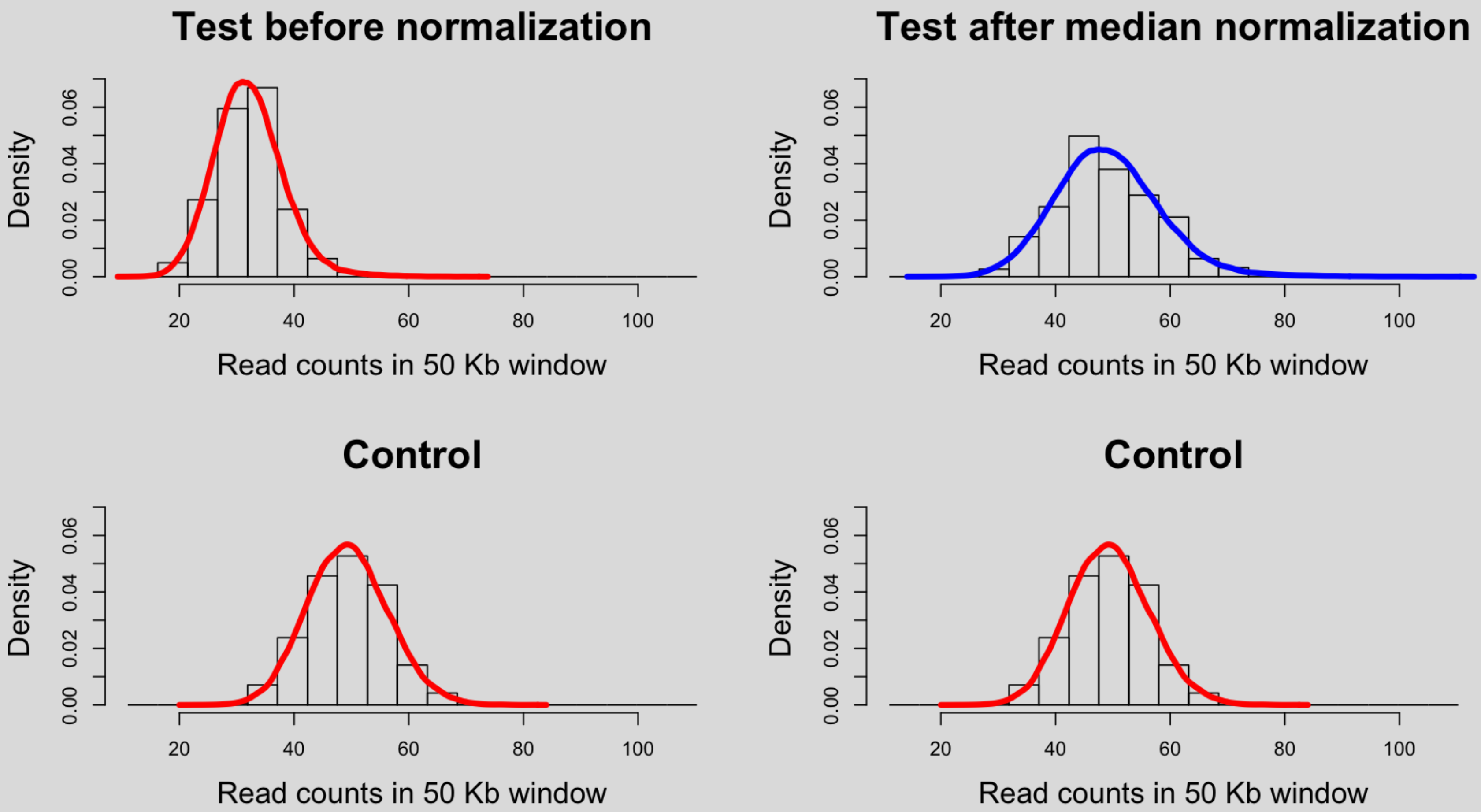

Test before normalization

Test after median normalization

Control

Control

Normalization. A crucial step

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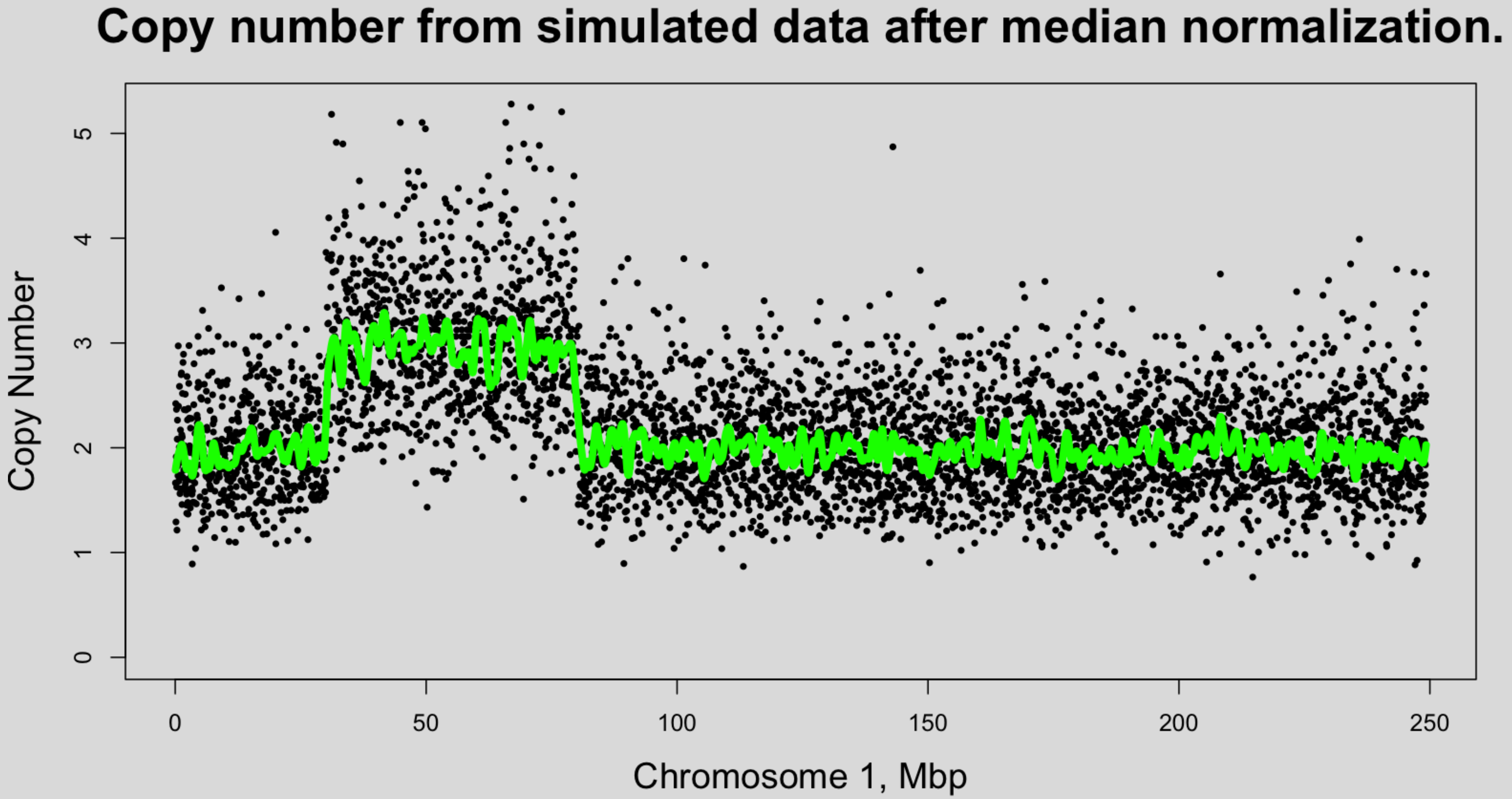

