

# Application Note

## Leica EM AMW – *Arabidopsis thaliana* (L.) accession Col

Courtesy of: Mag. Dr. Bernd Zechmann and Univ. Prof. Dr. Günther Zellnig  
Institut für Pflanzenwissenschaften  
Universität Graz, Austria

Living up to Life

**Leica**  
MICROSYSTEMS

# Leica EM AMW Application Note

## *Arabidopsis thaliana*

### **Arabidopsis thaliana (L.) accession Col-0**

Plants were grown in growth chambers under defined conditions. After stratification for 4 days at 4°C seeds were grown in pots with soil with 9/15 hours day/night photoperiod. Day and night temperatures were 22°C and 18°C, respectively, the relative humidity was 60% and the plants were kept at 100% relative soil water content. Light intensity varied between 110 and 140  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Six weeks after stratification the youngest fully developed rosette leaves were harvested two hours after the onset of the light period and prepared for electron microscopy with the Leica EM AMW. Leaves at this stage were approximately 3.5cm in length and 1.5cm in width. Samples were taken from the middle of the leaves close to the middle vein. Small sections of leaves (1mm<sup>2</sup>) were cut with a razor blade on a modelling wax plate in a drop of 3% glutaraldehyde in 0.06M Sørensen phosphate buffer at pH 7.2. Sections were then evacuated with a water jet vacuum pump for a maximum of 10 seconds in a vial filled with the above described fixative solution. Subsequently, the specimens were transferred into small baskets with a mesh width of about 200 $\mu\text{m}$ . These baskets were then stacked on top of each other and transferred into the mono-mode chamber of the Leica EM AMW which already contained a vial filled with the above mentioned fixative solution. Microwave fixation was then started about 2 minutes after the cutting of the samples by starting the previously programmed protocol.

Sample preparation for transmission electron microscopy (TEM) was performed in order to develop a standard protocol that would reduce sample preparation time for TEM-investigations. Therefore the overall and fine structure of leaf cells prepared with the Leica EM AMW were compared with leaf cells that were prepared with a conventional fixation protocol at room temperature. Additionally, the diameter of membranes from different cell compartments (chloroplasts, nuclei and plasmamembrane) was determined by using quantitative computer supported transmission electron microscopy.

### **Epon, Agar 100**

Standard mixture: Glauert Medium

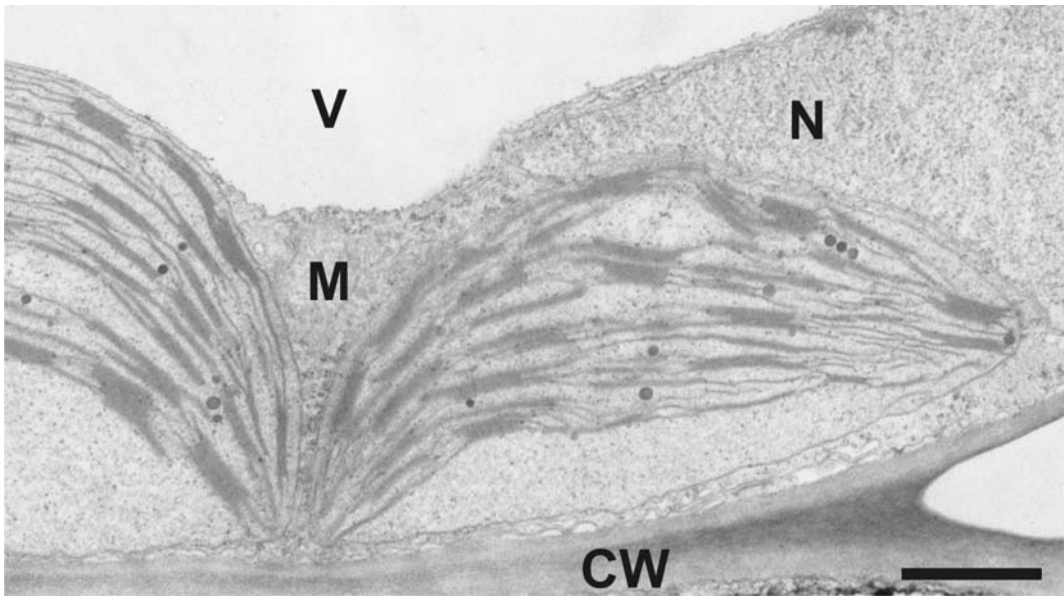
Epon (Agar 100 Harz, Fa. Gröpl)	20ml	<b>24g</b>
DDSA (Dodecenylsuccinic anhydrid)	16ml	<b>16g</b>
MNA (Methylnadicanhydrid)	8ml	<b>10g</b>
BDMA (Benzyldimethylamin)	1,3ml	<b>1,2g</b>

