

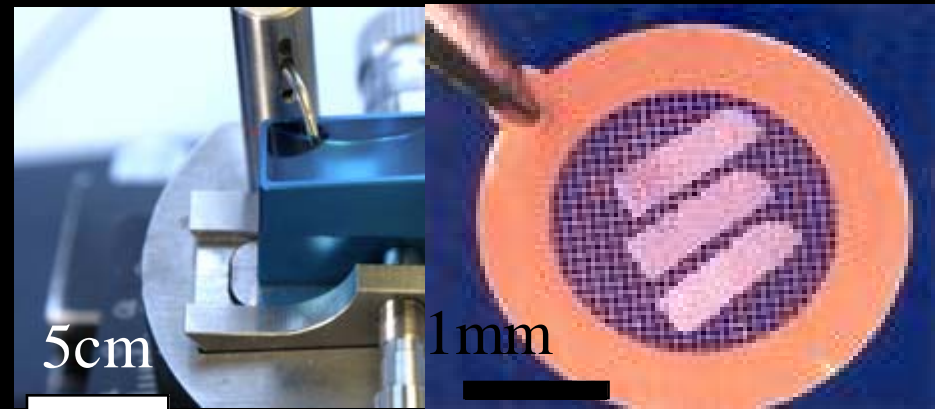
Ultrastructural 3D investigations of cells and cell organelles

G. Zellnig

Institute of Plant Sciences, University of Graz, Graz, Austria

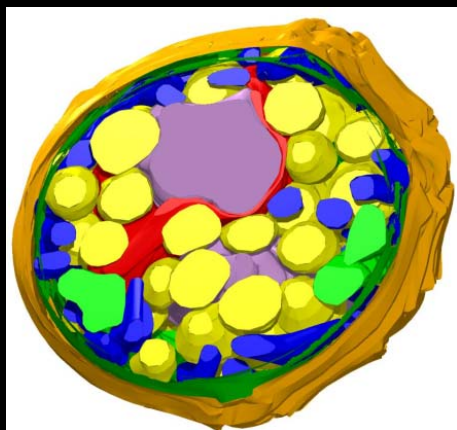
TEM-Preparation

- Fixation and stabilization of cell components
(Sample size around 1 mm²)
- Dehydration in alcohol
- Infiltration and embedding in plastic resins
- Ultrathin cutting:
block size 1mm x 200μm
section thickness 70-100nm
- Contrast of section
- TEM investigations

