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Implementation of Neodymium Acetate

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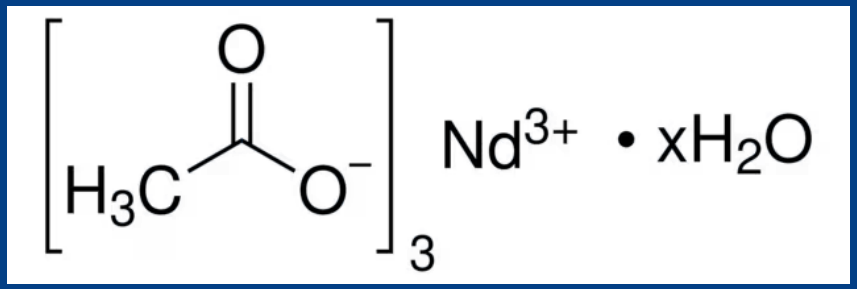
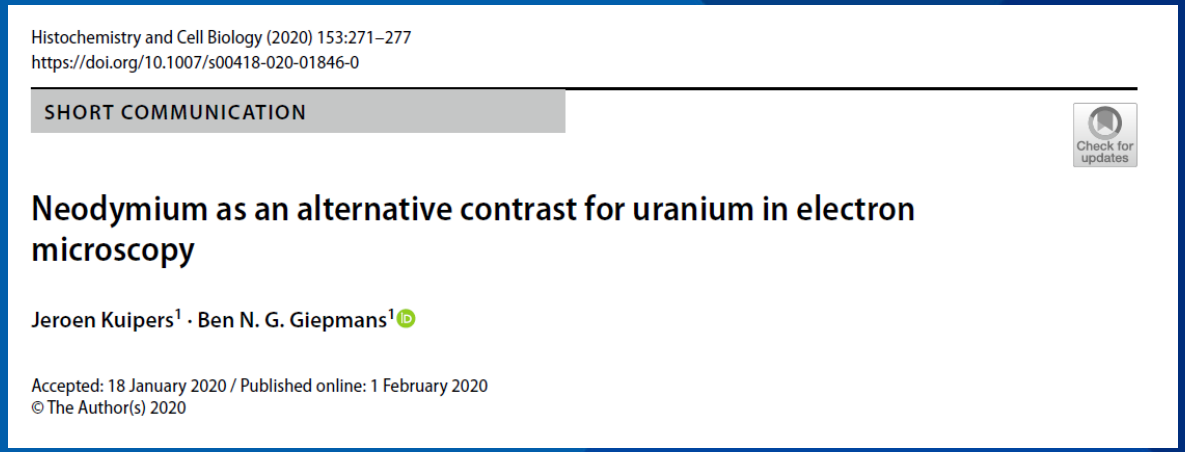
Neodymium (III) Acetate (NdAc)



Neodymium (III) acetate hydrate

- Neodymium is a lanthanide
- Situated above Uranium on the periodic table
- Proposed as an alternative as similar properties to uranium

- Journal demonstrated similar contrast to Uranyl acetate when used en block and via grid staining



Histochemistry and Cell Biology (2020) 153:271–277
https://doi.org/10.1007/s00418-020-01846-0

SHORT COMMUNICATION

Check for updates

Neodymium as an alternative contrast for uranium in electron microscopy

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Our labs experience of NdAc

- Make up working solution and optimise contrasting for TEM staining
- Verify use en block and via grid staining with multiple tissue types
- Stability test solution for future use

NdAc solution

- Tested 4%, 6%, 8% and 10%
- Difficult to dissolve stronger solutions and lots of precipitate

- 4% Methodology
  - Add 16g Neodymium (III) acetate in 400ml ultrapure water
  - 60 degree hotplate, stirring for at least 10 minutes or until fully dissolved
  - Filter when cooled

NdAc contrasting

4% NdAc grid staining

Protocol:

1. Stain grids with NdAc for 10 minutes
2. Rinse in 6 changes of ultrapure water
3. Stain grids with Lead Citrate for 5 minutes
4. Rinse in 6 changes of ultrapure water
5. Blot grids dry