Epon soft:		Epon hard:	
Epon	<b>24g</b>	Epon	<b>24g</b>
DDSA	<b>22g</b>	DDSA	<b>9g</b>
MNA	<b>6g</b>	MNA	<b>15g</b>

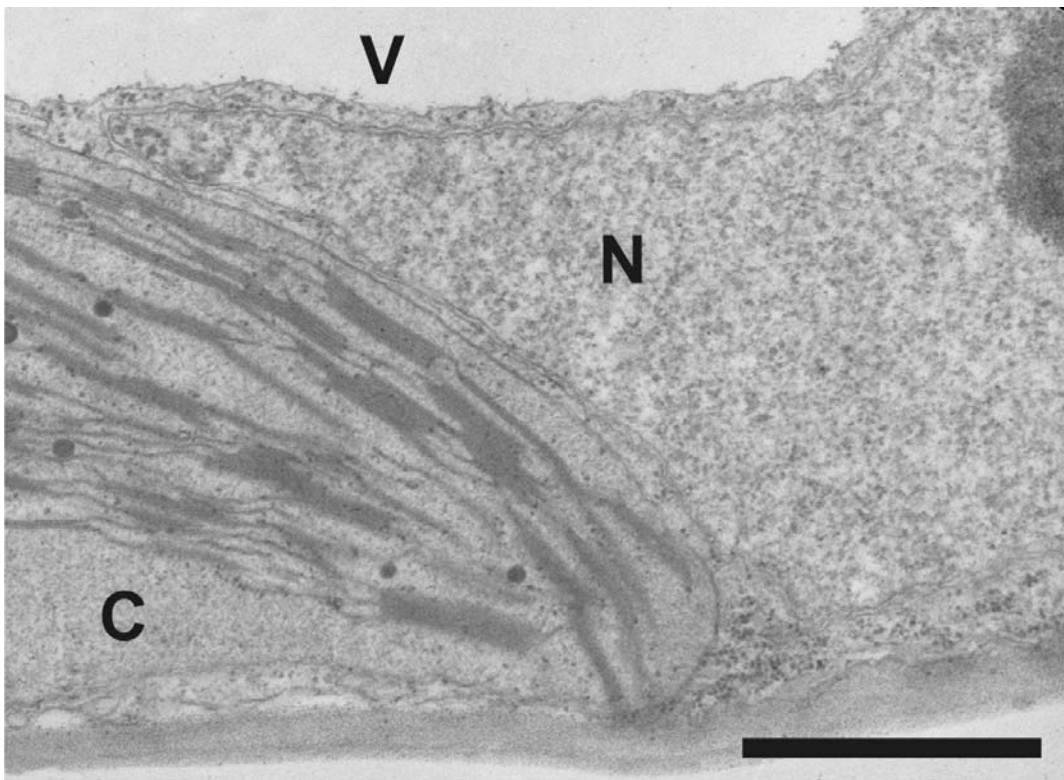
Sample: Arabidopsis thaliana

<b>Processing</b>						
vial	step	time	max. temp.(°C)	reagent	mode	max. power (W)
1	1	00:02:00	37	Buffer + glutar aldehyde	Cont.	15
1	2	00:02:00	20	Buffer + glutar aldehyde	Cont.	0
1	3	00:02:00	37	Buffer + glutar aldehyde	Cont.	15
1	4	00:02:00	20	Buffer + glutar aldehyde	Cont.	0
2	1	00:00:40	37	Buffer	Slope	20
3	1	00:00:40	37	Buffer	Pulse	15
4	1	00:00:40	37	Buffer	Slope	20
5	1	00:12:00	37	Buffer + OsO4	Cont.	15
6	1	00:01:00	37	Buffer	Cont.	15
7	1	00:01:00	37	Buffer	Cont.	15
8	1	00:01:00	37	Buffer	Cont.	15
9	1	00:01:00	37	Acetone 50%	Slope	20
10	1	00:01:00	37	Acetone 75%	Slope	20
