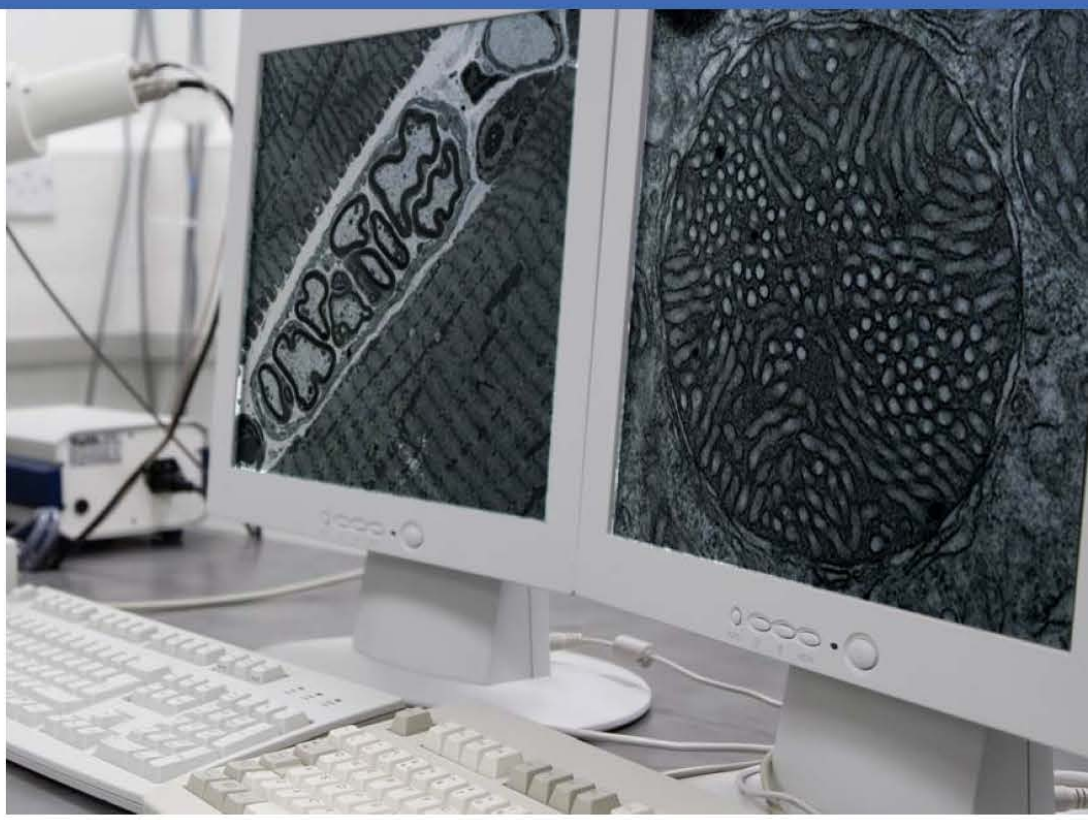
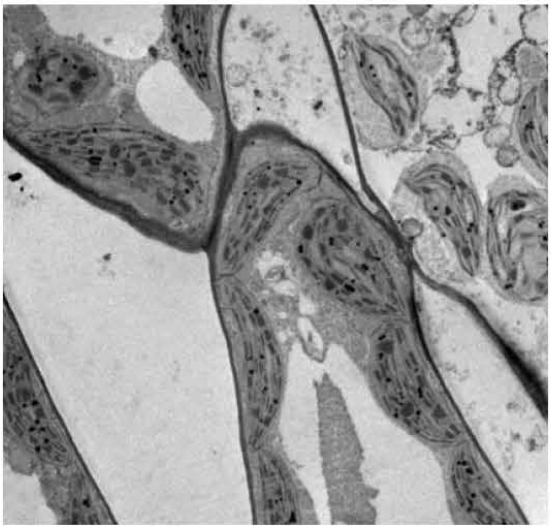


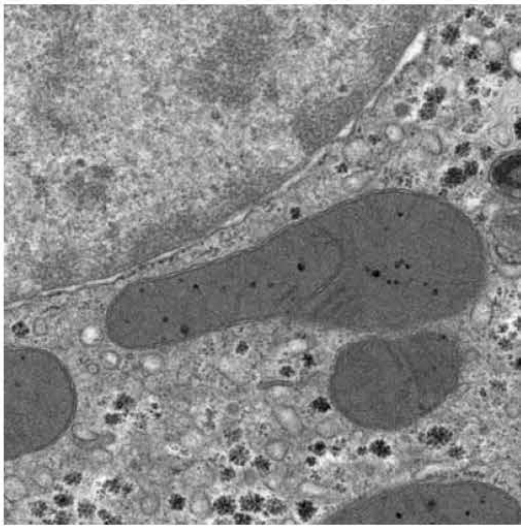
Introducing...