4% NdAc en bloc

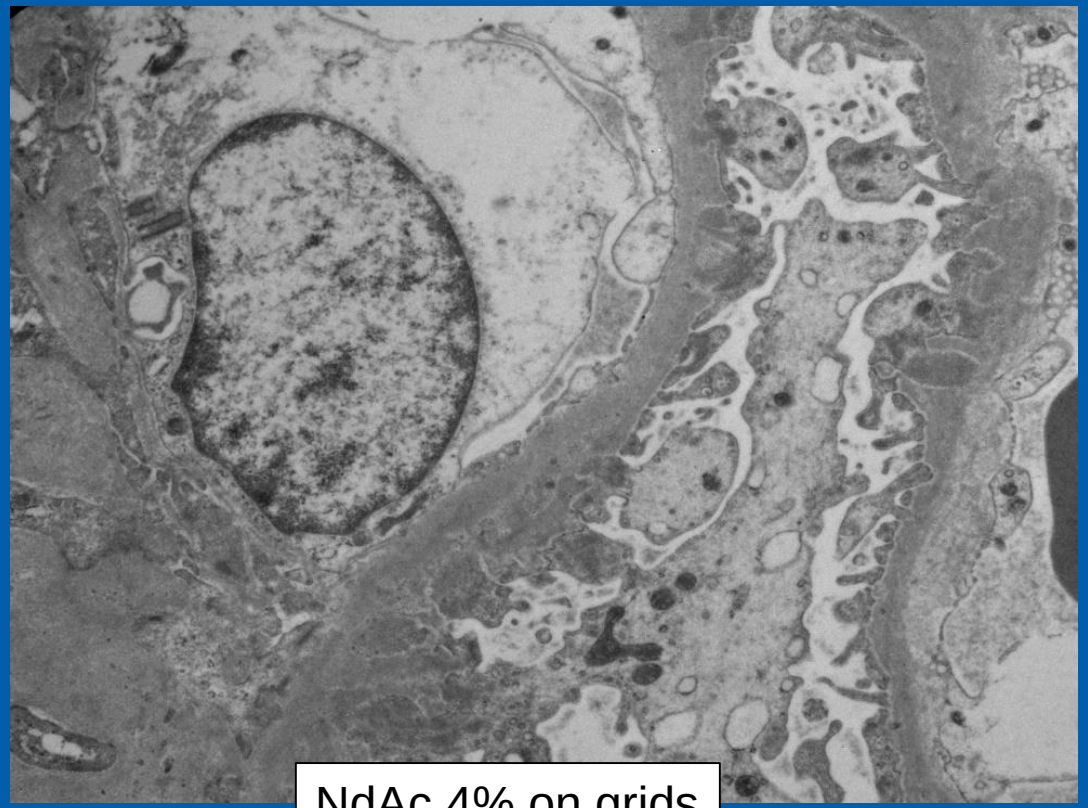
Protocol:

4% NdAc step to automated processing added after osmium and washes

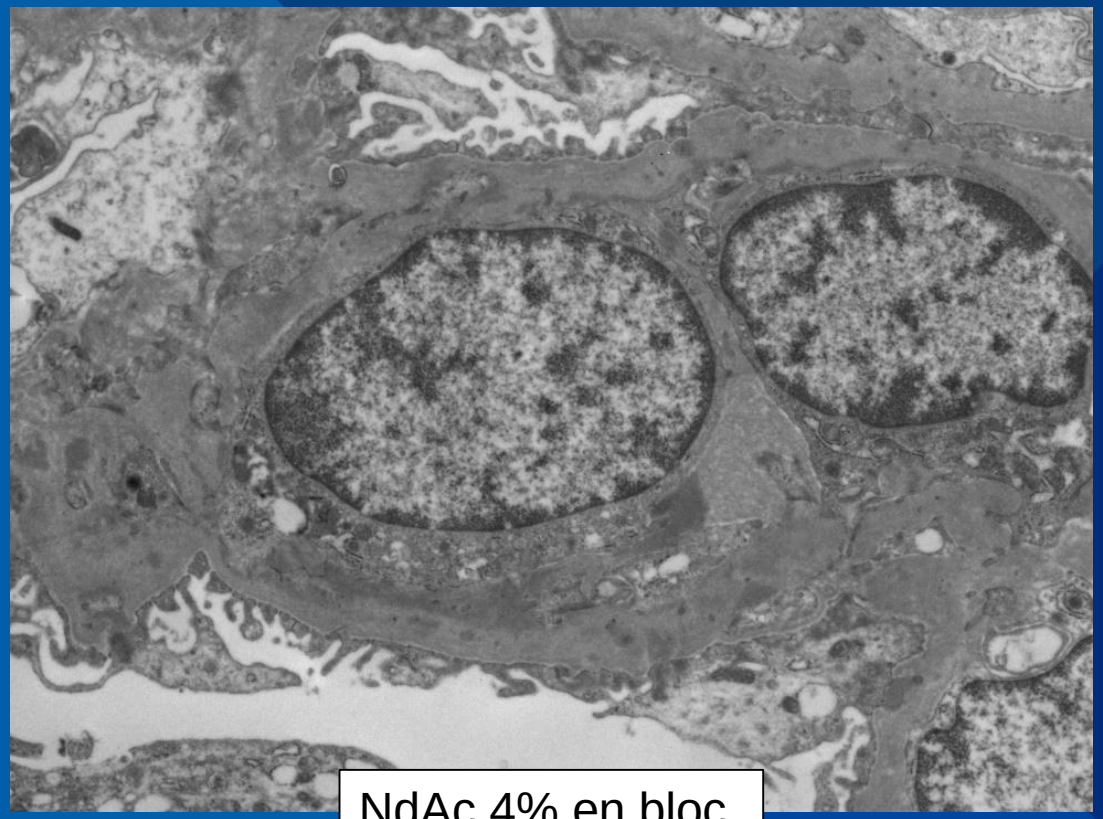
- 4% NdAc 30mins
- X2 wash steps in ultrapure water