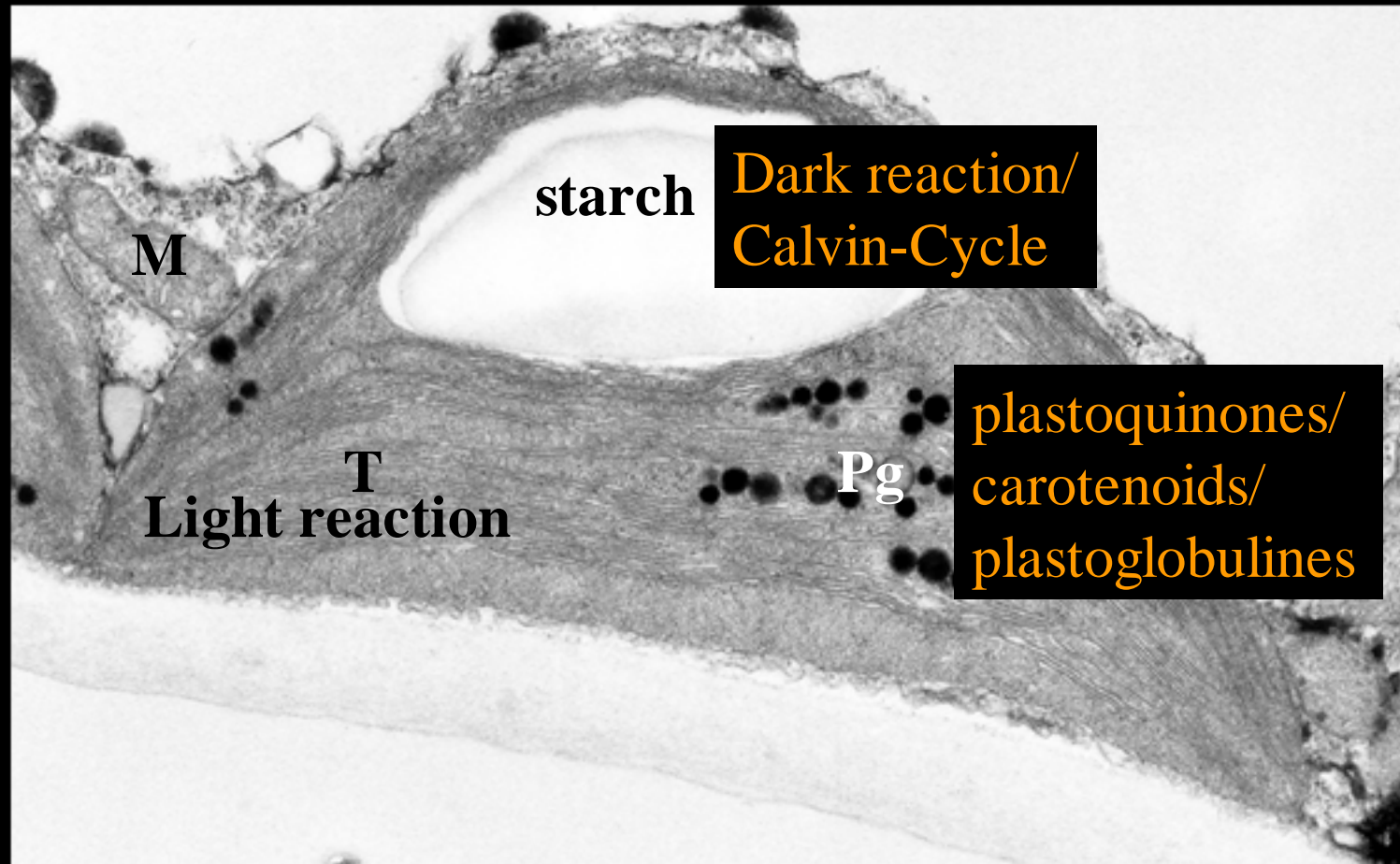


Content of the Presentation

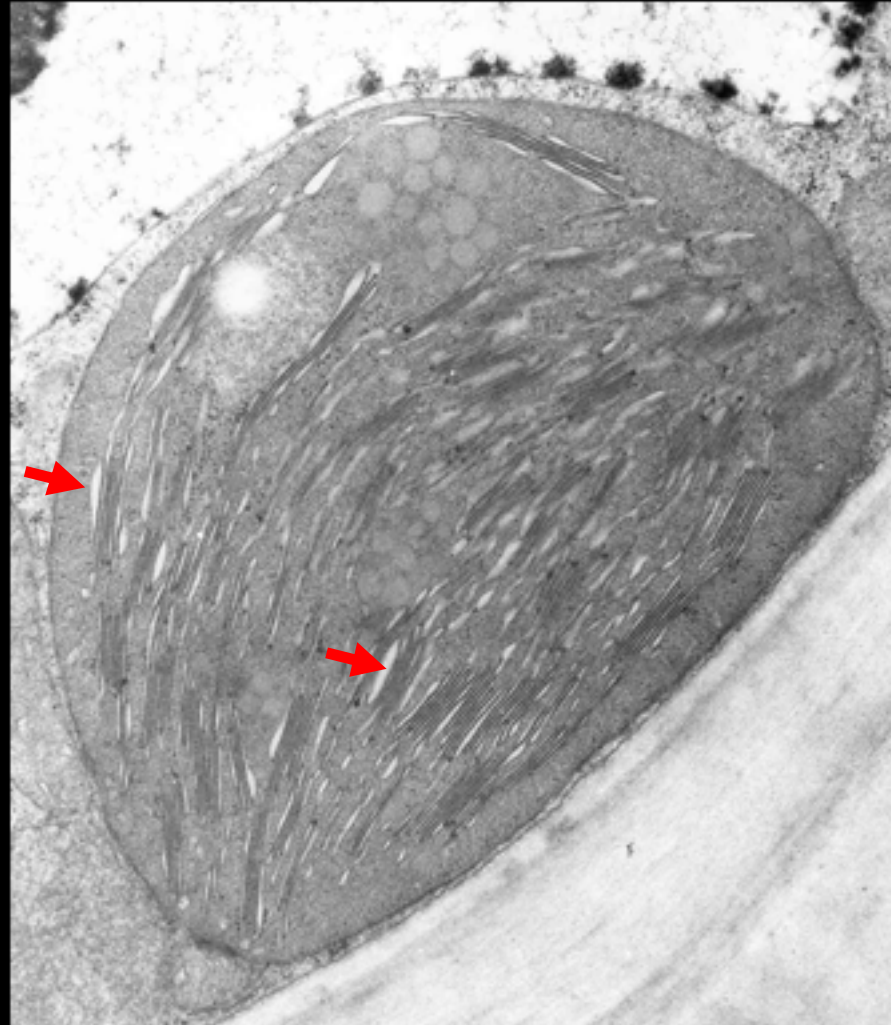
- **Methods:** Serial sectioning/quantification
- **Results:**
 - quantitative data of complete cells/organelles
 - related to chloroplasts/mitochondria of two plant species (spinach, spruce)
 - related to fixation methods (yeast cells)