The cancer genome is often aneuploid

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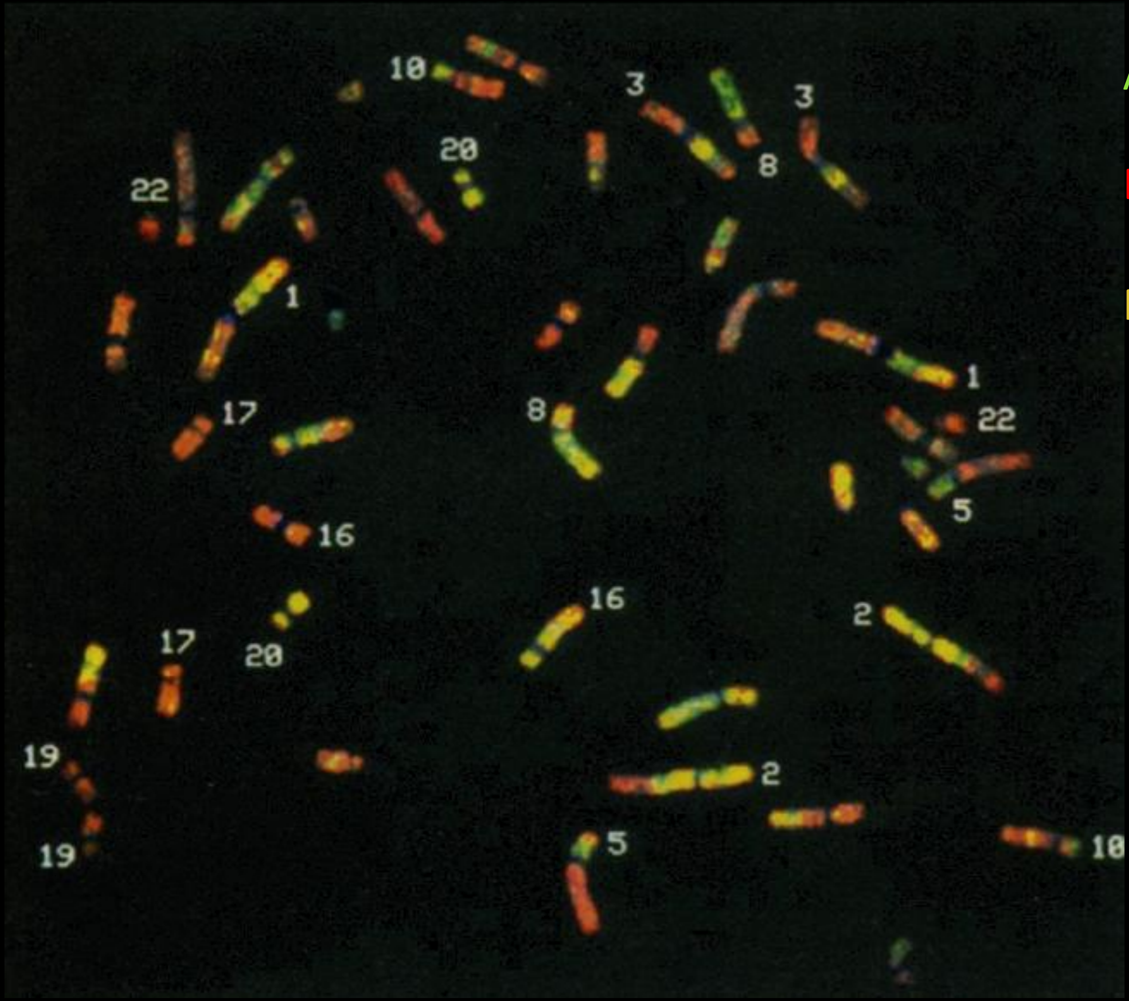


