Counting malaria parasites with digital image analysis

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Why count malaria parasites?

- Severity indicator: rough guide
- Response to treatment
- High transmission areas: clinical assessment of fever
- Case definition for clinical drug or vaccine trials eg fever + 2500 parasites/µl

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Parasitaemia problems

- Counting is tedious and error-prone
- Variation between slides – same reader
- Variation between readers – same slide
- Low counts: random distribution effects
- High counts: reader error

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Project aims

- Apply DIA to quantitation, not diagnosis/ID
- Thick blood films
- Ordinary microscope & camera system
- Readily-available software: Image J (NIH)
- No custom programmes or IT expertise
- Suitable for limited-resource environment

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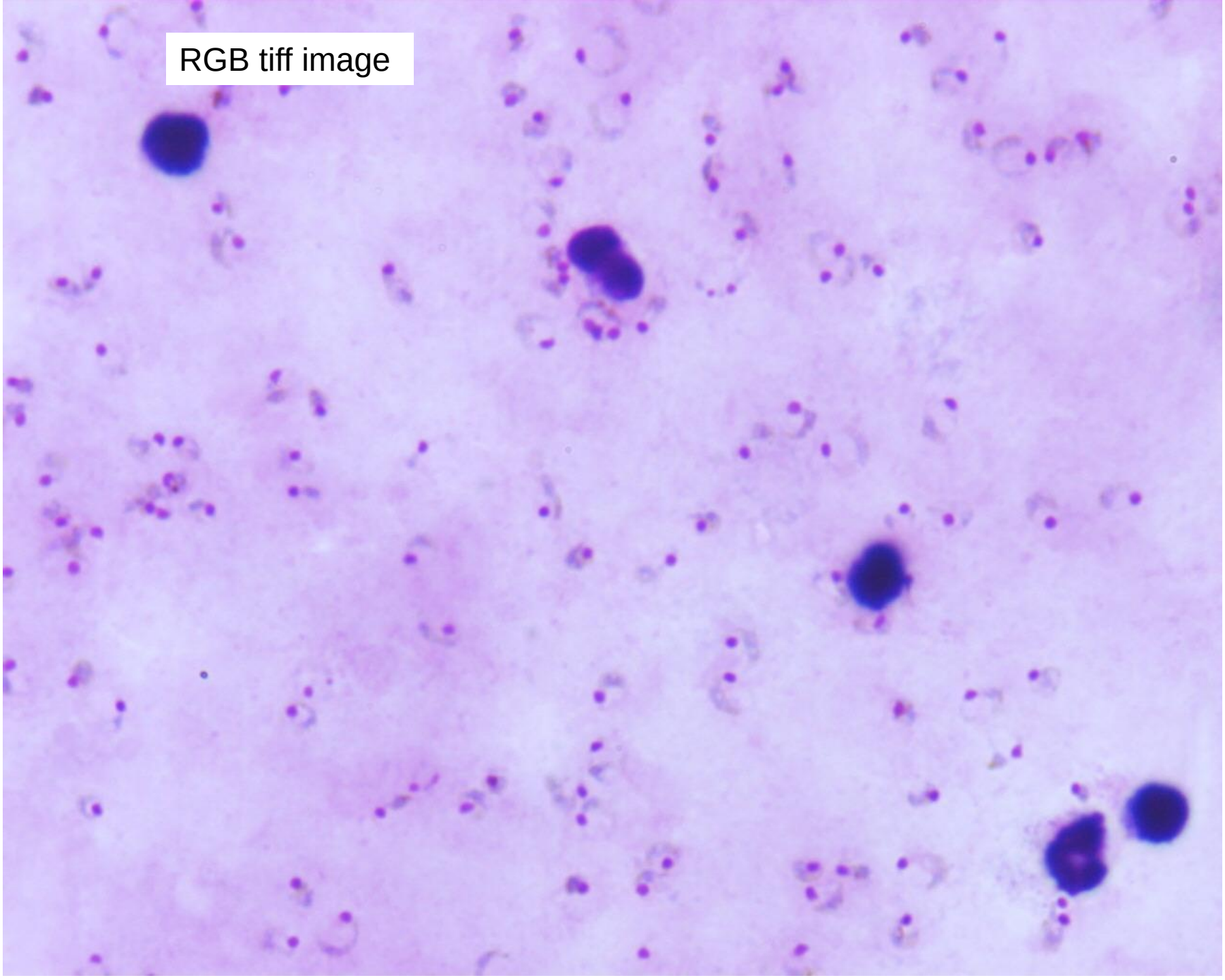
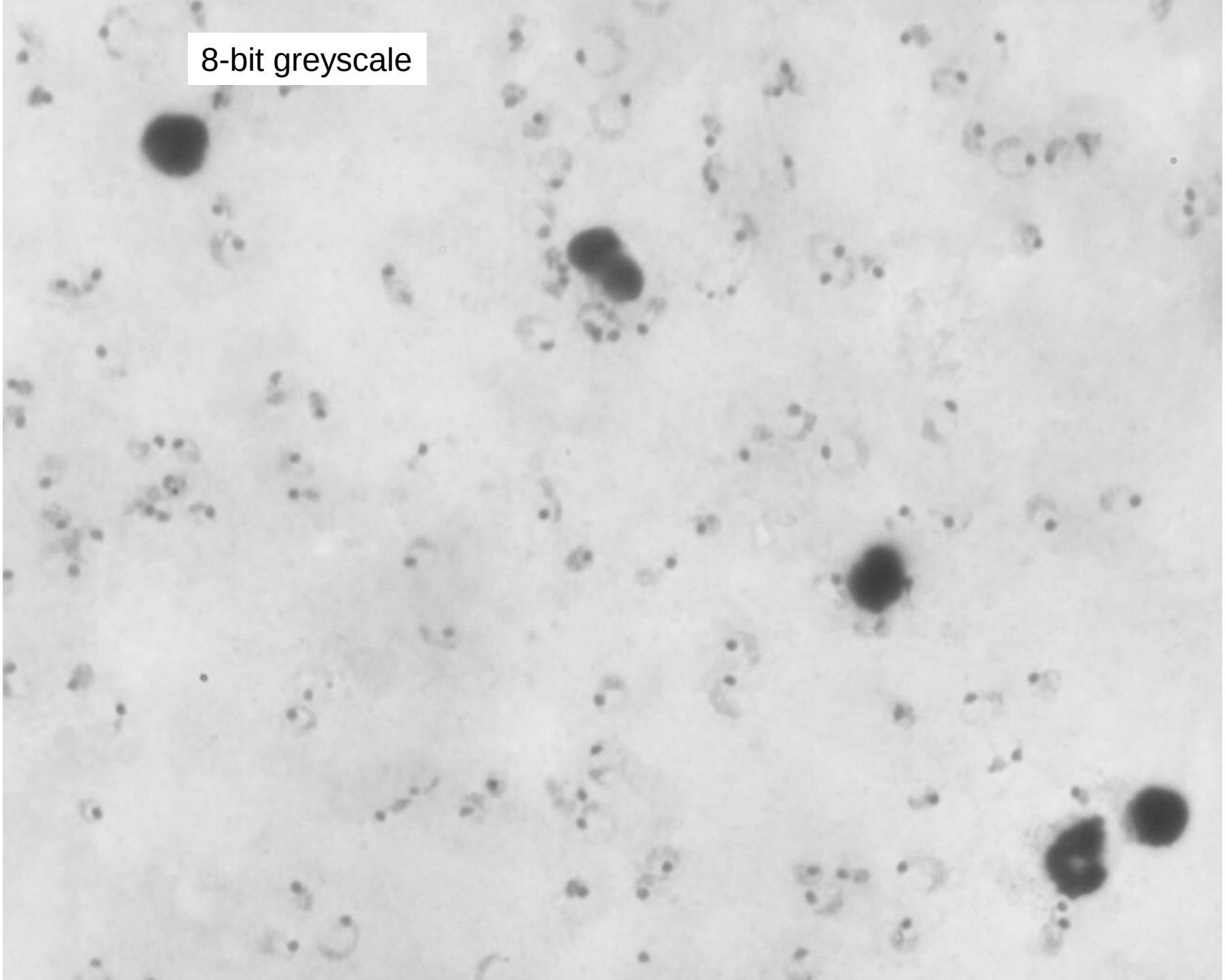
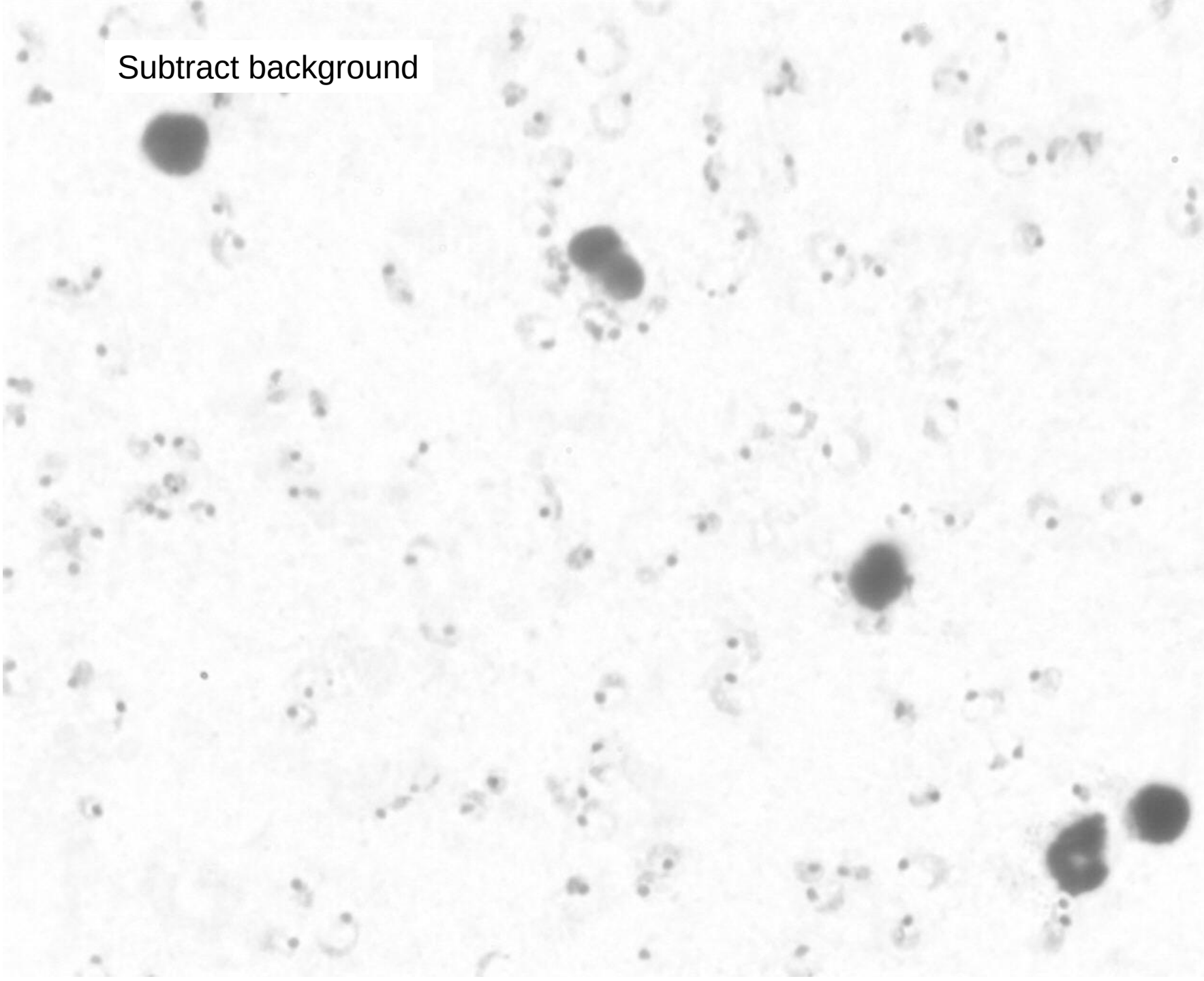
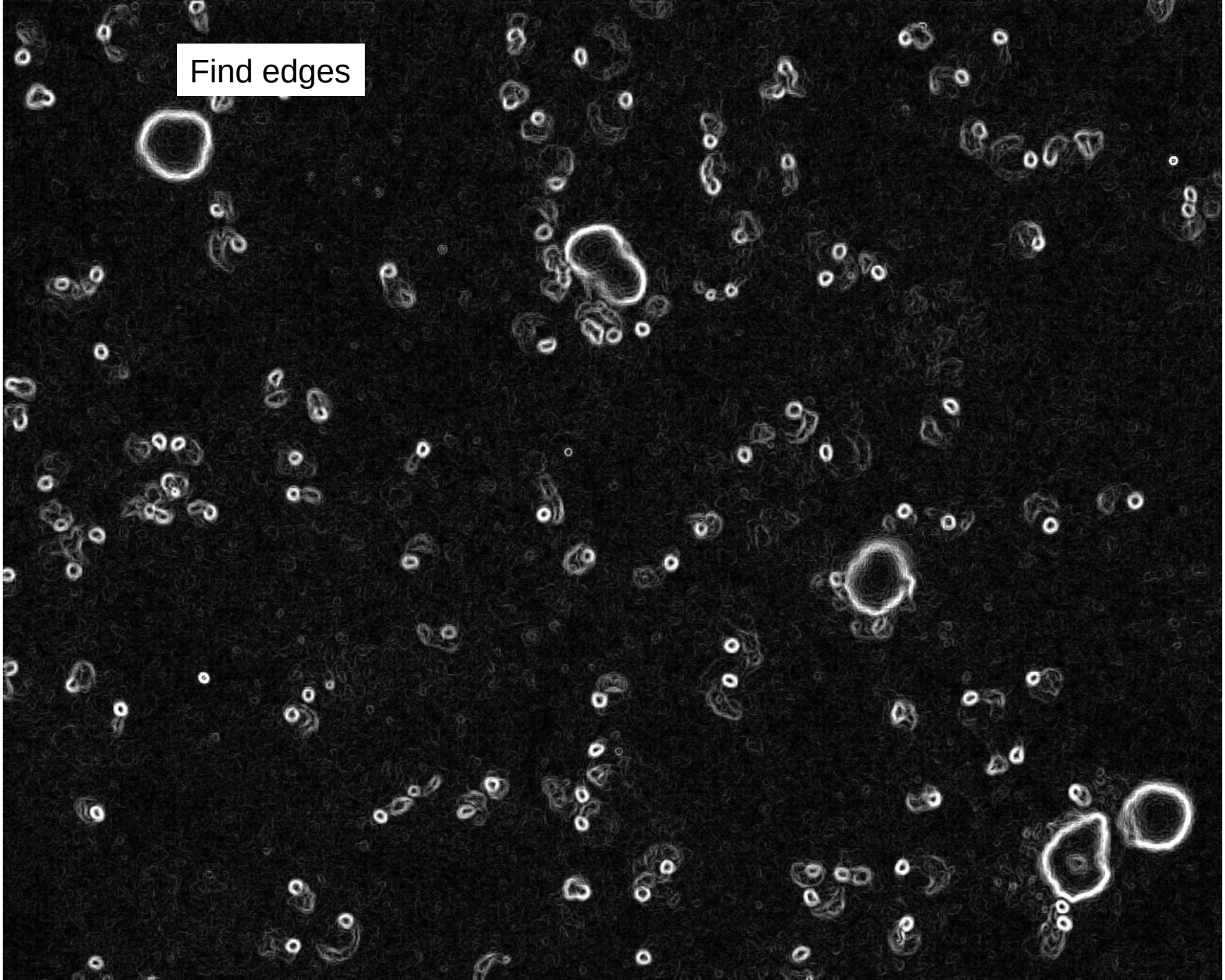
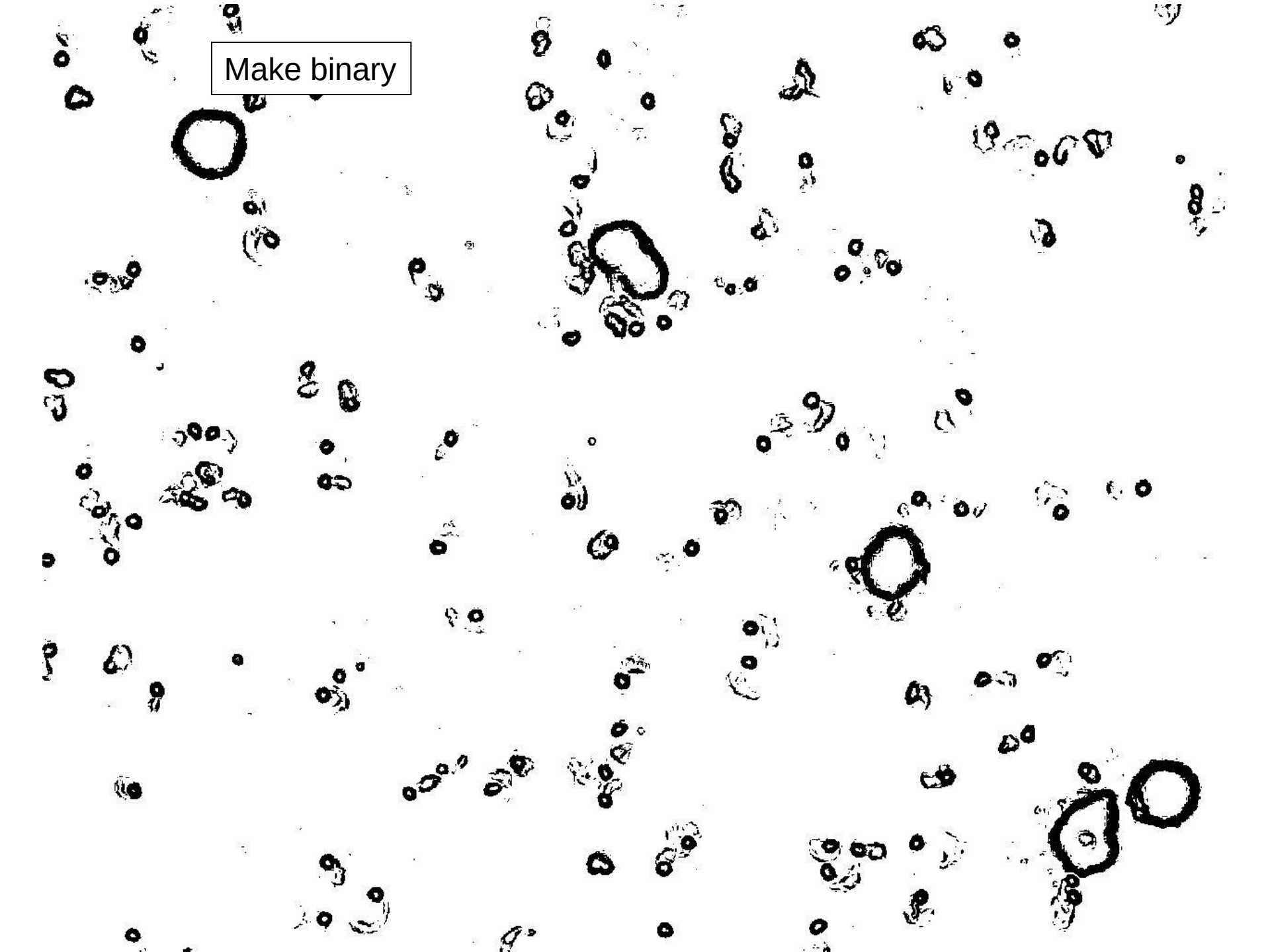
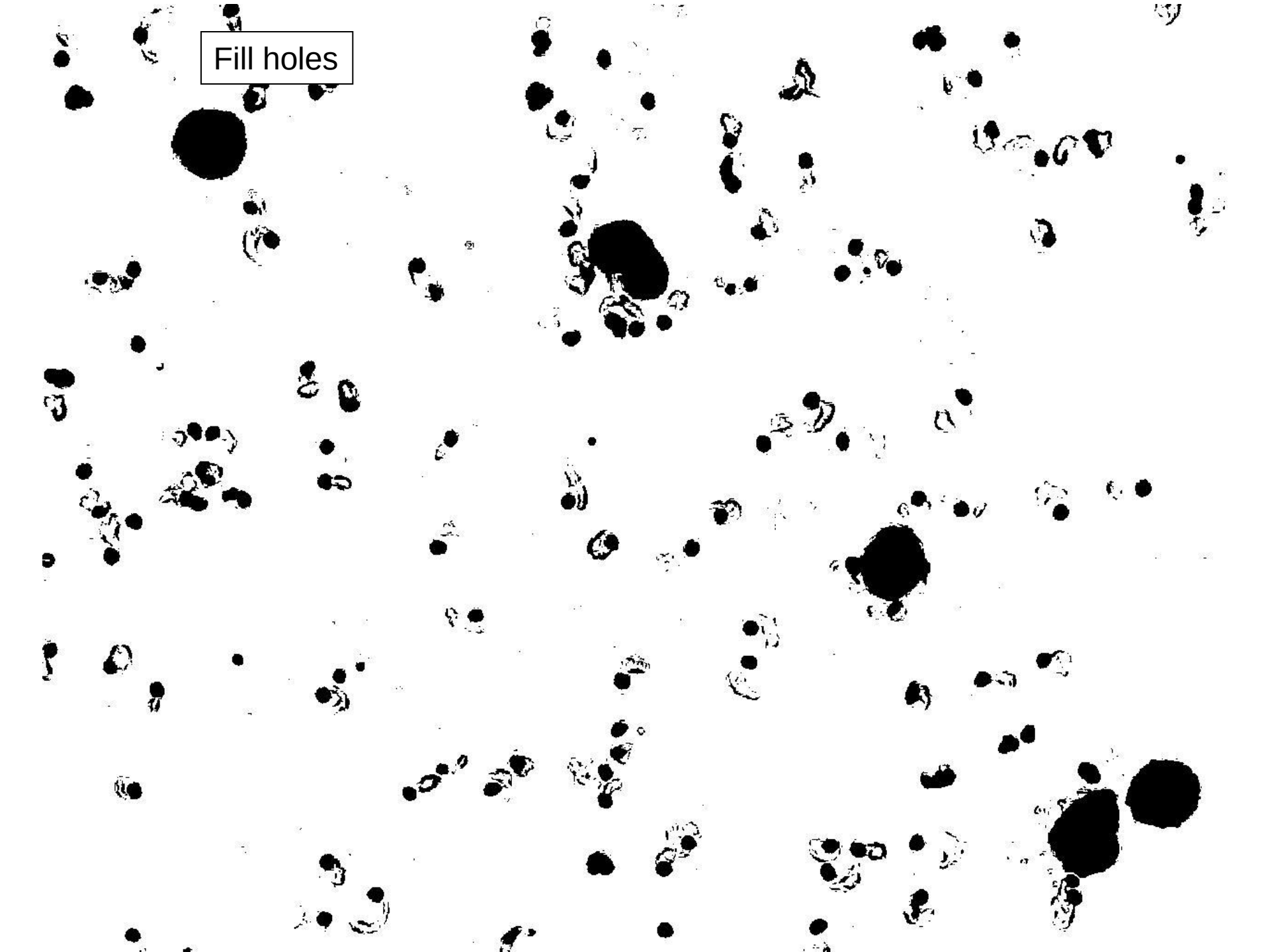