- Grids stained with Lead Citrate for 5 minutes

Our lab experience of NdAc Results

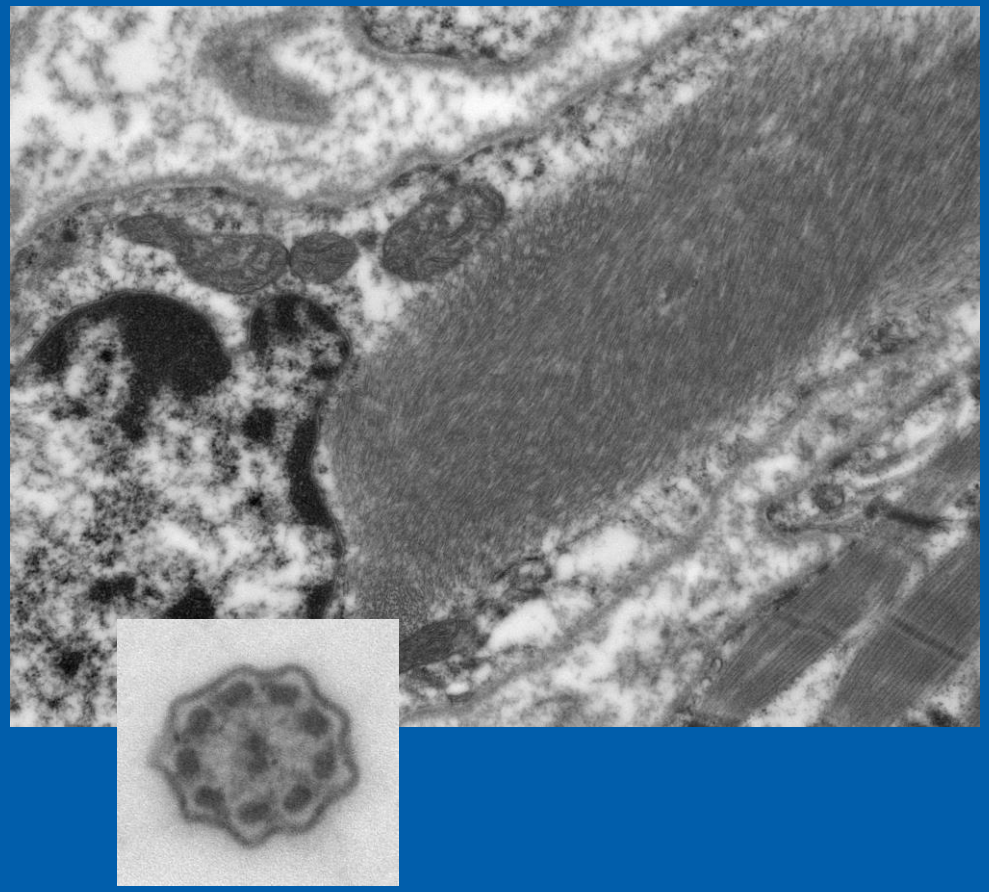


NdAc 4% on grids



NdAc 4% en bloc

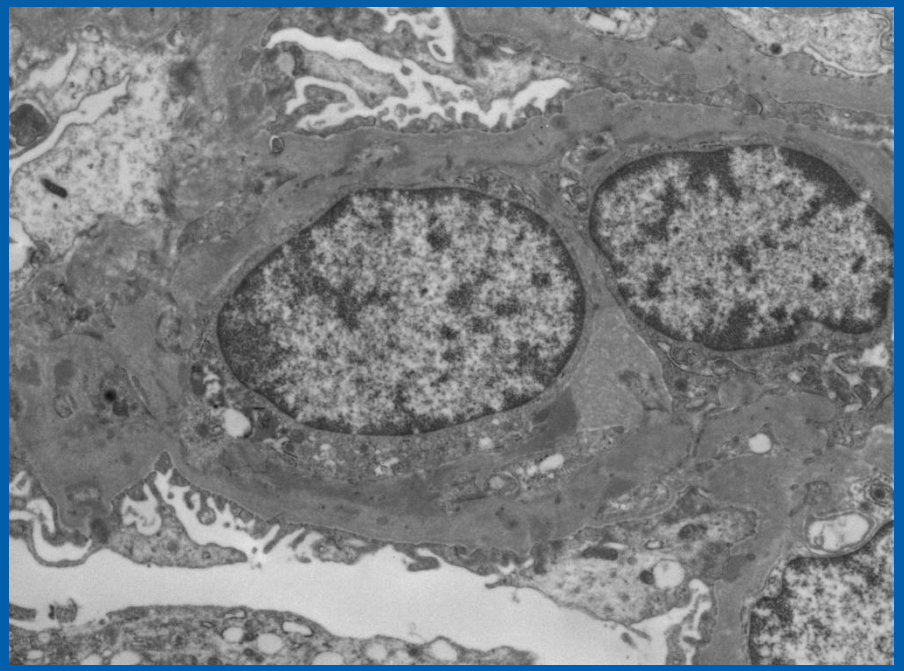
TEM images