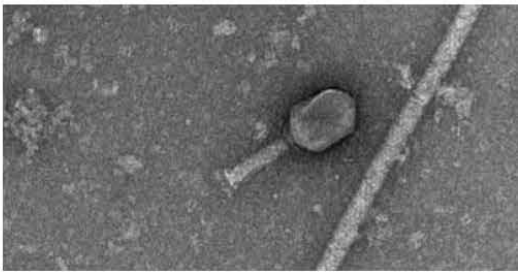
UranyLess

EM STAIN

A Substitute for Uranyl Acetate







DELTA MICROSCOPIES

A Substitute for Uranyl Acetate...

UranyLess EM Stain

EMS is proud to introduce UranyLess, a new contrast stain solution for TEM, for all of your negative staining applications. It is an amazing substitute for Uranyl Acetate with similar results.

After only a minute of contact, UranyLess' fast-acting, non-radioactive lanthanide mix is finished staining your sections or deposits (see protocols below). If needed, lead citrate is recommended to increase the contrast.

UranyLess's pH level is about 6,8 to 7. The 30ml airless bottle will stain approximately 1500 grids. The airless bottle increases the shelf life, eliminates CO_2 contamination, and produces less waste — the solution pumps out in perfect amounts without leaking or spilling. UranyLess is also available in a larger amount for use in automated staining equipment. When using UranyLess for automated staining, do not wash longer than 10 minutes or you run the risk of losing all contrast.

UranyLess has been tested on many biological tissue (animal and plant): intestine, skeletal and cardiac muscle, liver, kidney, adrenal gland, nerve, cell culture, plant tissue, and also on negative staining of bacteriophage, bacteria, and polymers. UranyLess is ideal because of its ability to stain any kind of material and results are reproducible.

30ml Airless Bottle

How does an airless bottle operate?

Its use is very simple; simply push on the head of the bottle to get a drop. When you release, the bottle back pump actuator lifts up. It prevents any air inlet in the bottle.

What is the advantage of an airless bottle?

It is a bottle in which air never enters. Some products, such as lead citrate, are atmospheric CO_2 sensitive. Thanks to this system, those products have a longer shelf life. It also allows the product to be deposited drop by drop, quickly, cleanly and in any position.

References

"Easier and Safer Biological Staining: High Contrast UranyLess Staining of TEM Grids"

1. Delta Microscopies, 22, B route de saint Ybars, La côte blanche, 31190, Mauressac, France
2. Université Toulouse, CMEAB Faculté Medecine, 118 route Narbonne, 31062, Toulouse, France
3. Microscopy Innovations LLC, 213 Air Park Rd, Suite 101, Marshfield, WI, 54449, USA

"C-Nap1 mutation affects centriole cohesion and is associated with a Seckel-like syndrome in cattle." Nature Communications. Published 23 Apr 2015. Sandrine Floriot, all.

200ml Bottle

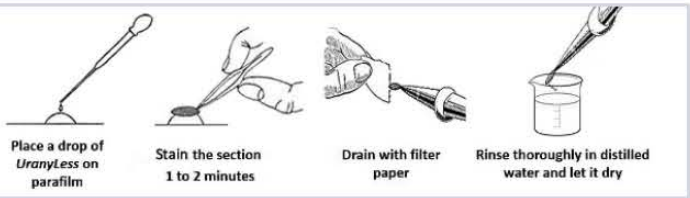
PROTOCOLS OF USE

Classic Contrast

This protocol is used for double staining with UranyLess/Lead citrate on ultrathin sections. This protocol is adapted to biologic samples that have been fixed with glutaraldehyde, osmium, or ruthenium and embedded in an epoxy type resin (Epon, Araldite, Spurr) or acrylic type (LRWhite, HM20).

Staining Protocol:

- Place a drop of UranyLess on parafilm or any other hydrophobic slide.
- Place the grid on the UranyLess drop for 1 to 2 minutes.
- Blot the grid on a filter paper and then wash in distilled water.
- Let it dry.
- After drying, go to the lead citrate staining according to Reynolds method (1963).
- Place the grid on the lead citrate drop according to the Reynolds method, for 1 minute.
- Blot the grid on a filter paper before rinsing with distilled water.
- Let it dry.



Technical Tip:

UranyLess is not air or light sensitive, unlike Uranyl Acetate.

After lead citrate, drain immediately in a freshly prepared distilled water bath or wash with 0.01N of NaOH solution.

If there is a precipitate in the solution, filter it prior to use.

If solution was refrigerated, allow solution to return to room temperature prior to use.

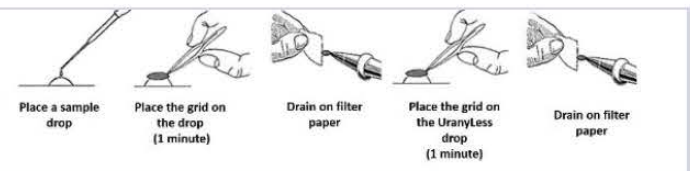
Do not keep lead citrate refrigerated.

Negative Staining

Negative staining is a very useful technique in electron microscopy. It allows characterization of isolated particles of morphology as bacteria, virus, protein, nanoparticles, liposomes, exosomes, etc.