RGB tiff image

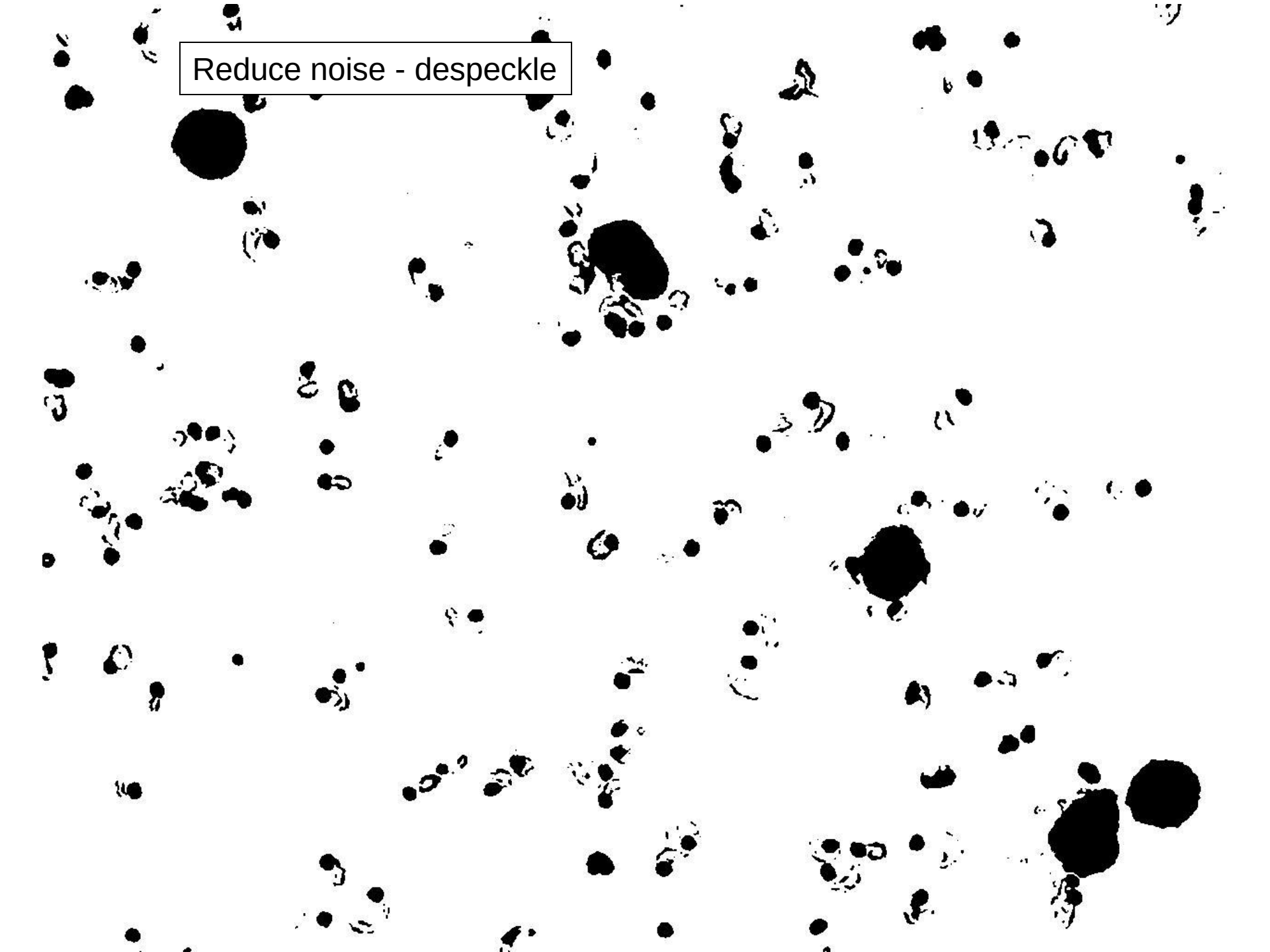

8-bit greyscale

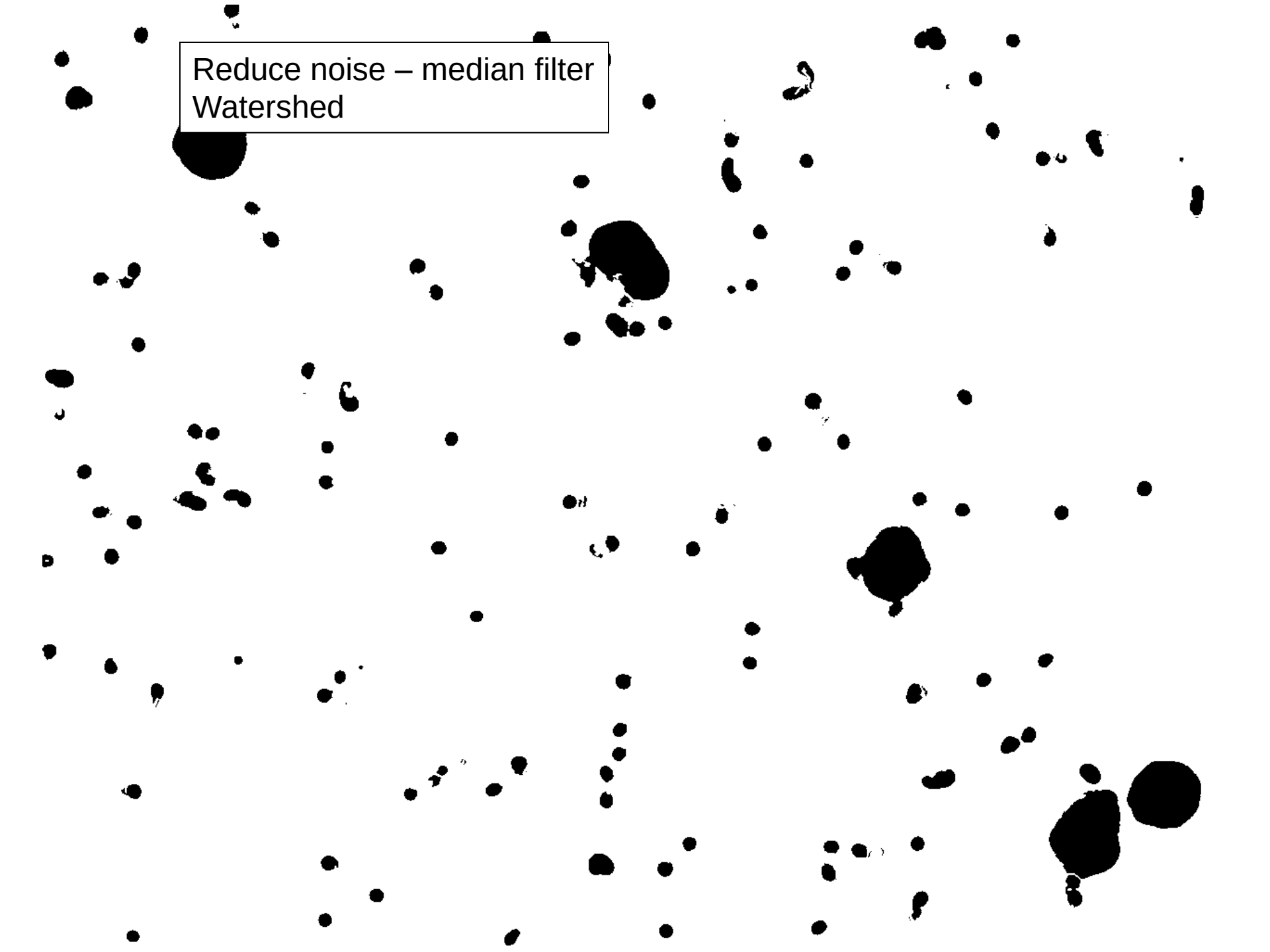

Subtract background


Find edges

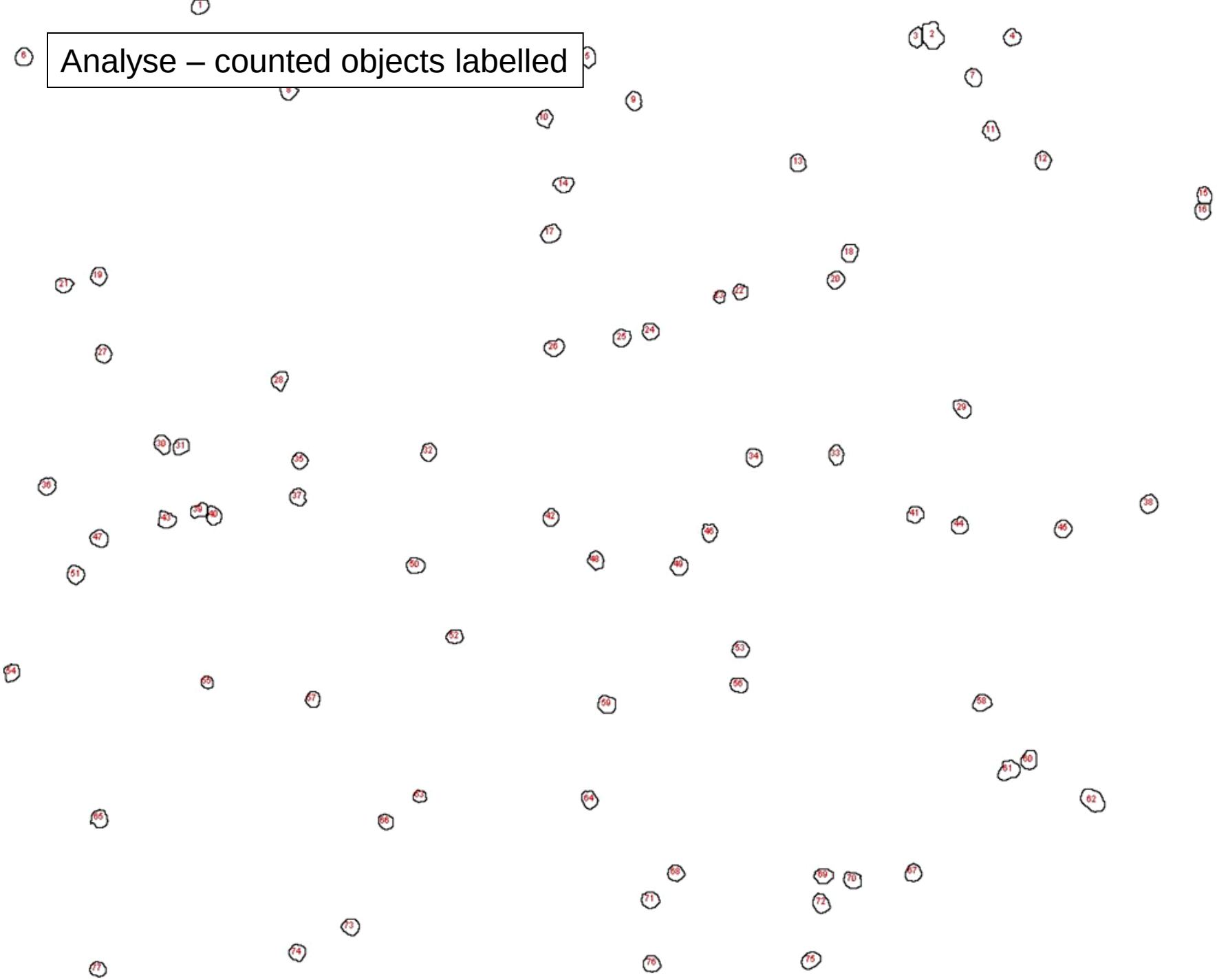

Make binary


Fill holes


Reduce noise - despeckle


Reduce noise – median filter
Watershed

Analyse – counted objects labelled



Problem

- Tweaking algorithm: very accurate counts
- Parasites on different slides look different:
  - ages
  - sizes
  - staining features
  - densities
- So one basic algorithm does not do for all
- Tweaking ⟶ bias & non-uniformity

