

Linda Graham, Anatomical Pathology, LabPlus.

Milestone's KOS Microwave Trial

- **2.5 day training by Jim Milios**
- **PROCURE 812 test runs**
- **LR White test runs**
- **1 mm³ tissue: tumour**

Renal PM*

Renal biopsy

- **Large tissue format:**
- Muscle**
- Nerve**

*PM tissue used for renal trials until satisfactory outcome of initial runs; portions of current biopsies used at end of trial.

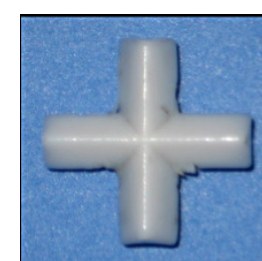
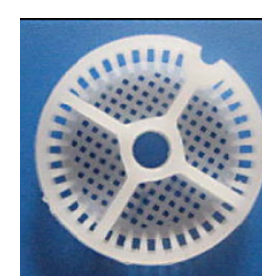


MATERIALS

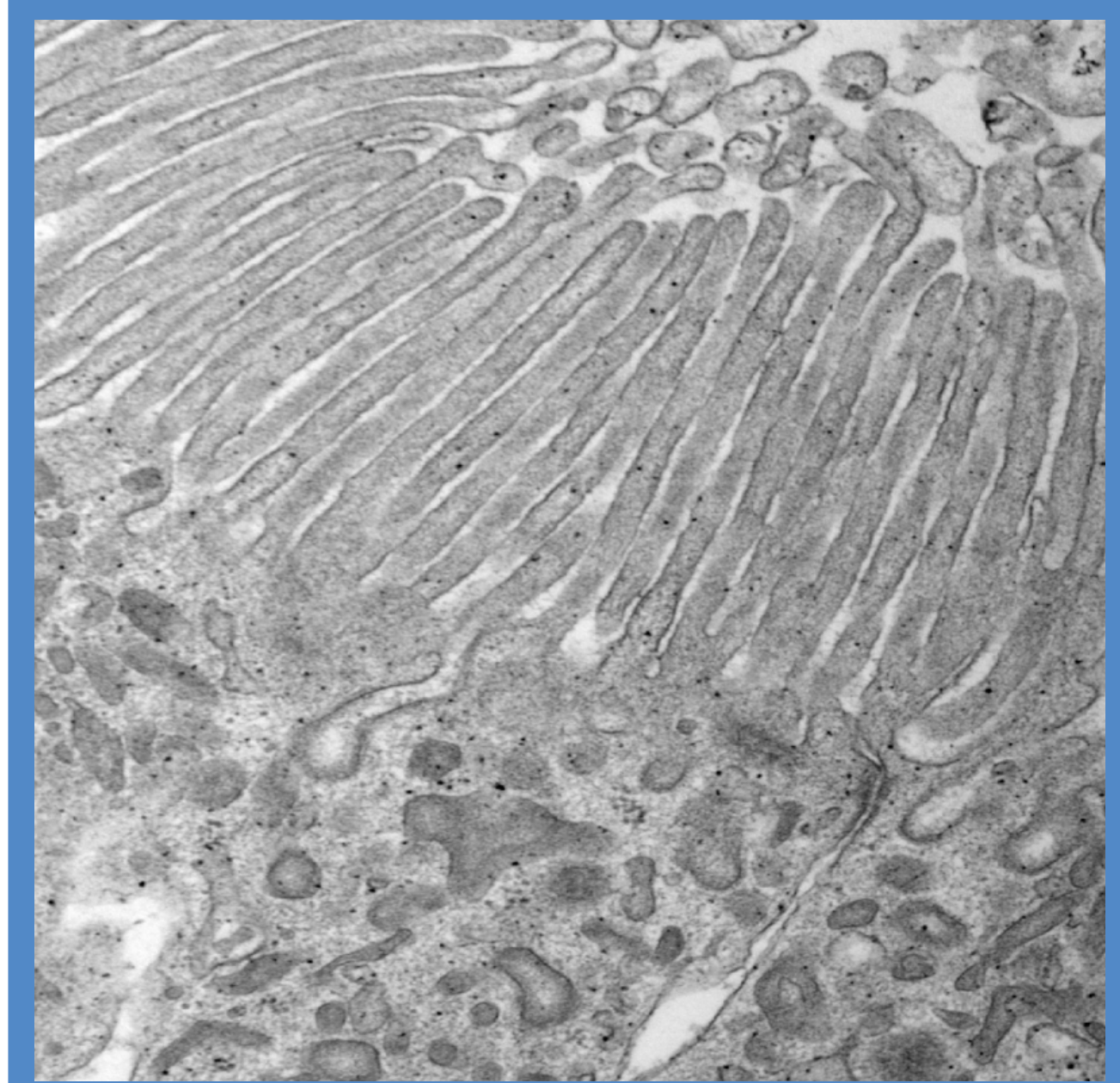


spindle

basket



stir bar



Rinse step included

Renal proximal tubule

*Post osmium rinse step omitted from later trial runs due to osmium precipitate

Introduction

LabPlus EM trial of the KOS microwave commenced in March 2012 and trials continued for another 6 weeks after the initial training. The most time consuming aspect was the loading & unloading of tissue into the processing baskets. Hopefully this is something that would speed up with more practice.

At this point in time only the small tissues have been viewed on the TEM. Only a few LR white processed blocks had curing & sectioning problems. Preliminary results show improved infiltration (& consequently improved sections) in larger tissues.