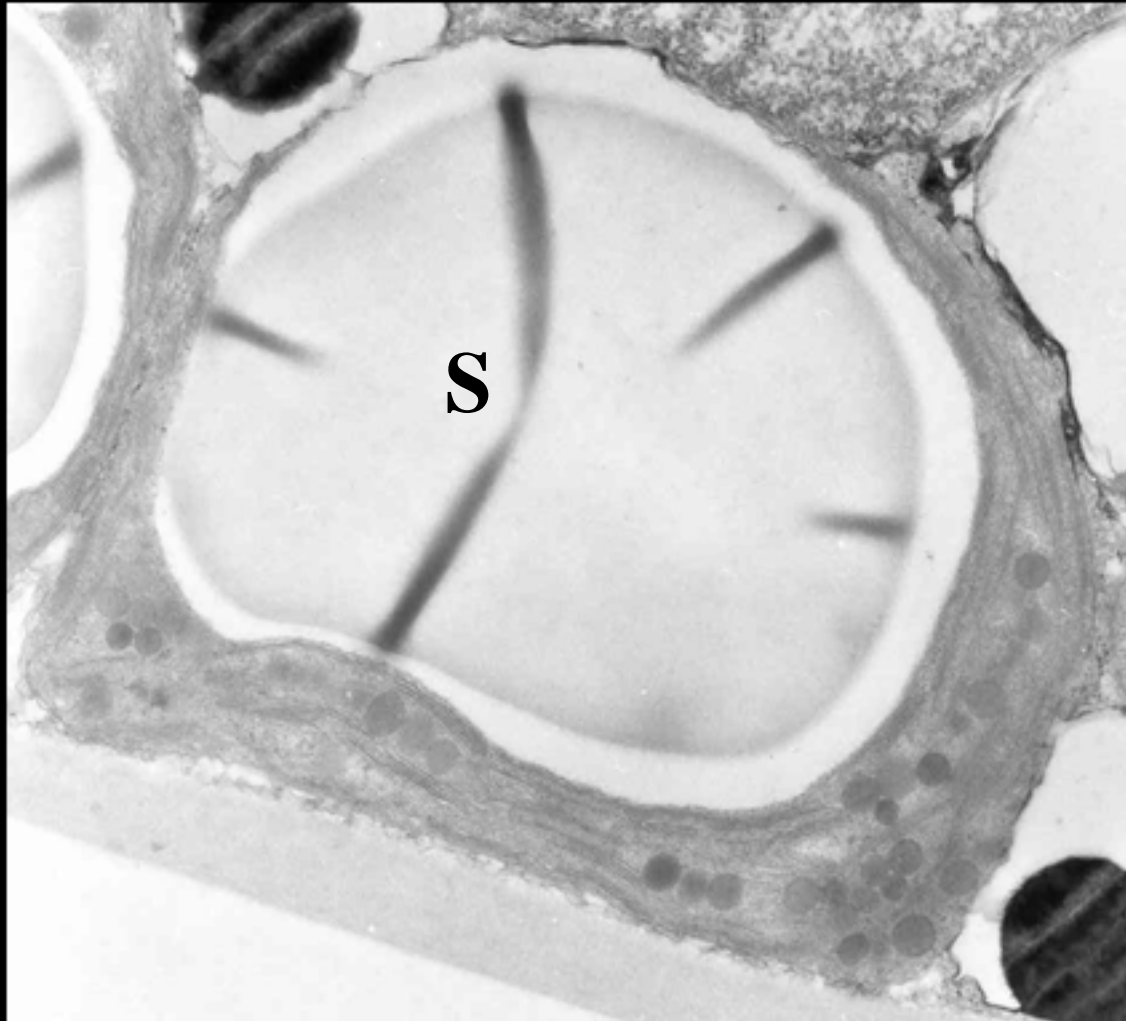
Chloroplast in TEM (80nm ultrathin section)