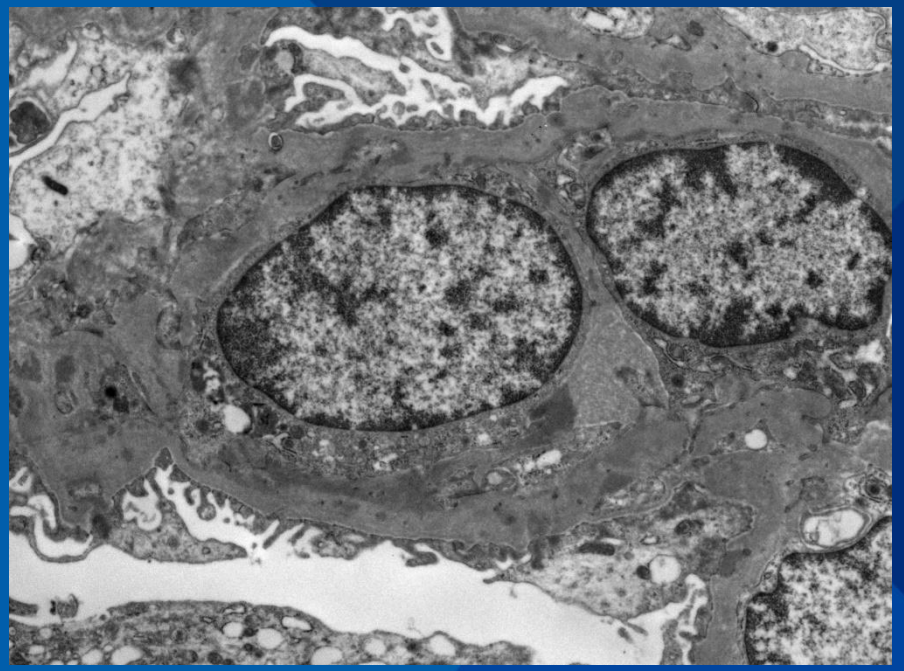
- Contrast acceptable, not as good as original UA
- En bloc better than on grids
- Digital enhancements improve overall image contrast without losing too much detail
- NdAc acceptable for all tissue types