Staining Protocol:

- On a piece of parafilm or any other hydrophobic carrier, place a drop of your solution (~10µl) and a UranyLess drop.
- Using our fine tweezers, place your sample drop on a formvar-carbon coated grid. for about 1 minute.
- Blot your grid using filter paper.
- Place your grid on the UranyLess solution for 1 minute.
- Blot, let it dry for 5 minutes and observe under the microscope.



Technical Tip:

If the staining is too intense, wash with water for 1 minute.

Ordering Information

RT	22409	UranyLess EM Stain*	30 ml
RT	22409-20	UranyLess EM Stain	200 ml

* in airless bottle

DELTA MICROSCOPIES

FREQUENTLY ASKED QUESTIONS...

What is UranyLess made from?
UranyLess is a solution ready for use, a mix of lanthanides (rare-earths).

How is UranyLess sold?
In an aqueous solution (water).

What is its shelf life?
One year.

What are the storage conditions for UranyLess?
Store it at room temperature away from direct sunlight.

Does it need to be diluted?
No, it is sold ready for use.

What is its pH?
UranyLess pH is 6.8–7.

How to stain with UranyLess?
Simply drop UranyLess on your grid, and wait a minute. Dry, then contrast with lead citrate according to Reynolds method.

Is it the same protocol for every kind of tissue (animal, plant, marine)?
Yes it is - a double stain of UranyLess plus Lead citrate.

Does it adjust to every kind of resin?
Yes, it operates with every kind of resin (Epon, Araldite, Spurr).

Can it be used on negative staining?
Yes, it can be used on negative staining.

Can it be used for bloc contrast?
Some tests are in progress.

Is it adapted to a cryo use?
No, because it is prepared in water. However, we are currently developing many formulations of UranyLess, including ethyl UranyLess and acetone UranyLess, the latter being the best-adapted to cryogenic use.

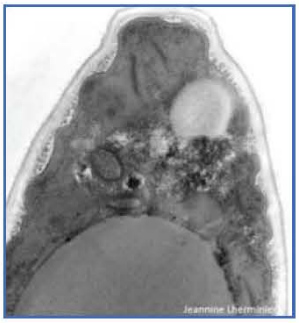
Is it efficient on marine material?
Yes.

How is UranyLess packaged?
We sell UranyLess in an airless 30ml bottle and also in a brown 200ml bottle.

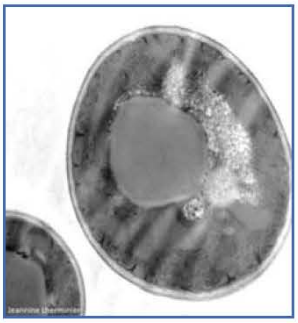
Can it be used with automated staining equipment?
Yes, the 200ml bottle is available for use with automated staining equipment. When using UranyLess for automated staining, do not wash longer than 10 minutes or you run the risk of losing all contrast.

UranyLess Applications
All photos taken on Transmission Electron Microscope.

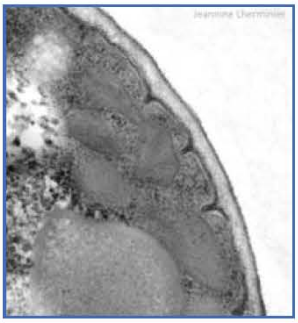
Yeasts, Preparation of the sample using the following protocol:
- Classic Fixation Glutaraldehyde - Osmium - Included in Epon
- Contrast the UranyLess monitoring Lead Citrate



Yeast. Photo: Jeannine Lherminier (INRA - Dijon).

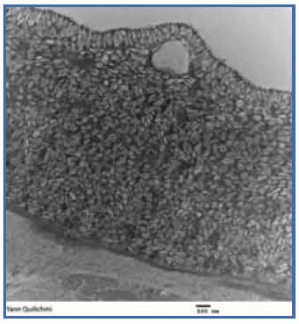


Yeast. Photo: Jeannine Lherminier (INRA - Dijon).

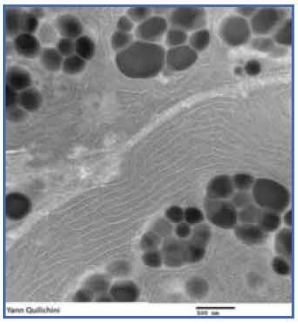


Yeast. Photo: Jeannine Lherminier (INRA - Dijon).