Amplifications

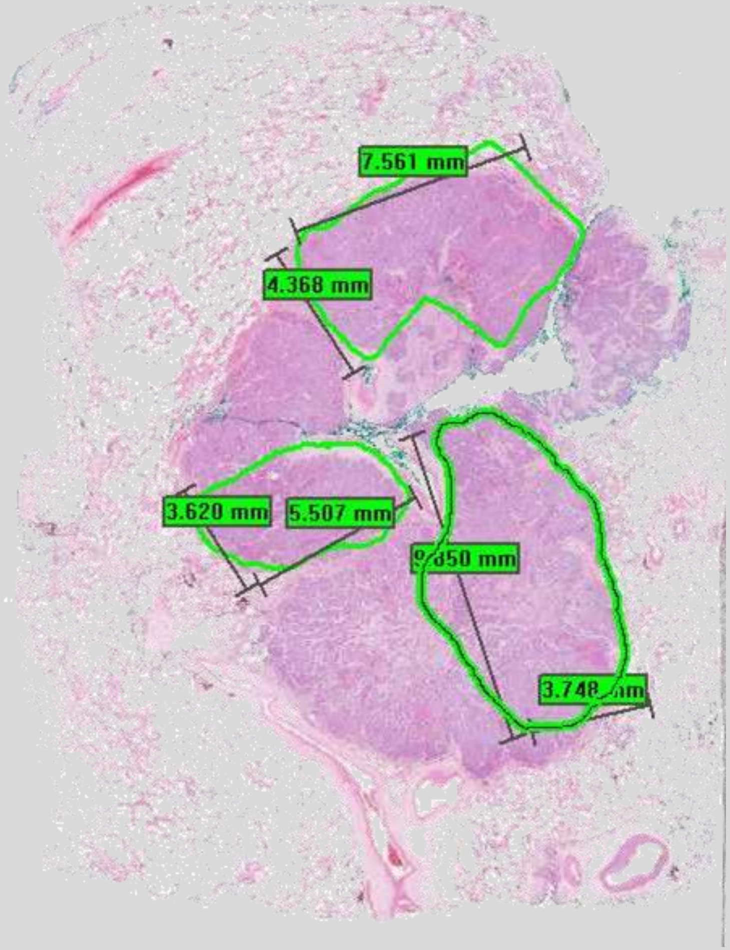
Deletions

Normal

Many amplifications and deletions!

Hartwell and Kastan. Science, 1994

Patient’s tumour samples



- Contamination with stroma, inflammatory cells...

Lung tumour

The real samples. A lot noisier

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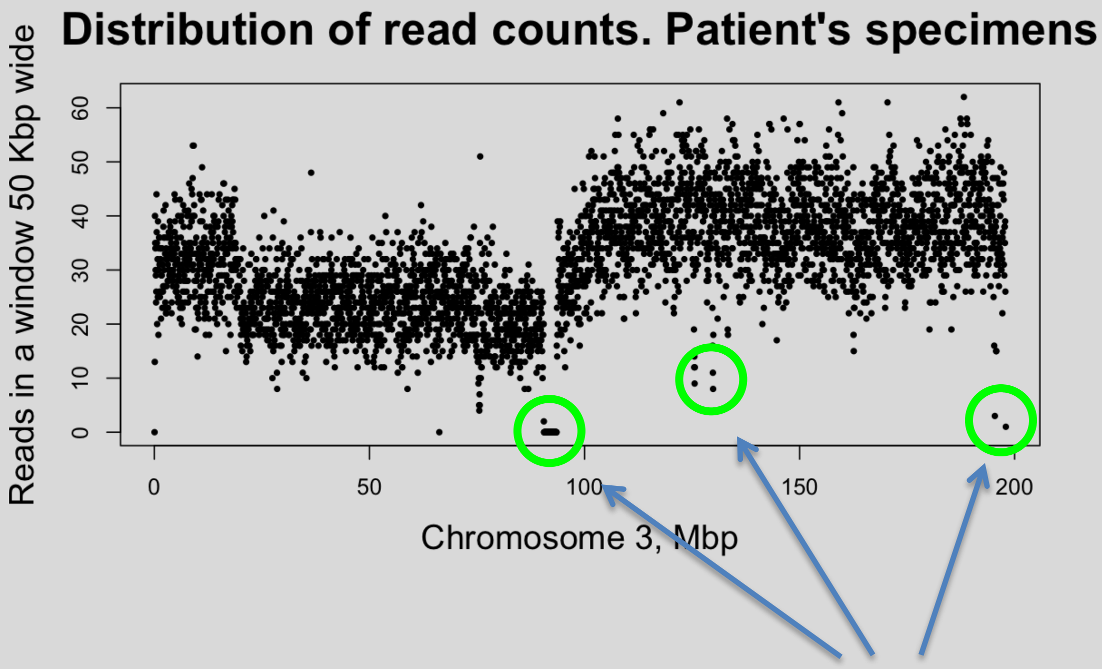

Aligning artefacts

- Some sequences cannot be aligned (repeated regions)
- GC content bias
- Unequal number of total reads.
- Extra noise of unknown origin