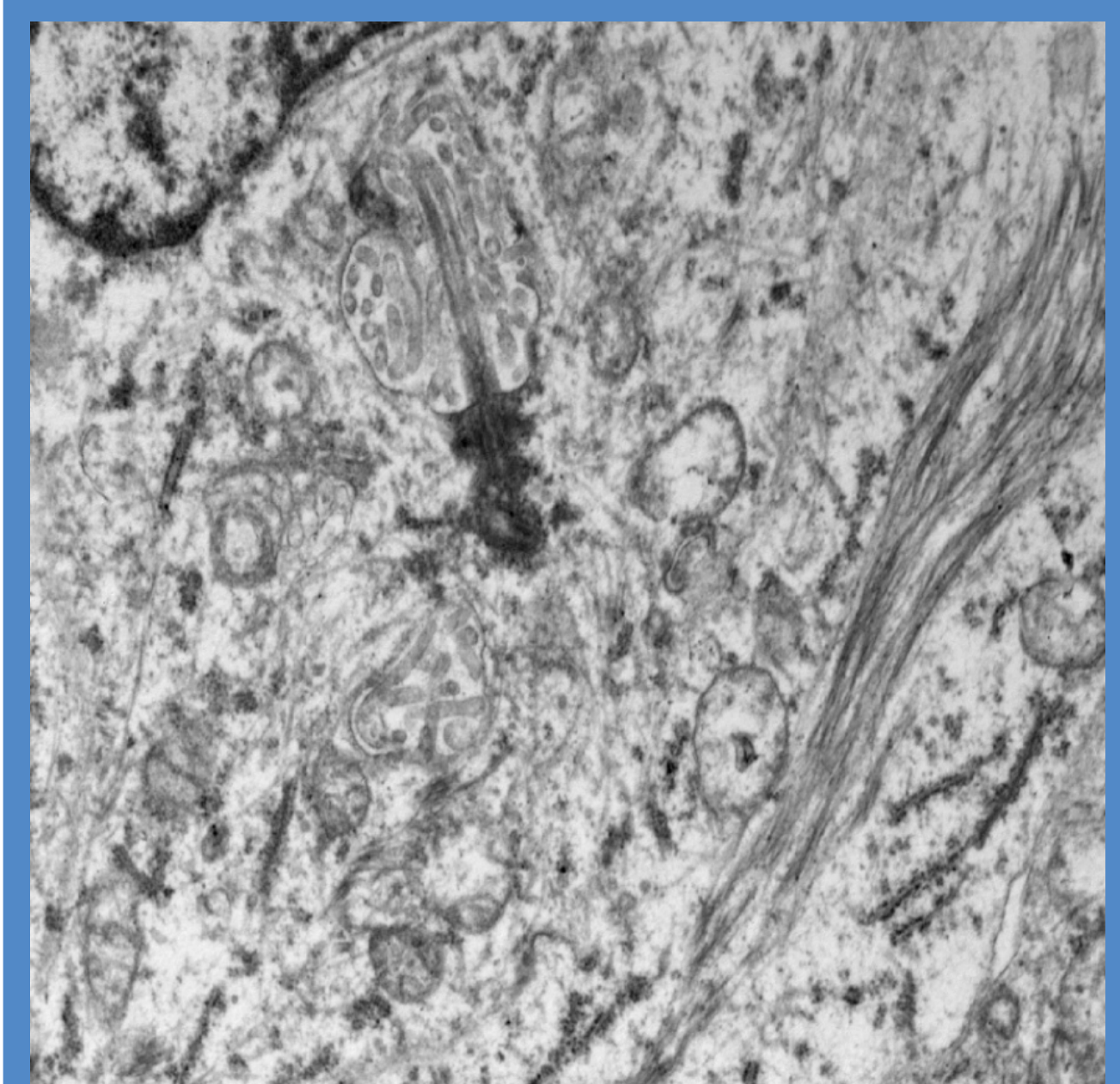
EPOXY RESIN PROCESSING PROTOCOLS

Standard Protocol	Microwave Protocol
Glutaraldehyde (fixation prior to arrival in EM)	Glutaraldehyde (fixation prior to arrival in EM)
Buffer rinses 3 x 5 mins	Buffer rinses 3 x 5 mins
Osmication 30 mins	Osmication 10 mins (3 x 3 min rinses in water)*
Dehydration 7 x 5 mins	Dehydration 7 x 5 mins
50:50 Infiltration 30 mins	50:50 Infiltration 20 mins
75:25 Infiltration 2 hrs	75:25 Infiltration 45 mins
100% Infiltration overnight	100% Infiltration 60 mins
3 hrs 50 mins + overnight	3 hrs 5 mins

Afternoon microwave processed tissue could then be cured overnight in a conventional oven as usual or could be microwave cured (under water) for 1 hour 45 minutes.