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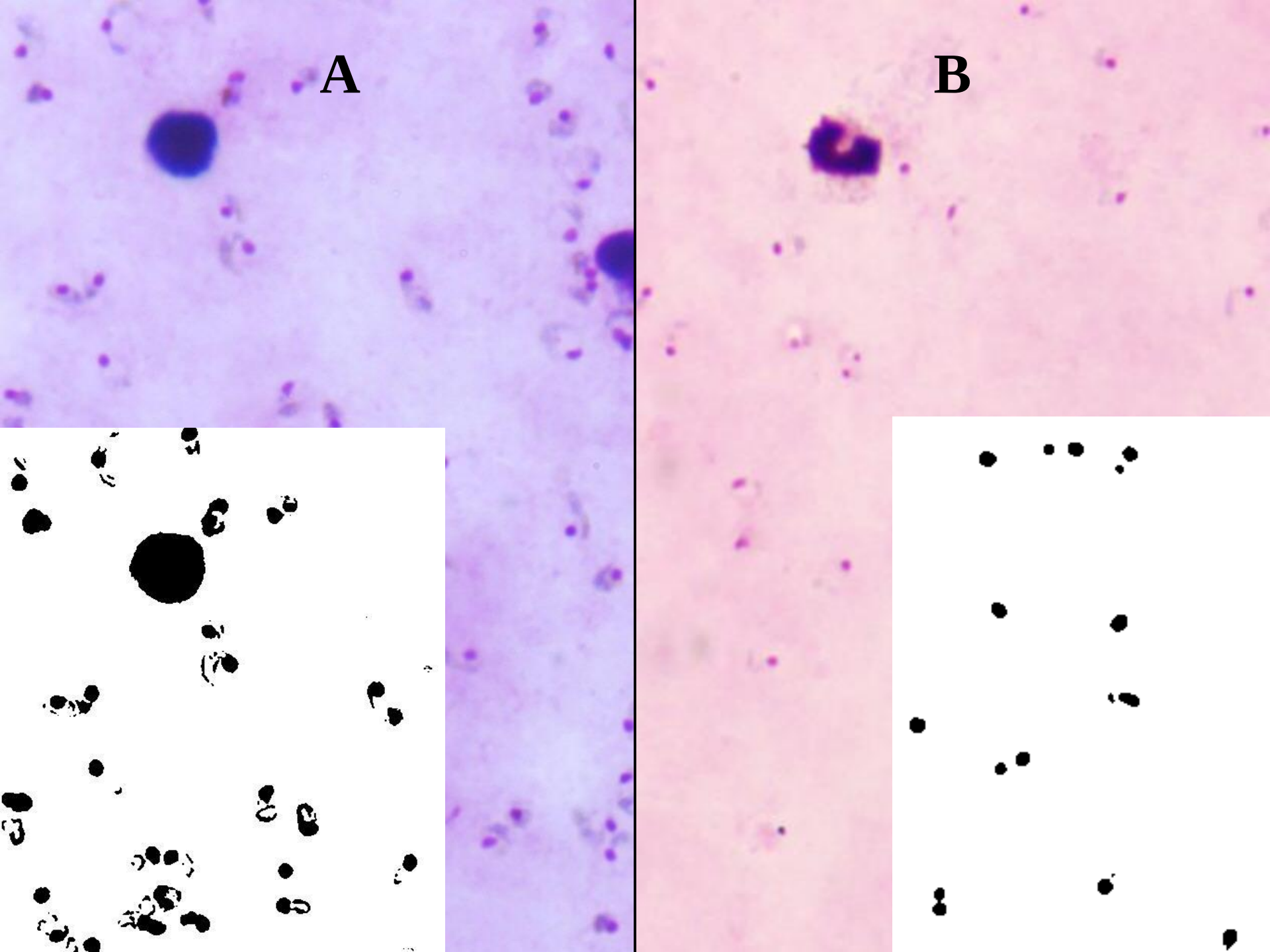
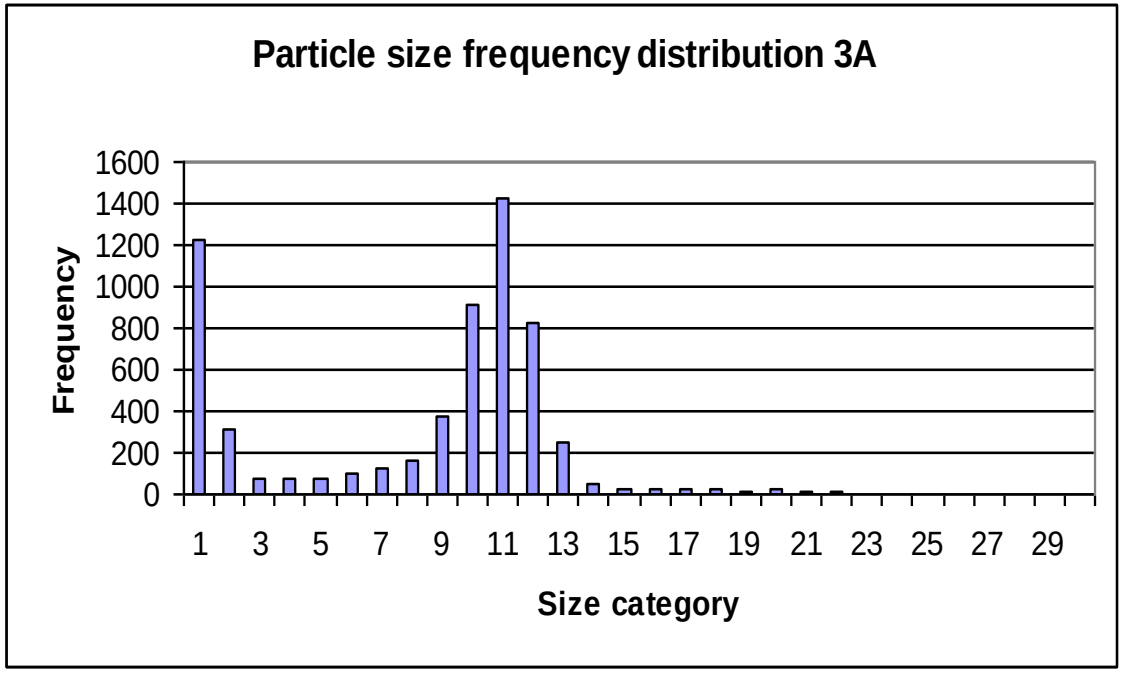