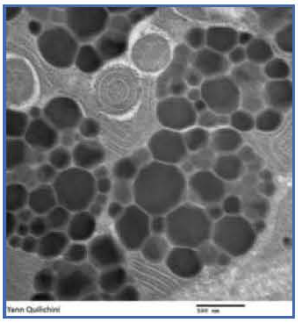
Trematodes, Preparation of the sample using the following protocol:
- Classic Glutaraldehyde Fixation, Osmium, Inclusion in Spurr Resin
- Contrast the UranyLess monitoring Lead Citrate



Trematodes. Photo: Yann Quilichini (Microscopy Platform of the University of Corsica - Corte)



Trematodes. Photo: Yann Quilichini (Microscopy Platform of the University of Corsica - Corte)

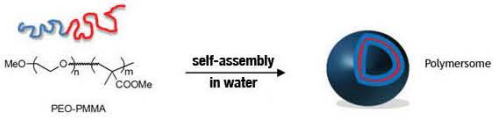


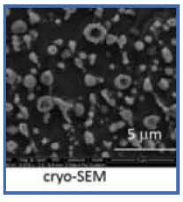
Trematodes. Photo: Yann Quilichini (Microscopy Platform of the University of Corsica - Corte)

Polymersomes

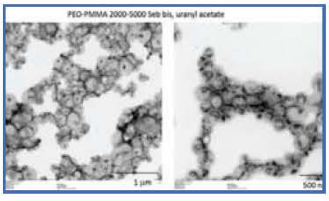
UranyLess was tested in comparison with uranyl acetate, which is at acidic pH 4 (seems to disrupt the organization of the molecular structure) in comparison also the comments by the technique Cryo SEM (scanning electron microscopy).

The chemical structure is organized as follows:

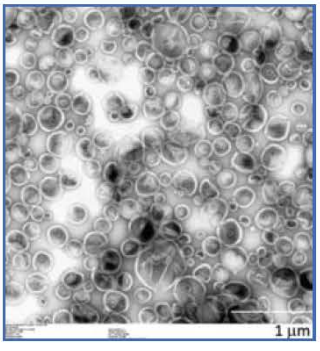




Polymersome, Observation Microscopy Scanning in Freeze Mode. Photo: The Toulouse Laboratory IMRCP, team Anne-Françoise Mingotaud.



Polymersomes, Negative Staining in Uranyl Acetate pH 4. Photo: The Toulouse Laboratory IMRCP, team Anne-Françoise Mingotaud.



Polymersomes, Negative Staining 1 mn in UranyLess in pH 7 Aqueous Solution. Photo: The Toulouse Laboratory IMRCP, team Anne-Françoise Mingotaud.

DELTA MICROSCOPIES

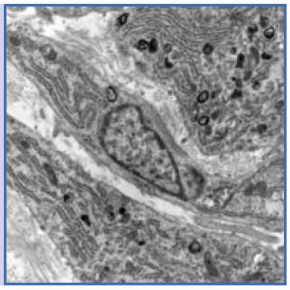
UranyLess Applications

All photos taken on Transmission Electron Microscope.

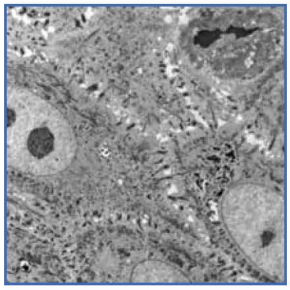
Reconstituted Epidermis

Preparation of the sample using the following protocol:

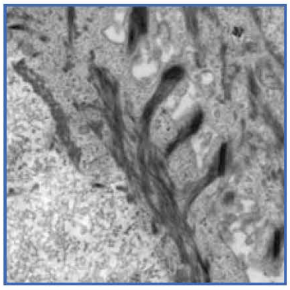
- Fixing Classic Glutaraldehyde, Osmium, Epon / Araldite
- Cutting Ultra-Thin, Double UranyLess Contrast and Lead Citrate



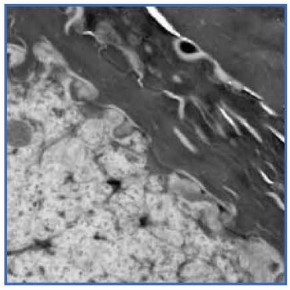
Epidermis. Photo: Audrey Houcine (CMEAB Toulouse)



Epidermis. Photo: Audrey Houcine (CMEAB Toulouse)



Epidermis. Photo: Audrey Houcine (CMEAB Toulouse)

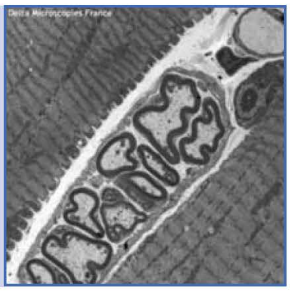


Epidermis. Photo: Audrey Houcine (CMEAB Toulouse)

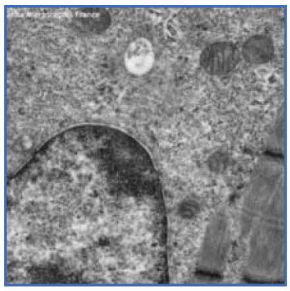
Muscle - Nerve - Mice

Preparation of the sample using the following protocol:

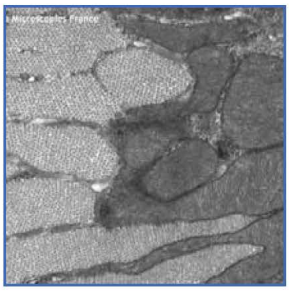
- Classic-Glutaraldehyde Fixation PFA, Osmium, Epon
- Ultrafine Cup - UranyLess Contrast 1 minute followed lead Citrate 1 minute