The median might be meaningless

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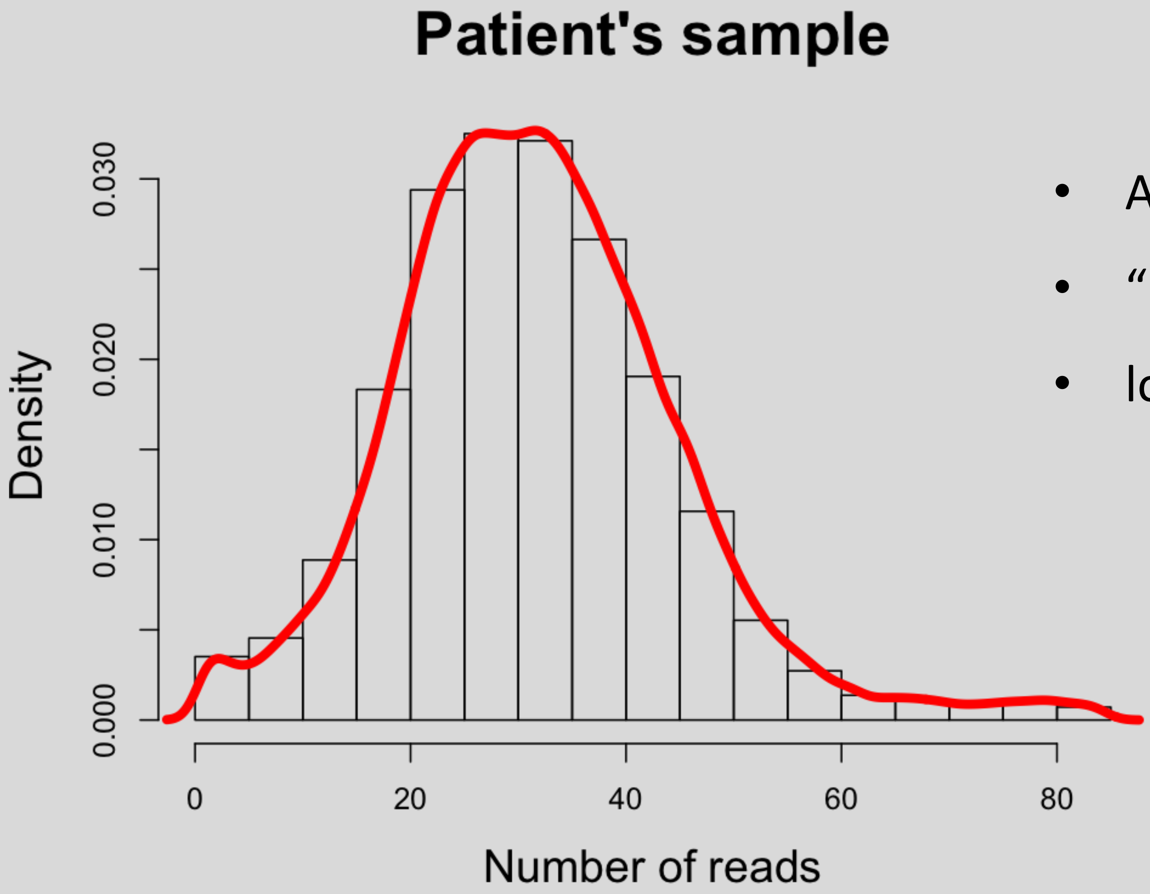
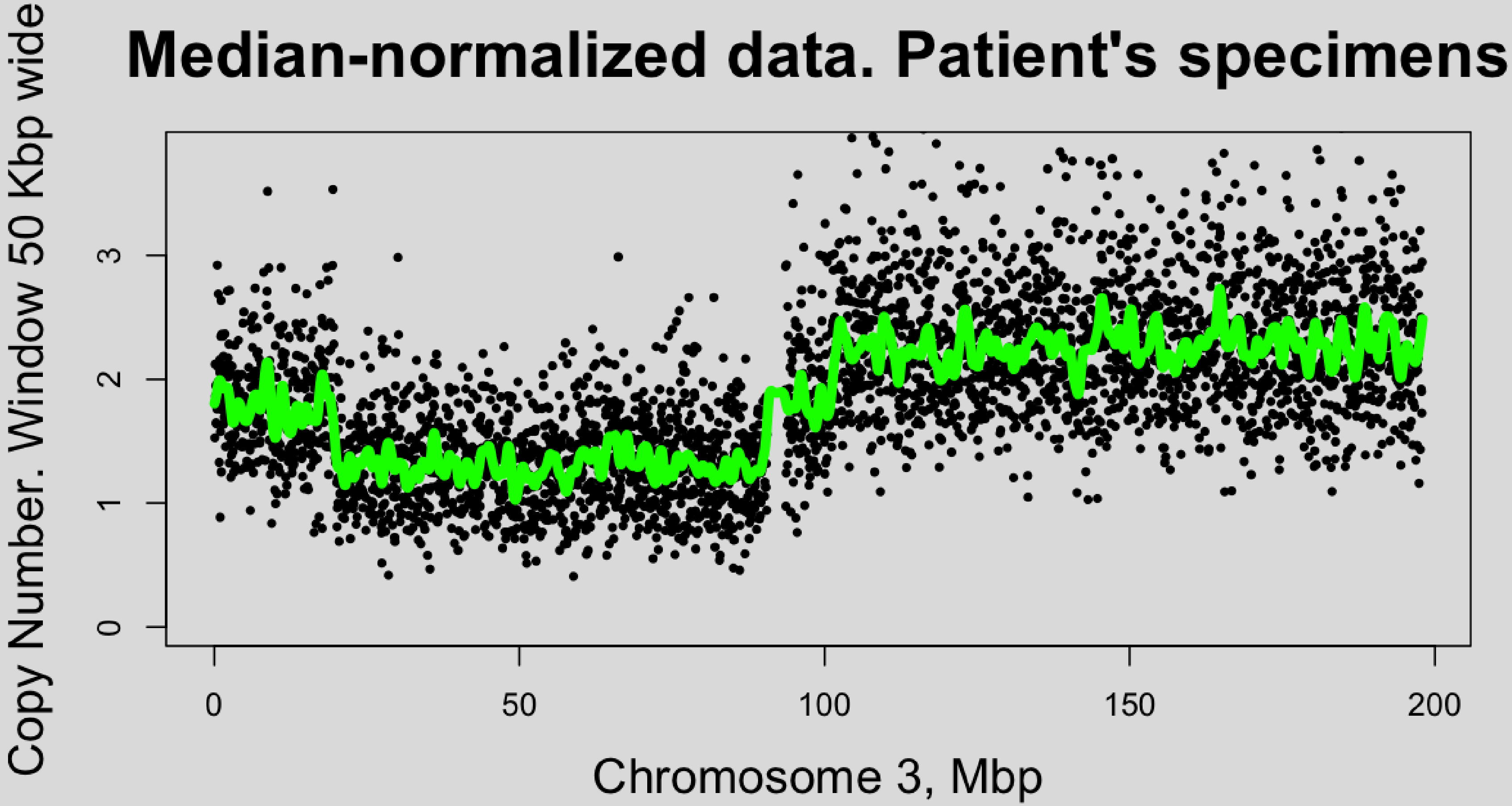
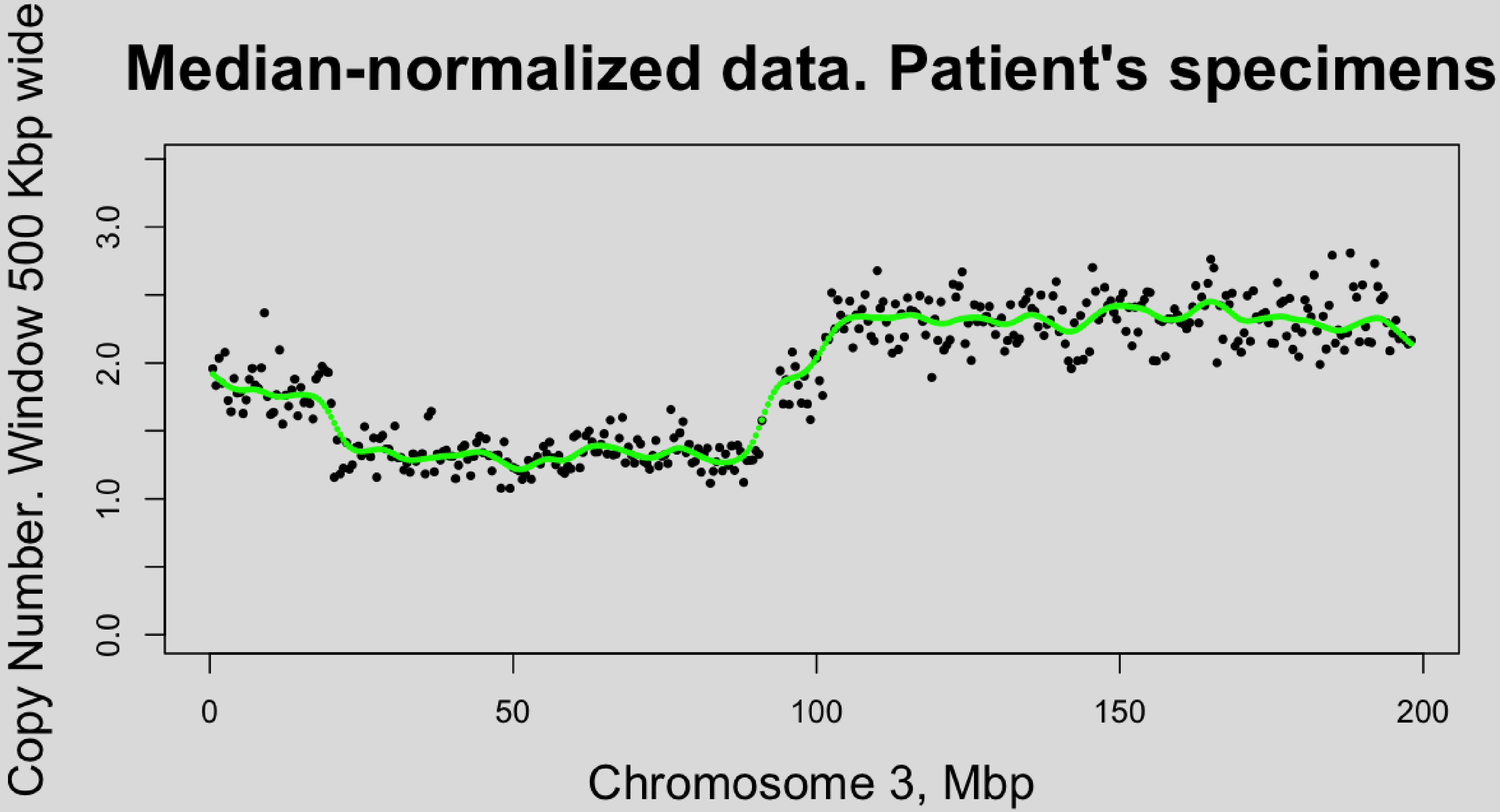