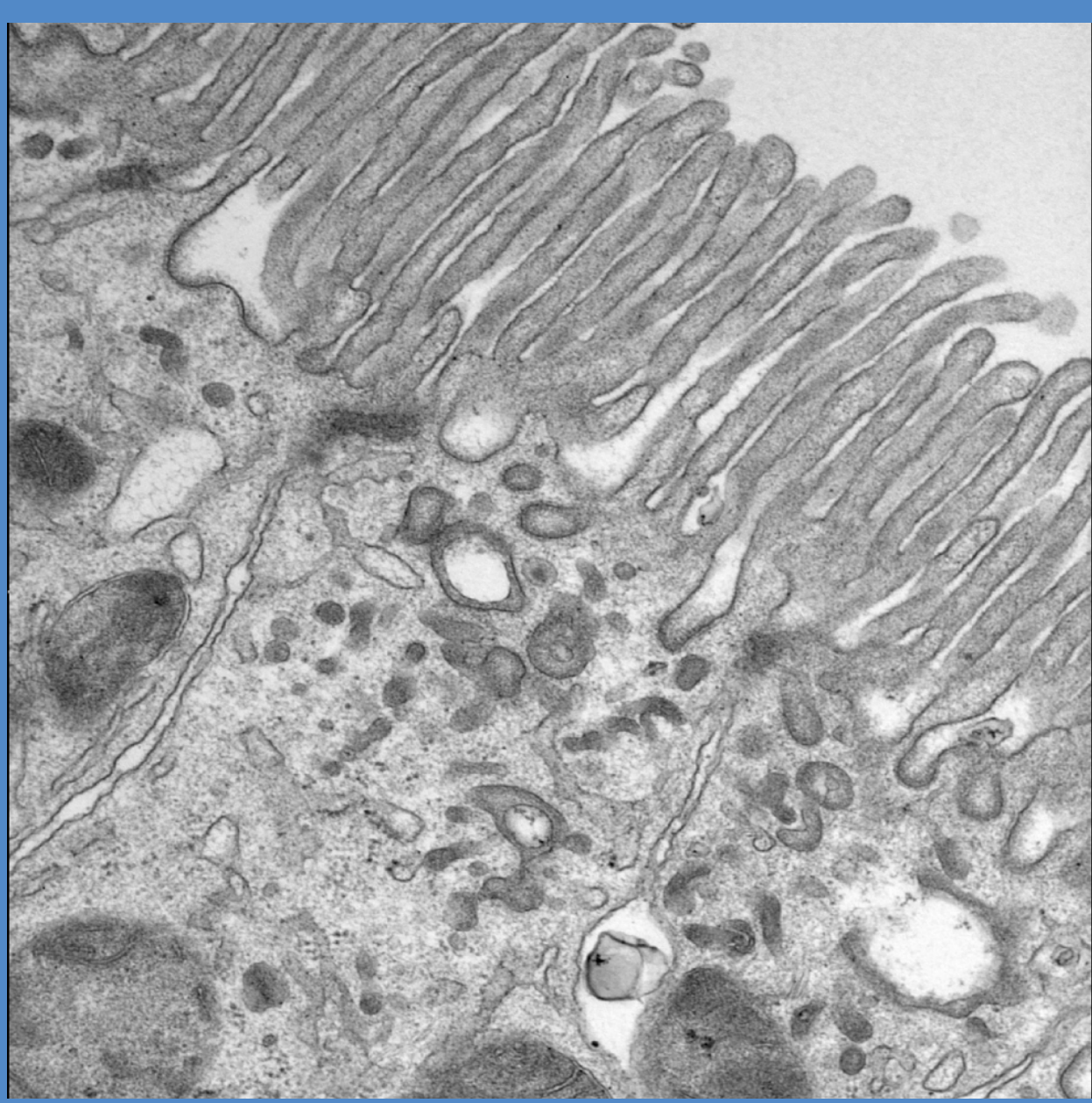
Conventional Oven Cured

Overnight



Ependymoma

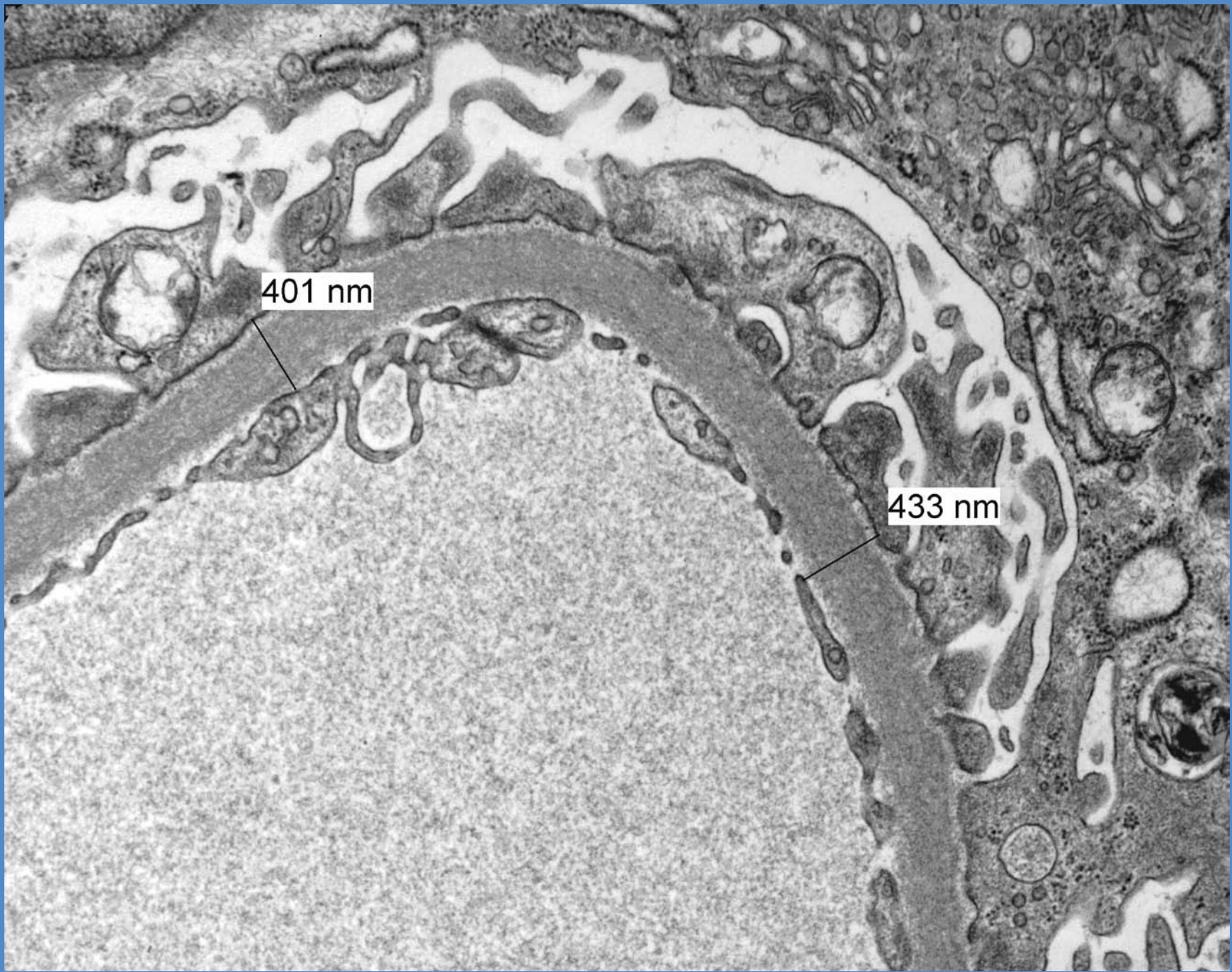
Ultrastructurally there is no difference in the microwave processed tissue compared to our standard processing.



