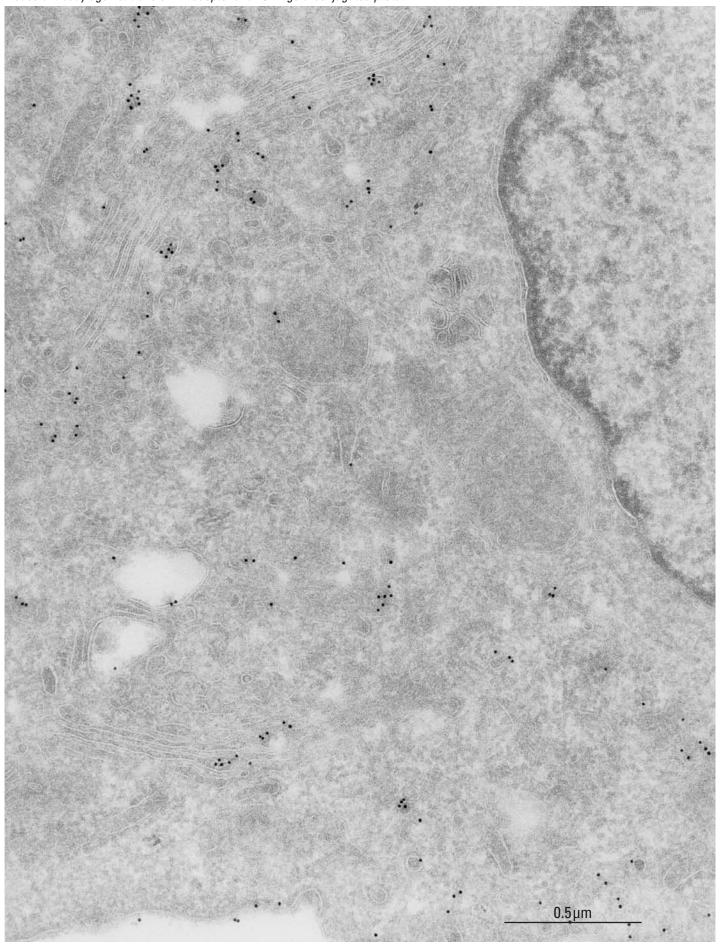


Image front page:

Cryo-section of rat pancreas. Golgi area in an exocrine cell, double immuno-labeled for the coat protein COP II (15 nm gold) and the SNARE protein rBet 1 (10 nm gold). JW. Slot, Cell Biology. University Medical Centre Utrecht.

Peter J. Peters and Erik Bos, Netherlands Cancer Institute, Amsterdam. Ultrathin cryosection of fixed rat tumor cell (9L3.9) incubated with mouse antibody against transferrin receptor and 10nm gold-conjugated protein A.



Introduction

In 1981 Diatome was the first manufacturer to introduce a diamond knife especially developed for cryo techniques.

In the meantime major advances in immuno-cytochemistry and for the sectioning of frozen hydrated specimens have been realized using our cryo diamond knives (1, 2, 3).

In 1999 we presented the cryo P knife with the epoxi platform. The improved version of this knife type, the *cryo immuno*, has been developed in collaboration with reputed cryo ultramicrotomists for the Tokuyasu technique.

In immuno-cytochemistry it has been found that a considerable reduction of structural damage in tissues and cells can be obtained with a modified pick-up method using sucrose/methyl cellulose (4, 5, 6).

Our diamond plateau allows an easy and gentle section pick-up. The sections are collected now directly from the platform with a loop and sucrose/methyl cellulose droplet.

This method reduces the stress applied to the sections and leads to better structural preservation.

References

Ref 1: H. Sitte: Advanced Instrumentation and Methodology related to Cryoultramicrotomy: A Review. Scanning Microscopy Supplement

Scanning Microscopy Supplement 10, pp. 387-466, 1996.

Ref 2: M. Michel, H. Gnägi and M. Müller: Diamonds are a cryosectioner's best friend. Journal of Microscopy, Vol. 166, Pt 1, 43-56, 1992.

Ref 3: K. Richter: Cutting artifacts on ultrathin cryosections of biological bulk specimens. Micron, Vol. 25, No. 4, pp. 297-308, 1994.

Ref 4: K. T. Tokuyasu: A technique for ultramicrotomy of cell suspensions and tissues. Journal of Cell Biology, Vol. 57, pp. 551-565, 1973.

Ref 5: W. Liu, H. J. Geuze, J. W. Slot: Improving structural integrity of cryosections for immunogold labeling.

Histochemistry and Cell Biology, Vol. 106, pp. 41-55, 1996.

Ref 6: P. J. Peters: Cryo-Immunogold Electron Microscopy.
In Current Protocols in Cell Biology (J.S. Bonifacino, M. Dasso, J.B. Harford, J. Lippincott-Schwartz and K.M. Yamada, eds) pp. 4.7.1-4.7.12, 1999. John Wiley & Sons, New York.

Ref 7: E. van Donselaar. Cell Biology. University Medical Centre Utrecht: Personal communication.

Specifications

Knife angle: 35°

Cutting edge length: 3mm

Cutting range: 30nm-1µm

Order no: DCIMM3530



Handling and Use

- 1 Start sectioning with the cryo immuno knife: ionizer on Pos. 10, distance of electrode knife approx. 10mm.
 After 2 3 sections: distance of electrode approx. 25mm.
- 1a If sections lift up from the knive surface: reduce voltage.
- 1b If sections stick at the cutting edge: increase voltage.
- 2 Use special care when pickingup the sections: Do not touch the cutting edge with any solid objects.
- 3 Remove knife from the chamber (before heating up!).

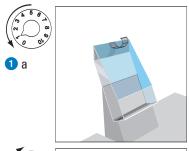


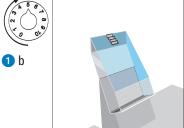
- Bevel one of our polystyrene rods to an angle of approx. 60°.
- Dip in ethanol 50% and shake off the excess. Gently run the rod across the cutting edge without applying lateral pressure.
- If sections or debris dry on the knife edge:
 - Place the knife in a 2% Decon solution (7) for a few hours, rince with tap water.
 - Clean with the polystyrene stick and distilled water
 - Clean with the polystyrene stick and 50% ethanol

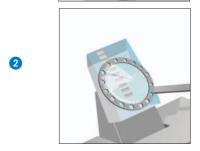


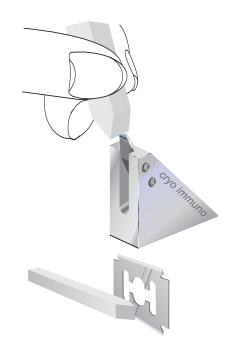
can be found in our publications:

- "Static Line II brochure".
- Flyer "Perfect Loop"
- Flyer "Trimming Blades"
- Video "cryo trimming" and "cryo sectioning", P. Peters, Netherlands Cancer Institute, Amsterdam











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