- Asymmetric distribution
- “Flat top”
- long tail

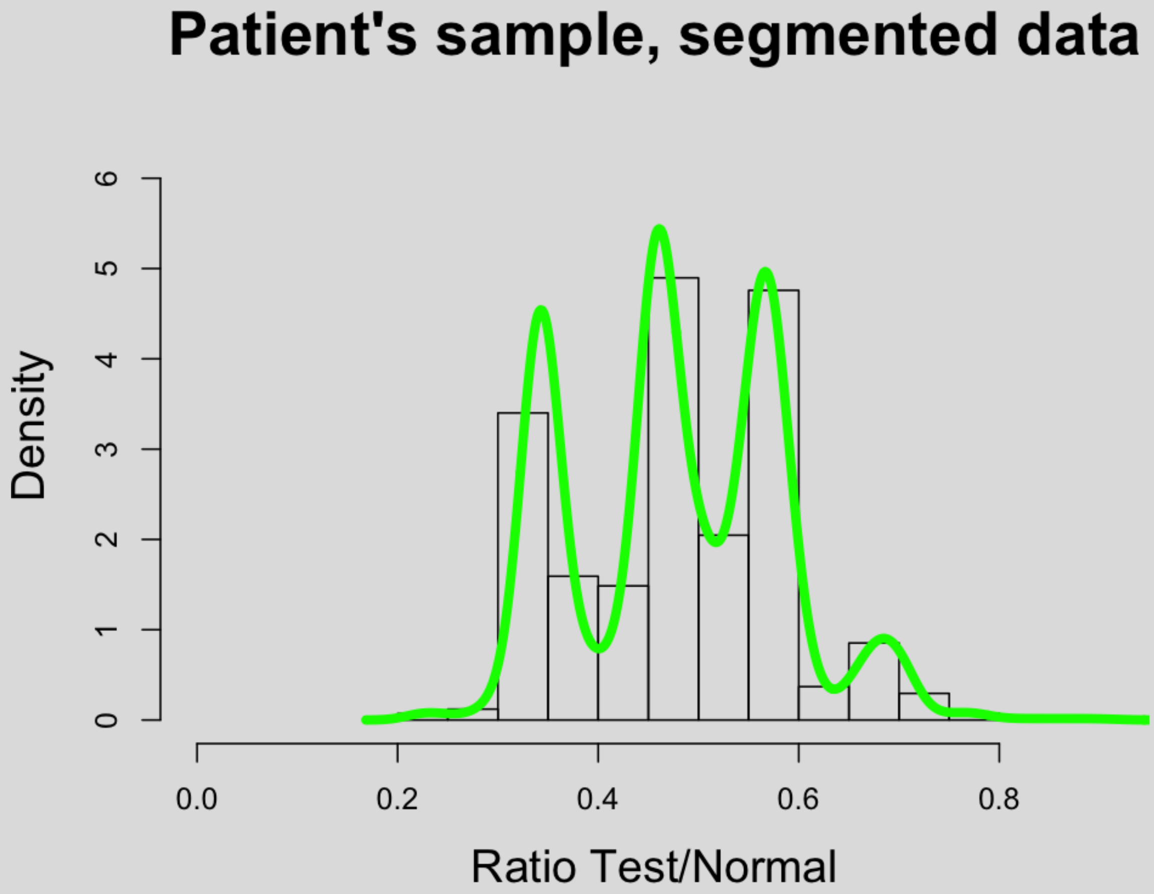
Median normalisation



Trade resolution for noise



Discrete data normalisation



Discrete data normalisation

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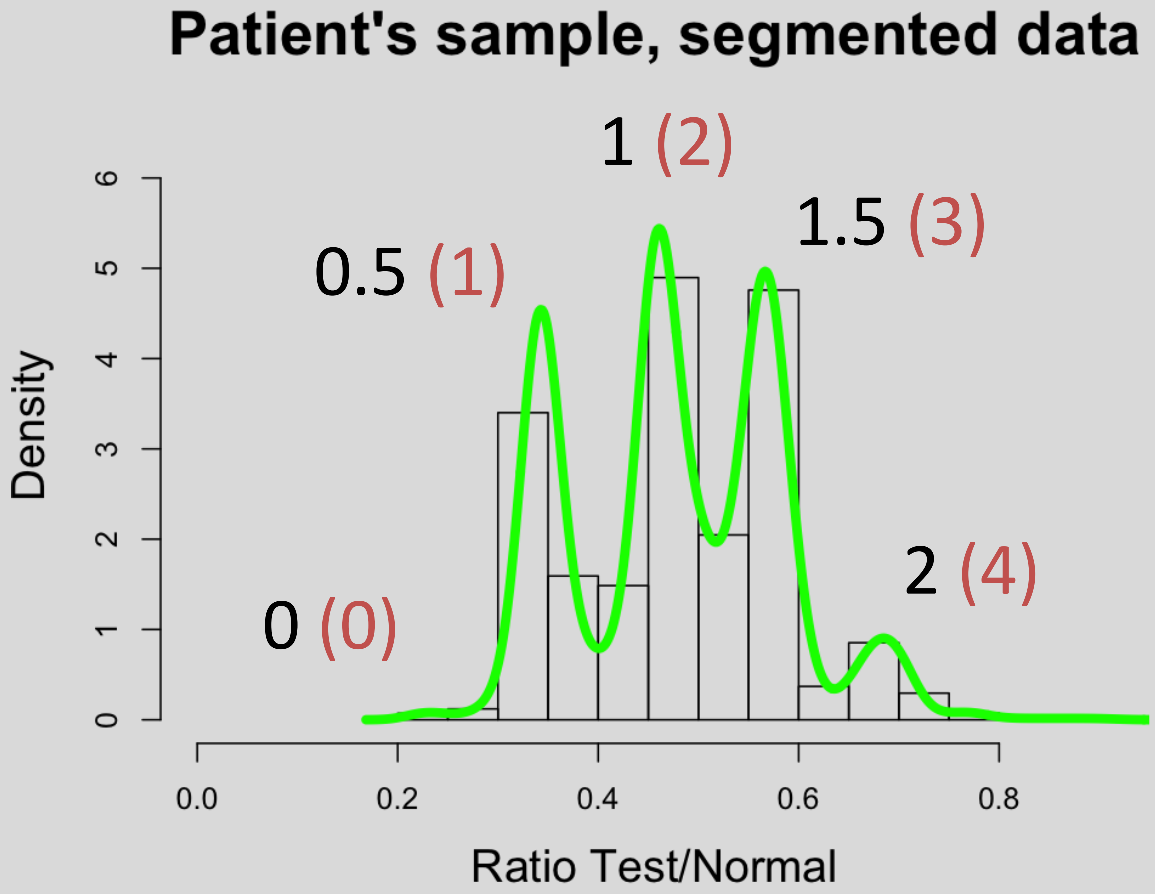

Ratio (Copy num)

Discrete data normalisation

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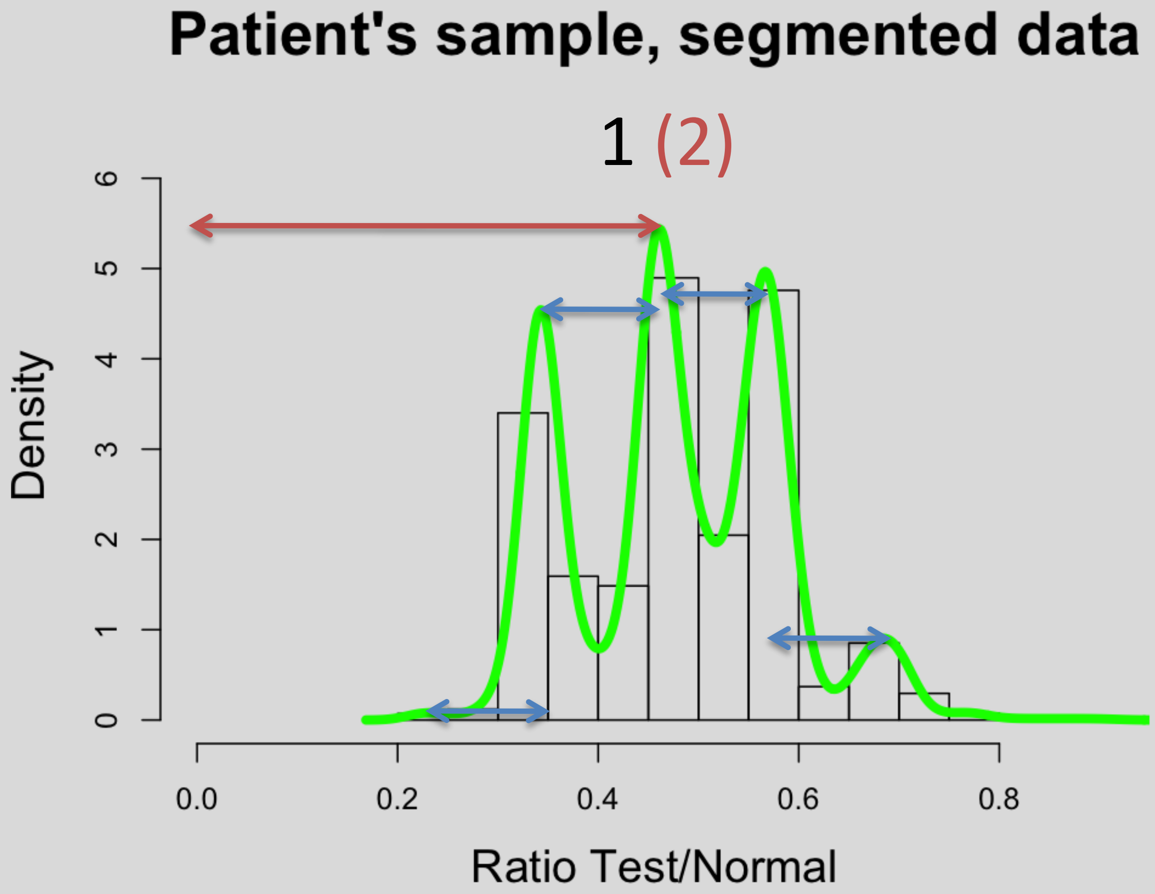