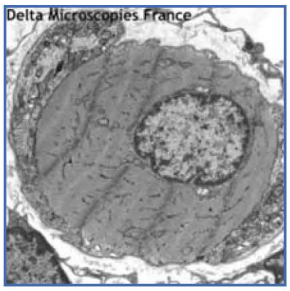
Longitudinal Section of Mouse Skeletal Muscle - Nerve Cup (dense area myelin sheath). Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



Detailed View of Myocytes. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



Cross Section of Muscle Fibers - Mitochondria. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)

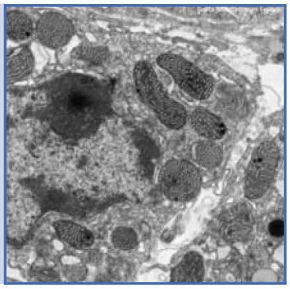


Myocyte. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)

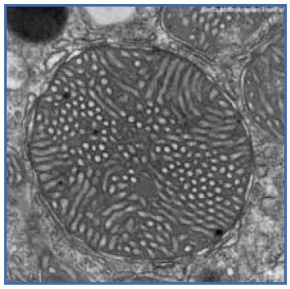
Mouse Ovarian Follicle

Preparation of the sample using the following protocol:

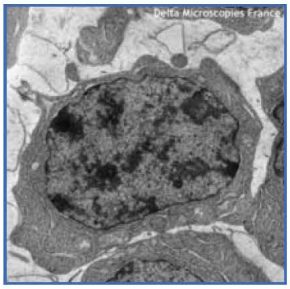
- Classic-Glutaraldehyde Fixation PFA, Osmium, Epon
- Ultrafine Cup - Contrast UranyLess Lead -Citrate



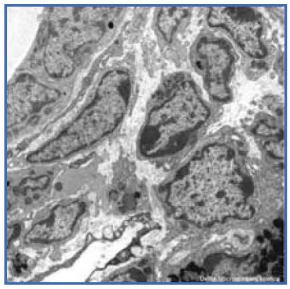
Theca Interna Mouse Ovarian Follicle. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



Mitochondria in Typical Finger Glove Steroid Synthesis in Cells (Internal Thèque Ovarian Follicle). Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



Follicular Cell of the Corona Radiata a Mouse Ovarian Follicle. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)

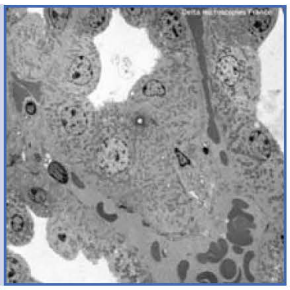


Cell of the External Library of Mouse Ovarian Follicle. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)

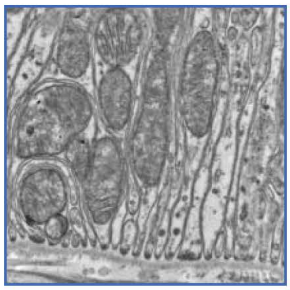
Mouse Kidney

Preparation of the sample using the following protocol:

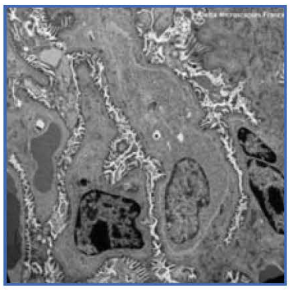
- Classic-Glutaraldehyde Fixation PFA, Osmium, Epon
- Ultrafine Cup - UranyLess Contrast 1 minute followed lead Citrate 1 minute



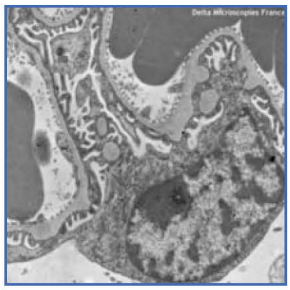
Mouse Kidney. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



Basal Invaginations - Hémidesmosome - Basal Lamina: Increase the Exchange Surface - Kidney. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



Glomerular area - Podocytes - Stalks - Kidney. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)

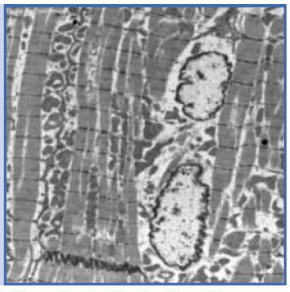


Podocyte - Pedicels - Kidney. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)

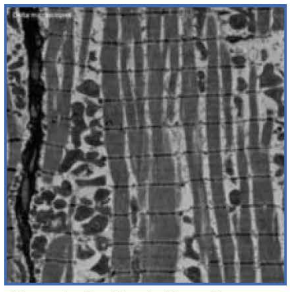
Mouse Cardiac Muscle

Preparation of the sample using the following protocol:

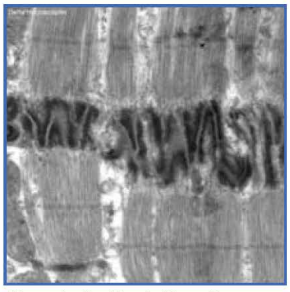
- Classic - Fixing Glutaraldehyde, Osmium, Epon Ultrafine
- Cups, Double UranyLess Contrast and Lead Citrate



Mouse Cardiac Muscle. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



Mouse Cardiac Muscle. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



Mouse Cardiac Muscle. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)

DELTA MICROSCOPIES

UranyLess Applications

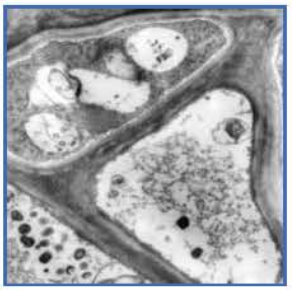
All photos taken on Transmission Electron Microscope.

Plant Tissue

Preparation of the sample using the following protocol:

- Glutaraldehyde Fixation Classic - Osmium - Included In Epon
- Contrast the UranyLess monitoring Lead Citrate

Plant Leaf. Photo: Jeannine Lherminier (INRA - Dijon)

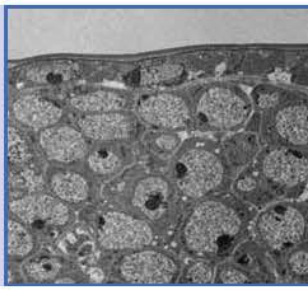


Plant Leaf. Photo: Jeannine Lherminier (INRA - Dijon)

Drosophila Larva

Preparation of the sample using the following protocol:

- Classic-Glutaraldehyde Fixation PFA, Osmium, Epon
- Ultrafine Cup - Contrast UranyLess lead -citrate

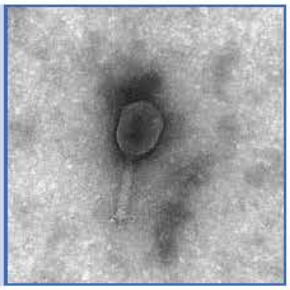


Drosophila Larva. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)

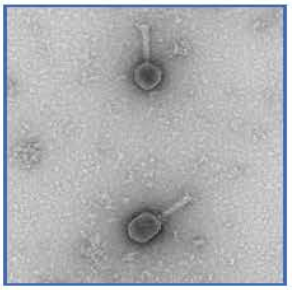
Phage T6

Preparation of the sample using the following protocol:

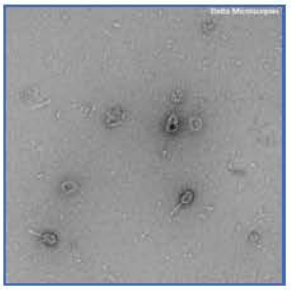
- Staggering Phage T6 on a G300-Cu grid Covered with a Carbon Formvar Film. Ionization 1 minute



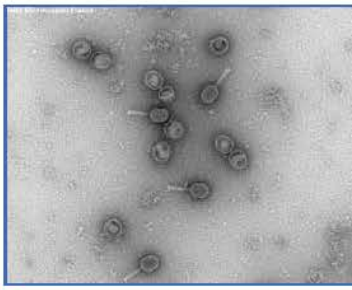
Phage. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



Phage. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



Phage. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)

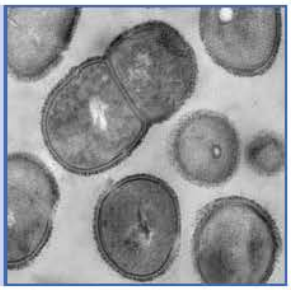


Phage. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)

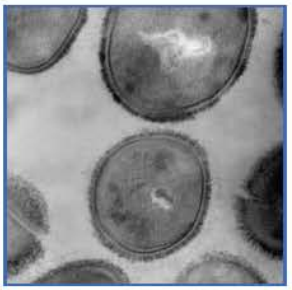
Cross-Sectional Bacteria

Preparation of the sample using the following protocol:

- Fixing Classic Glutaraldehyde, Osmium, EPON
- Cutting Ultrafine, Double Contrast UranyLess and Lead Citrate.



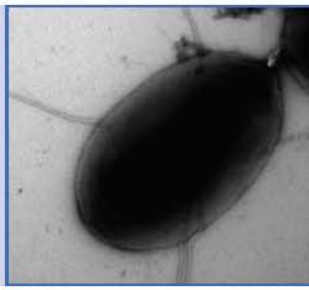
Bacteria. Photo: Christine Longin (INRA Jouy en Josas).



Bacteria. Photo: Christine Longin (INRA Jouy en Josas).

Bacteria E. Coli

Negative Staining for 2 Minutes UranyLess Bacteria Like E. Coli (Adherent and Invasive (ACSI) LF82) Which Have Pili and Flagella.