Our lab experience of Nd Results

4% en block without enhancements



Alter black/white histogram, adjust gamma and sigma



Stability testing

- Tested after 2 weeks and 4 weeks
- Acceptable staining (according to criteria)
- No significant precipitate

- Make up enough for 1 month, then dispose left overs

- Spare solution stored for 6 months - test to extend stability date

UK NEQAS EQA results

- Evaluation service for method change

- 8 for renal image stained on grid (+ lead)
- 9 x2 for renal and muscle image stained en block (+lead)

- Comment for 8 was ‘too little image contrast applied’
- Good scores, can always try to improve

Life after implementation…..

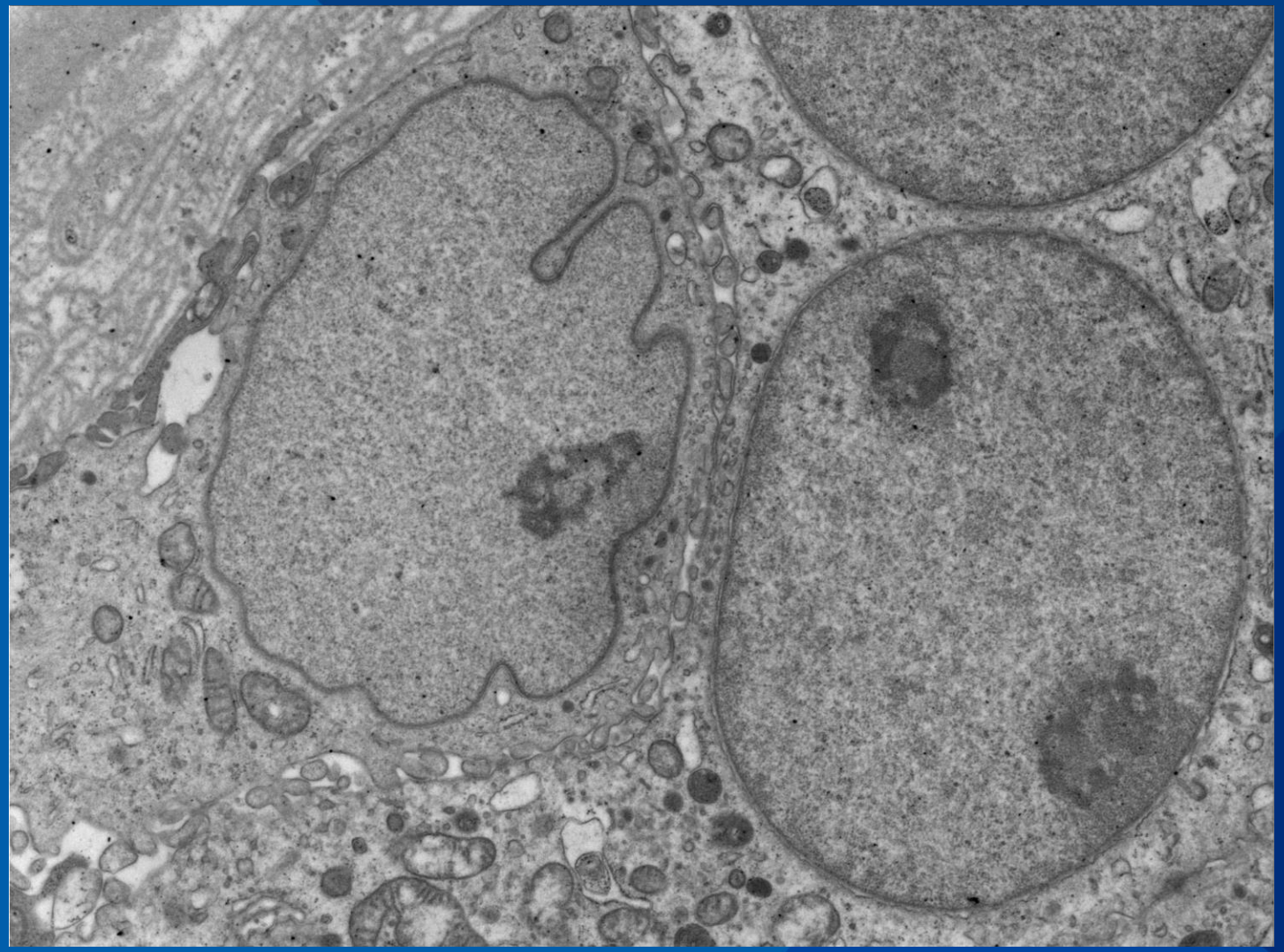
- Used from March 2024
- En bloc working well
  - Wet tissue
  - Blocks from external sources
    - not had en bloc contrasting
    - require staining on grid