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Discrete data normalisation

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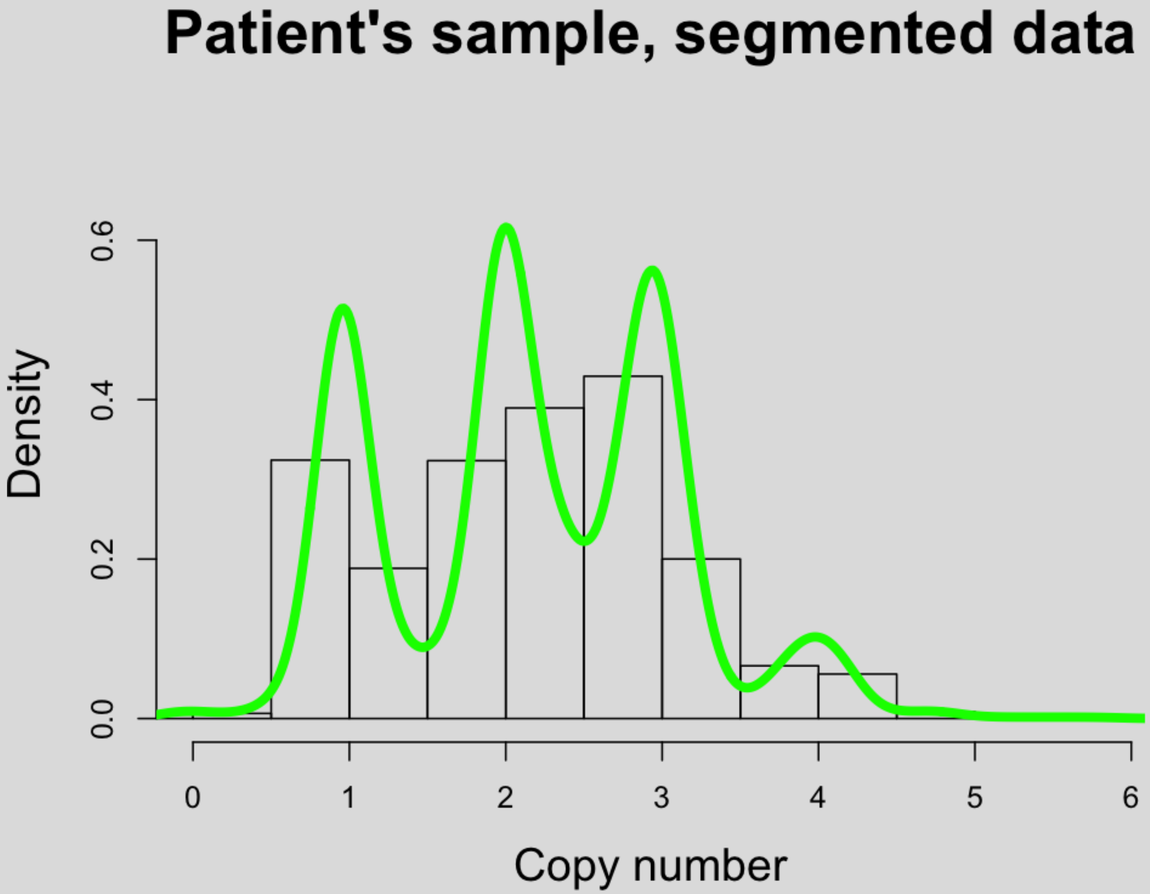