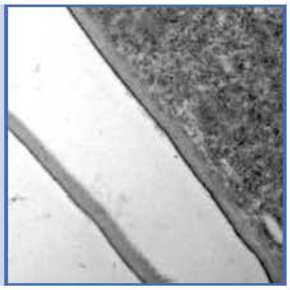


Bacteria. Photo: M2iSH team of Clermont Ferrand

Sacculina Crustaceans (Small Parasitic Crustacean)

Preparation of the sample using the following protocol:

- Classic Glutaraldehyde Fixation, Osmium, Epoxy Inclusion
- Fine Cups - Contrast to the Aqueous UranyLess to 60°C on a Hotplate without Lead Citrate Post Coloring

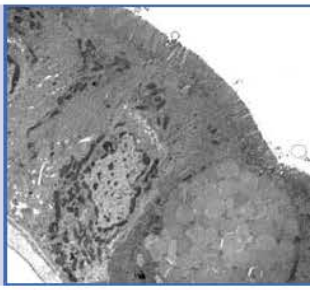


Sacculina (small parasitic crustacean) cuticle area. Photo: Djediat Chakib (Natural History Museum, Paris)

Intestine

Preparation of the sample using the following protocol:

- Classic-Glutaraldehyde Fixation PFA, Osmium, Epon
- Ultrafine Cup - Contrast UranyLess Lead -Citrate

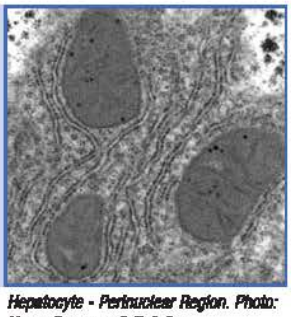


Intestine. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)

Liver Mouse and Gerbil Sahara

Preparation of the sample using the following protocol:

- Classic-Glutaraldehyde Fixation PFA, Osmium, Epon
- Ultrafine Cup - Contrast UranyLess Lead - Citrate

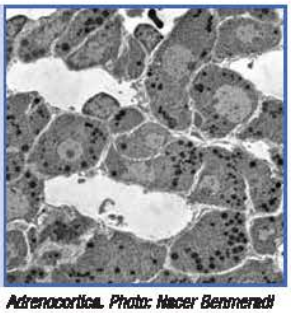


Hepatocyte - Perinuclear Region. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)

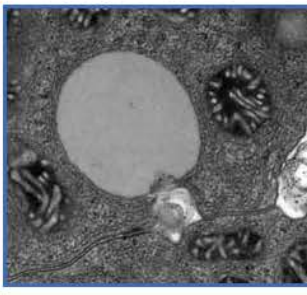
Adrenal Gland Gerbil Sahara

Preparation of the sample using the following protocol:

- Classic-Glutaraldehyde Fixation PFA, Osmium, Epon
- Ultrafine Cup - Contrast UranyLess lead -citrate



Adrenocortica. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



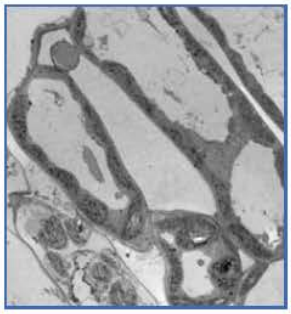
Adrenocortica. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)

DELTA MICROSCOPIES

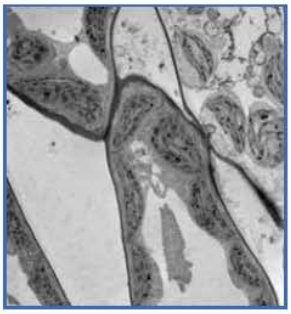
UranyLess Applications All photos taken on Transmission Electron Microscope.

Parsley and Rosebush, Preparation of the sample using the following protocol:

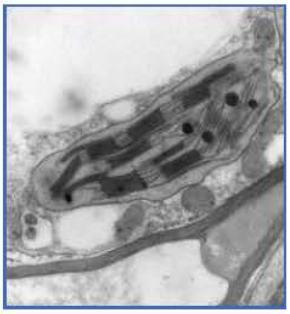
- Classic-Glutaraldehyde Fixation PFA, Osmium, Epon
- Ultrafine Cup - Contrast UranyLess lead -citrate



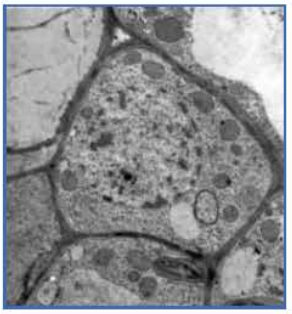
Parsley Leaf. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



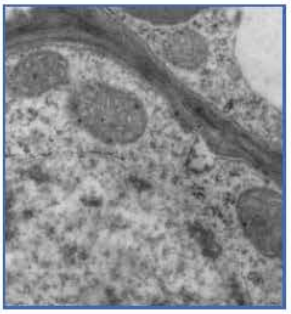
Parsley Leaf. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



Rosebush. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



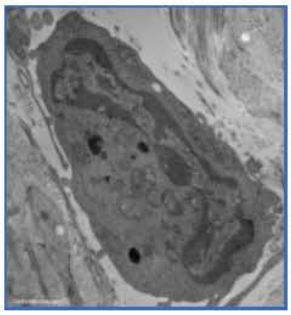
Rosebush Root. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