Not perfect with grid staining

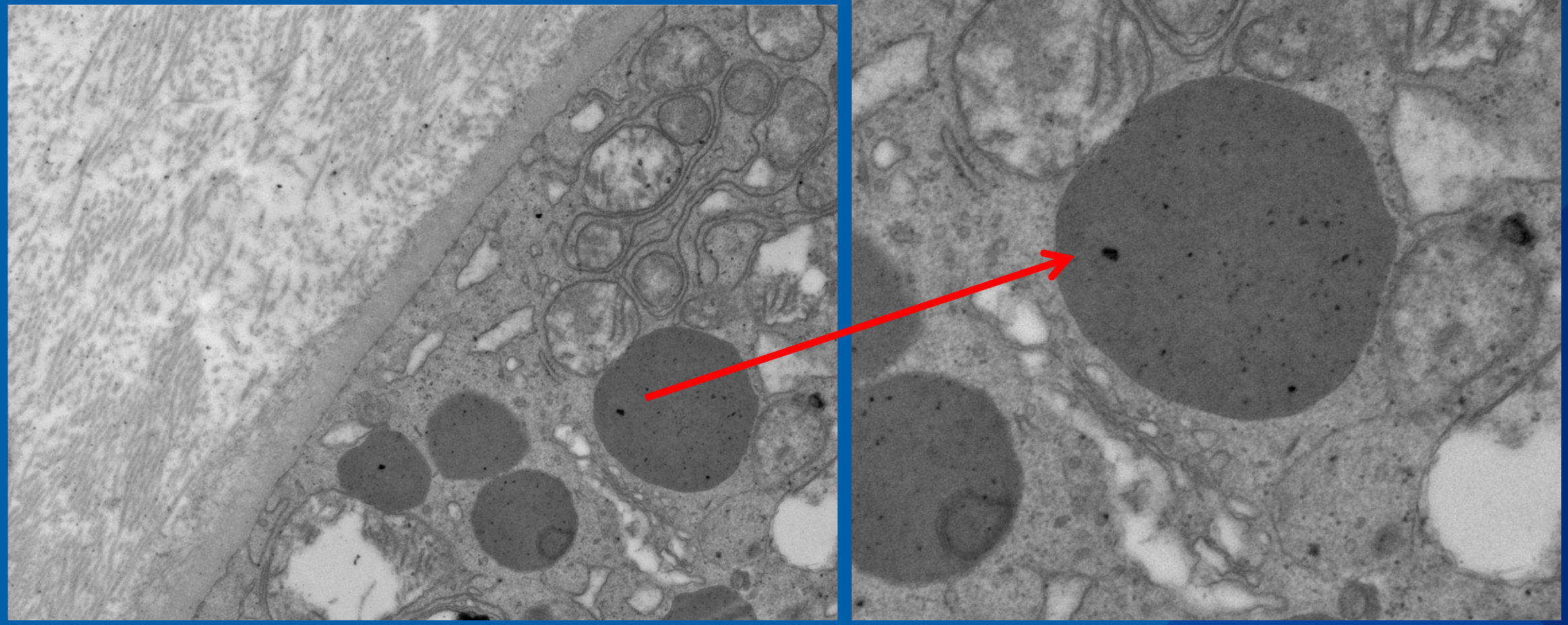
  - precipitate

NdAc precipitate

- Fine
- Barely visible until x4000 mag



NdAc precipitate



Currently…..

- Investigating ways to minimise precipitate
  - Perfect making up solution - properly dissolved, heated enough
  - Filtering
  - Extra washing
  - Validate 3% NdAc (?extend staining time with weaker solution)
- Plan to validate stronger osmium to enhance contrast further

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Thanks

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