Discrete data normalisation

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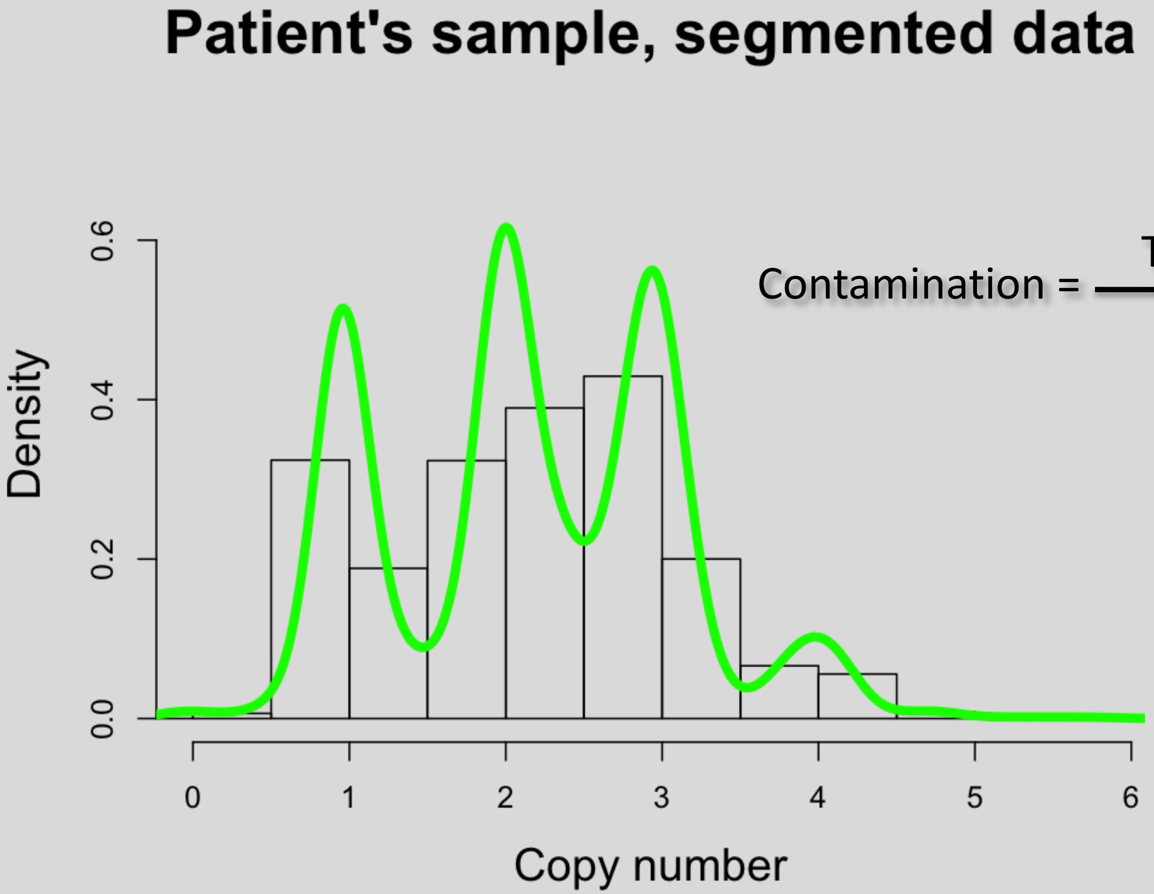

$$\text{Contamination} = \frac{\text{Tumour tissue}}{\text{Total tissue}} = 51\%$$

Keep high resolution and normalise

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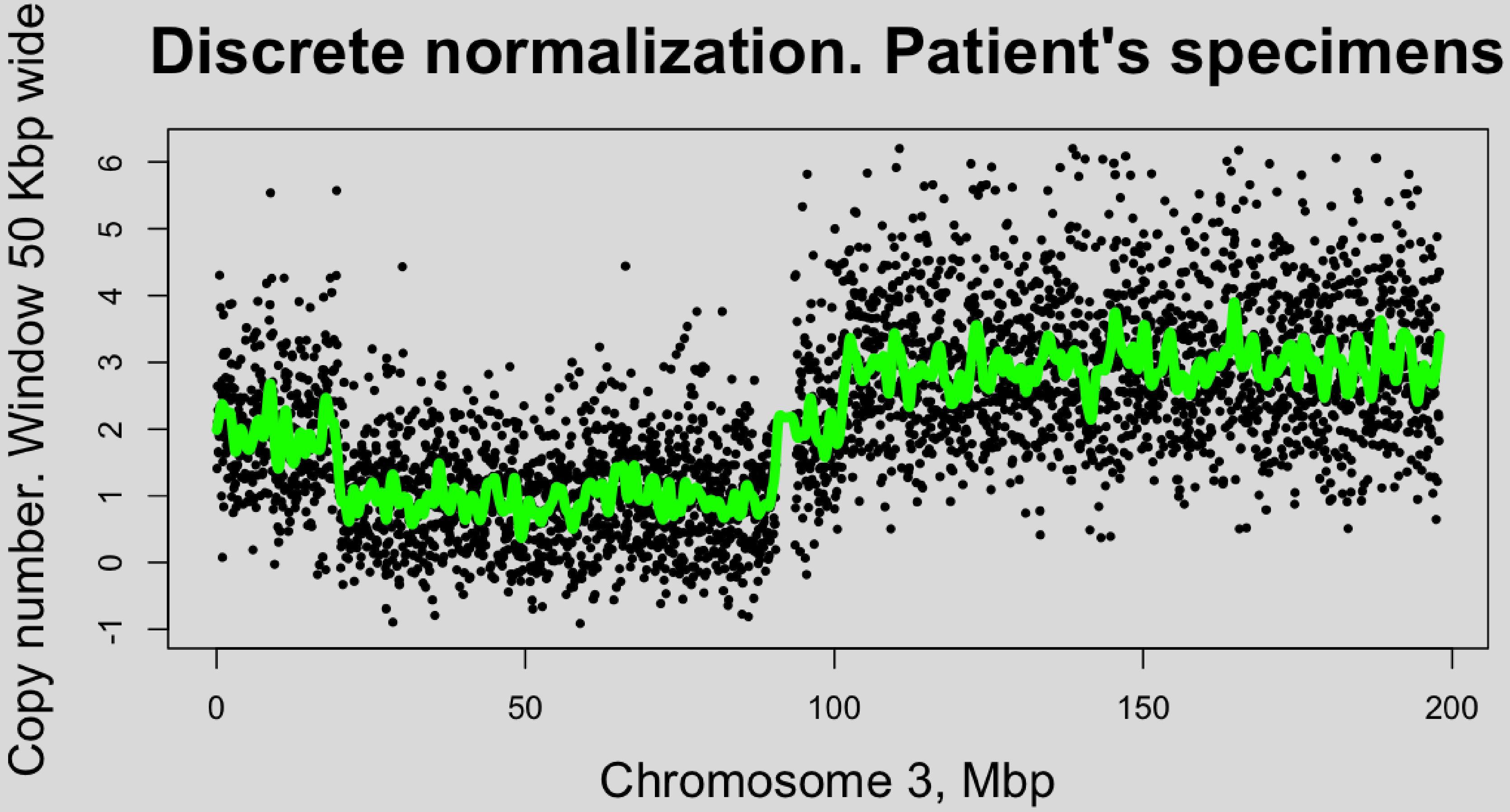

Conclusions

- Develop a novel normalisation method for “NextGen” data that can cope with
  - ✓ Highly abnormal genomes
  - ✓ Tumour samples contaminated by normal cells
- We can estimate contamination percentage.

Limits

- Contamination is allowed, but otherwise the tumour should be homogeneous.
- Process might require human supervision when calling discrete states.

Acknowledgements

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Arief Gusnanto

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