11	1	00:01:00	37	Acetone 90%	Slope	20
12	1	00:02:00	37	Acetone	Slope	20
13	1	00:02:00	37	Acetone	Slope	20
14	1	00:03:00	37	Resin 3:1	Cont.	10
15	1	00:03:00	40	Resin 1:1	Cont.	10
16	1	00:03:00	45	Resin 1:3	Cont.	10
17	1	00:03:00	50	Epon	Cont.	12
18	1	00:03:00	50	Epon	Cont.	12
19	1	00:03:00	50	Epon	Cont.	12
<b>Total time</b>		<b>00:49:20</b>				

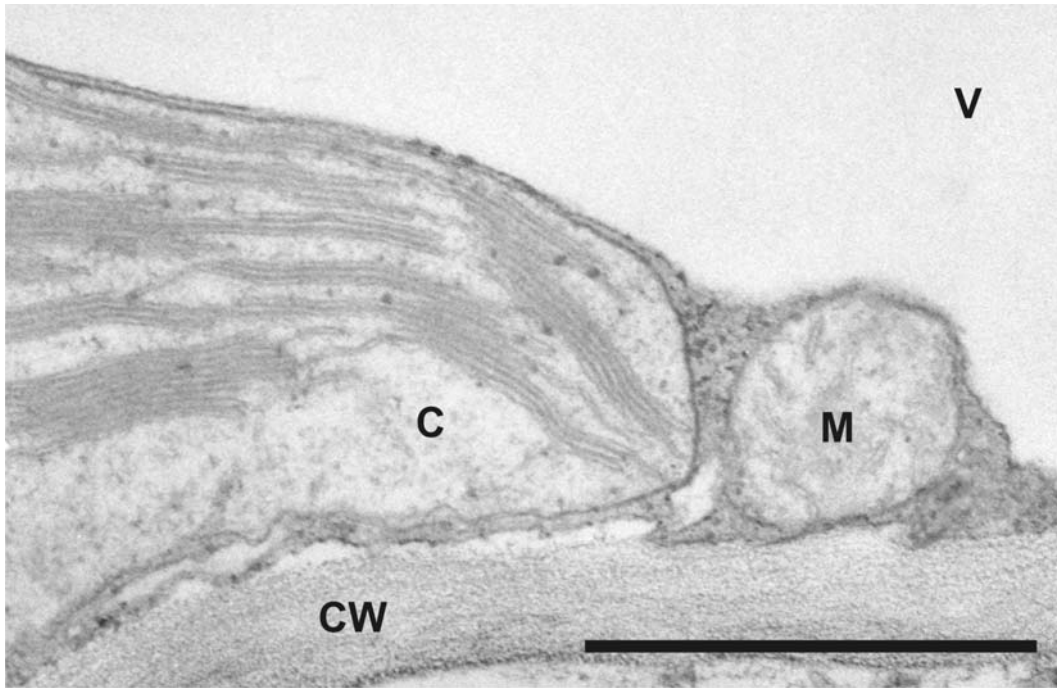
<b>Polymerisation</b>						
vial	step	time	max. temp.(°C)	reagent	mode	max. power (W)
1	1	00:05:00	65	Epon	Slope	30
1	2	00:05:00	75	Epon	Slope	30
1	3	00:15:00	85	Epon	Slope	30
1	4	02:35:00	85	Epon	Cont.	30
<b>Total time</b>		<b>03:00:00</b>				



Overview



Detail 1



Detail 2

M = Mitochondria  
N = Nucleus  
CW = Cell Wall  
V= Vacuoles

Scale Bar = 1  $\mu$ m

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