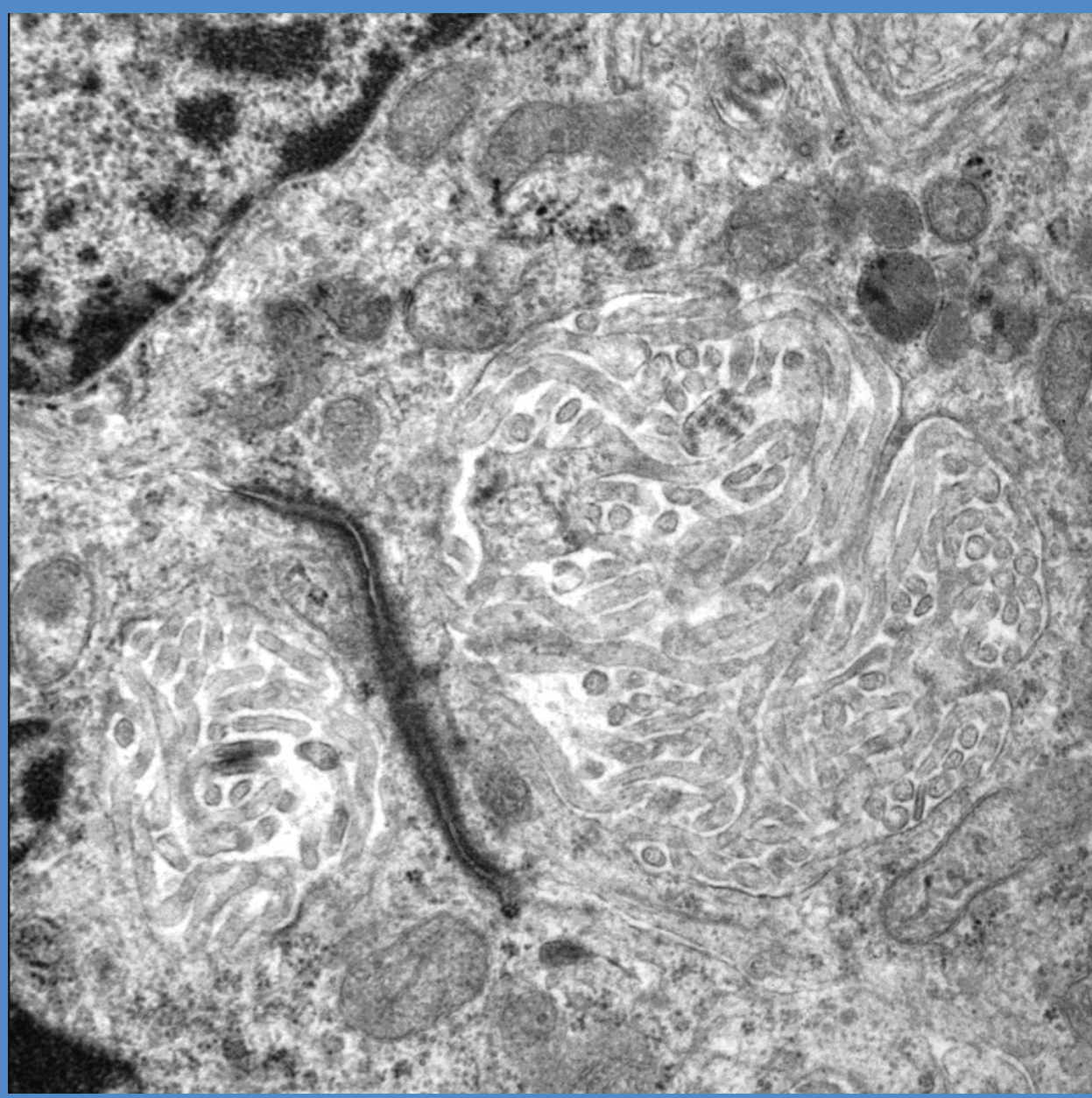
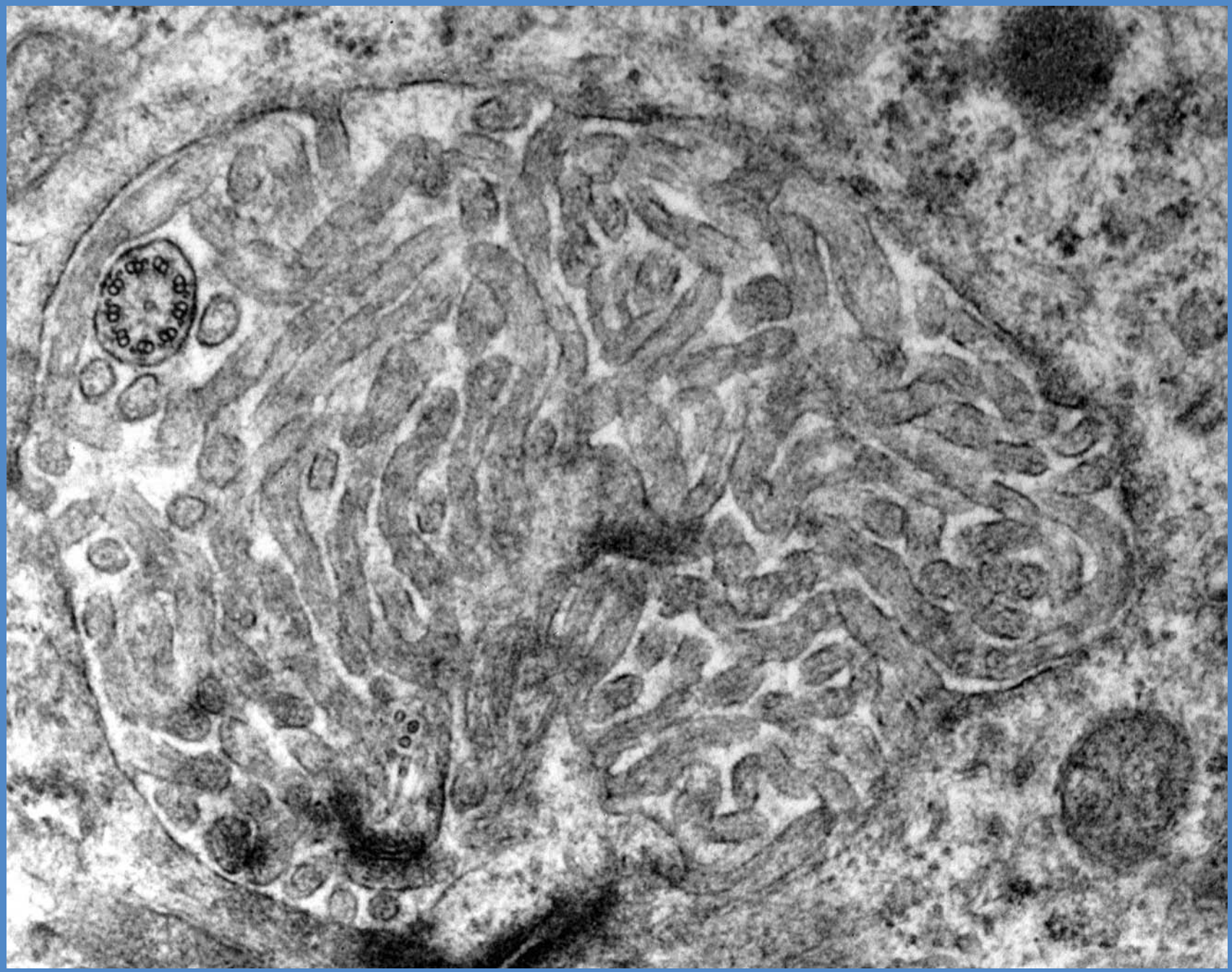
Rinse step omitted

Renal proximal tubule

which was eliminated in the tissue taken directly to 50% ethanol.



Both the renal capillary and the ependymoma tumour to the left were microwave processed.



Ependymoma

Results

Our standard staining protocols were used without modification and extracellular elements were also readily visualized.

Conclusions

- Microwave processing using our current reagents gives high quality results and could potentially improve our large tissue results.
- Same day processing of tissues that arrive prior to mid-day is achievable. Currently this would be between 1/3rd and 1/2 of renal biopsies in our laboratory.
- Daily processing would reduce TATS generally as our current practice of processing 2 x per week gives on average a 1.6 day delay between arrival of tissue and start of processing.
- In addition, microwave processing in the afternoon and overnight curing in a conventional oven would reduce processing TATs by one full day.
- This would definitely be an advantage for urgent specimens and would potentially bring routine EM results into the same timeframe as histology reports.

ACKNOWLEDGEMENTS

- I am indebted to Shirley Ling of ABACUS ALS for the KOS Microwave & components images and to Jim Milios of Milestone for adapting our standard processing protocol to the KOS microwave and for vital training.
- All LabPlus EM unit staff for sectioning & staining; Steve Cooke, Jaswin Narayan, Martin Michaels.