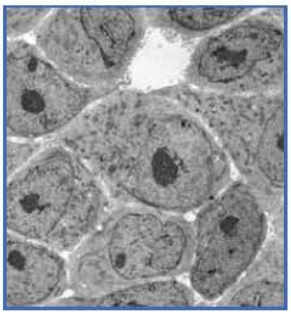
Rosebush Root. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)

Culture Cells, Preparation of the sample using the following protocol:

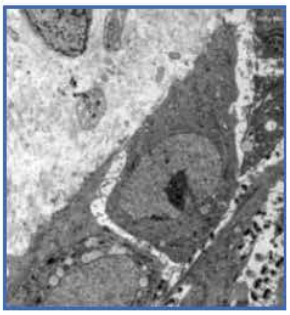
- Classic-Glutaraldehyde Fixation PFA, Osmium, Epon
- Ultrafine Cup - Contrast UranyLess lead -citrate



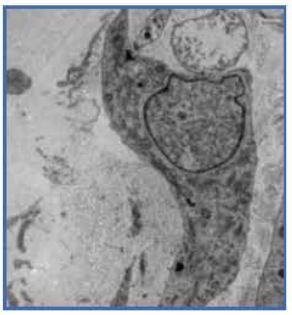
Culture Cell. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



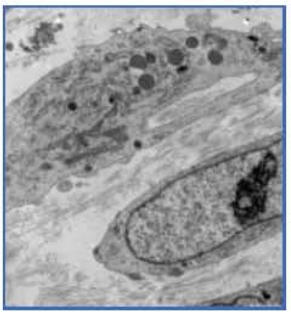
Culture Cell. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



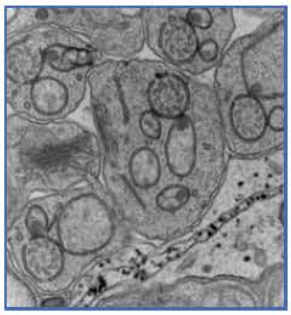
Culture Cell. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



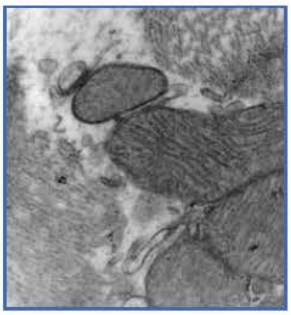
Culture Cell. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



Culture Cell. Photo: Nacer Benmeradi (R & D - DeltaMicroscopies-France)



Spermatides Drosophile. Photo: Chantal Cazevieille Montpellier



Heart Headset. Photo: Chantal Cazevieille CRIC / IURC la'INSERM Montpellier (R & D - DeltaMicroscopies-France)

PLC Contrast Leica EM Stain

Preparation of the sample using the following protocol:

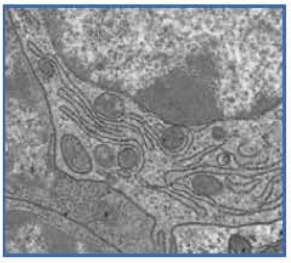
- Classic-Glutaraldehyde Fixation PFA, Osmium, Epon
- Ultrafine Cup - contrast Uranyless Lead -Citrate

Chantal Cazevieille CRIC / IURC INSERM Montpellier tested aqueous UranyLess in the Leica brand grid contrast controller on different tissues, Drosophila heart atrium, retina, cochlea and ileum (Gut). The tissues were fixed according to the standard protocol 2.5% Glutaraldehyde in PHEM buffer, the post fixation in 0.5% osmium in 0.8% potassium ferrocyanide in RT for 2 hours. The sections are collected on single-hole or 200 mesh grids.

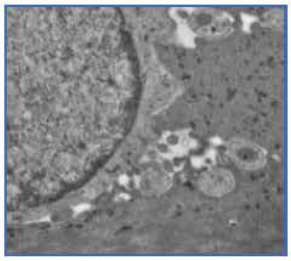
The treatment of the grids is UranyLess 7mn lead citrate followed 7 minutes.

We present here some images made by Hitachi transmission electron microscope with a digital camera AMT.

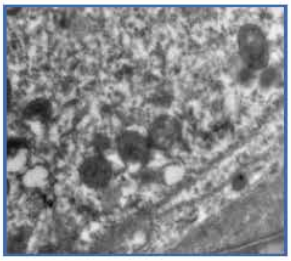
You will notice that the combined action of potassium ferrocyanide and UranyLess reveal a marked way the cyto-membranes in the ileum.



Drosophila. Photo: Chantal Cazevieille CRIC / IURC la'INSERM Montpellier



Drosophila. Photo: Chantal Cazevieille CRIC / IURC la'INSERM Montpellier



Drosophila. Photo: Chantal Cazevieille CRIC / IURC la'INSERM Montpellier

DELTA MICROSCOPIES