A
B

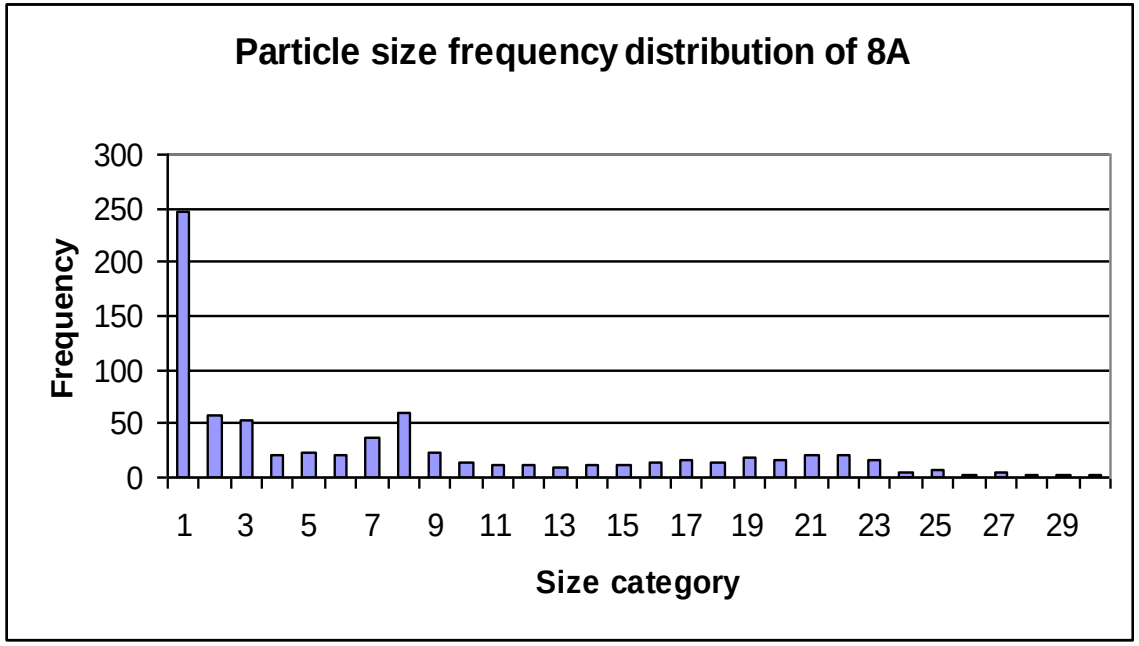
Younger parasites, higher counts

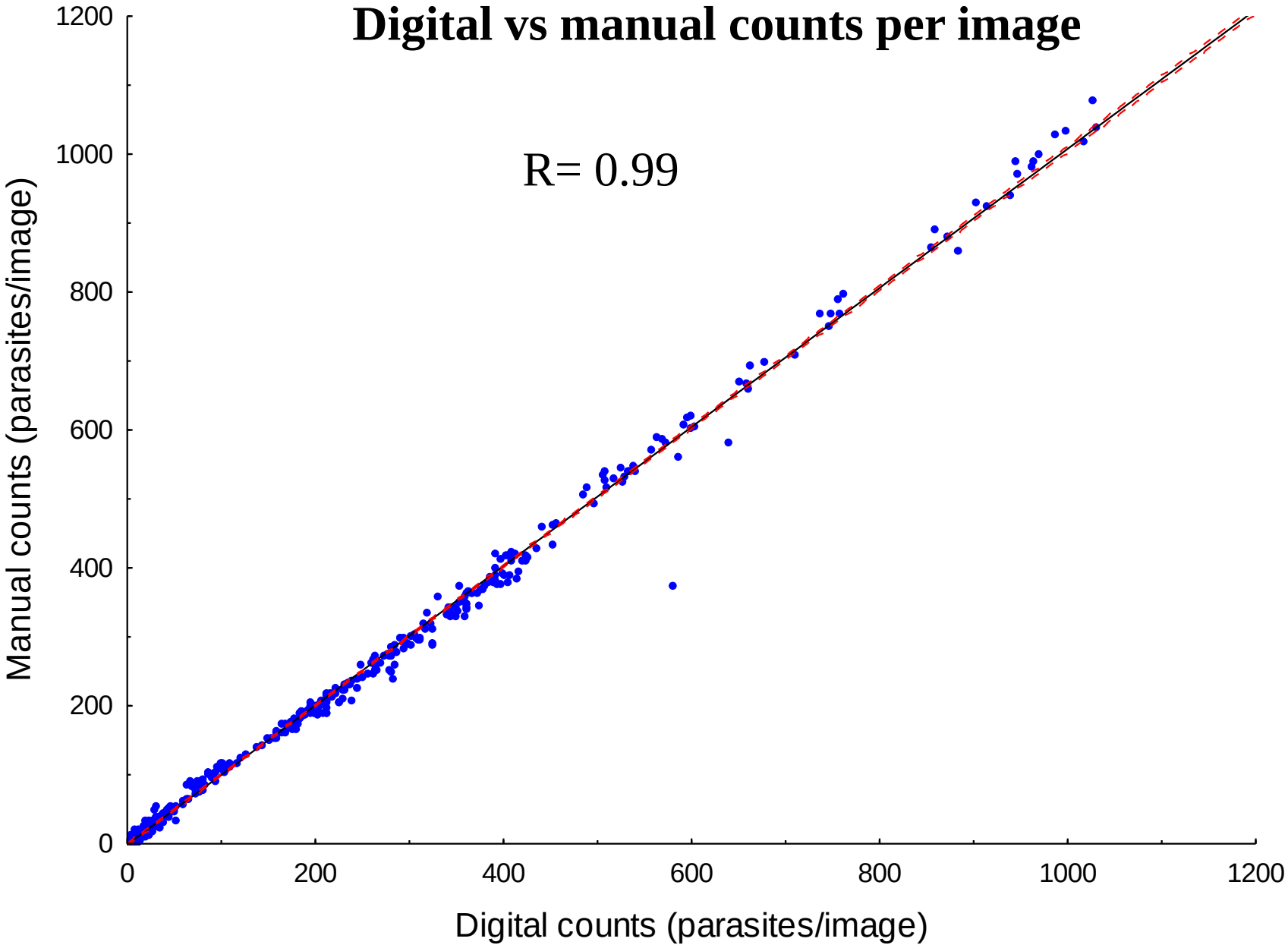
High S/N ratio;
Sk=2.27, K=4.33,
K/Sk=1.9, RN=5.2



Older parasites, lower counts

Low S/N ratio;
Sk=4.56, K=22.9,
K/Sk=5.02, RN=7




Digital vs manual counts per image
R= 0.99
Manual counts (parasites/image)
Digital counts (parasites/image)
0
200
400
600
800
1000
1200

Conclusion

- Simple, highly reliable for moderate to high counts
- Eliminates need for thin smear counts
- Does not replace conventional counting for low parasite densities
- Image capture is rate-limiting step
- Solution: automation!

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