Chloroplast



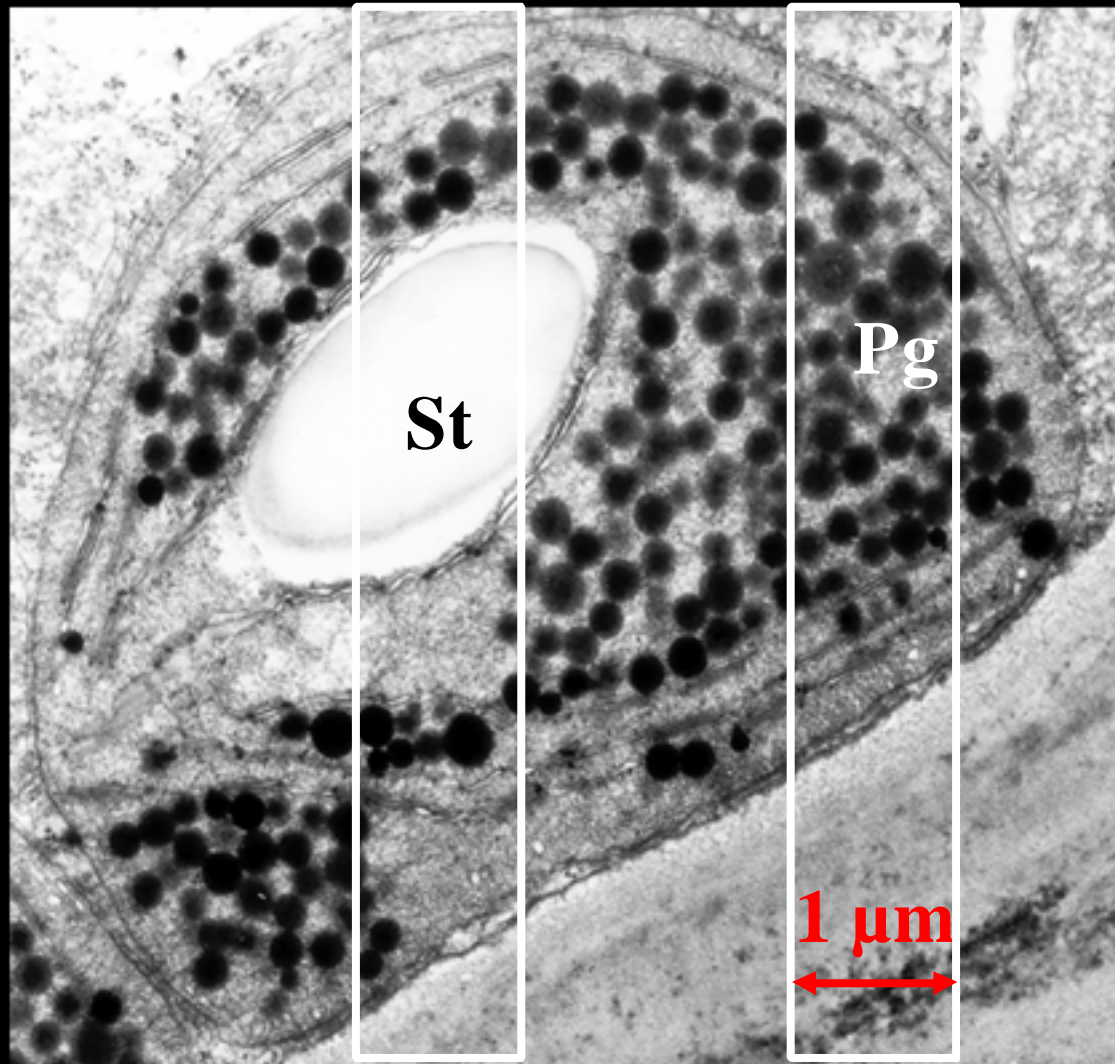
Chloroplast



Chloroplast

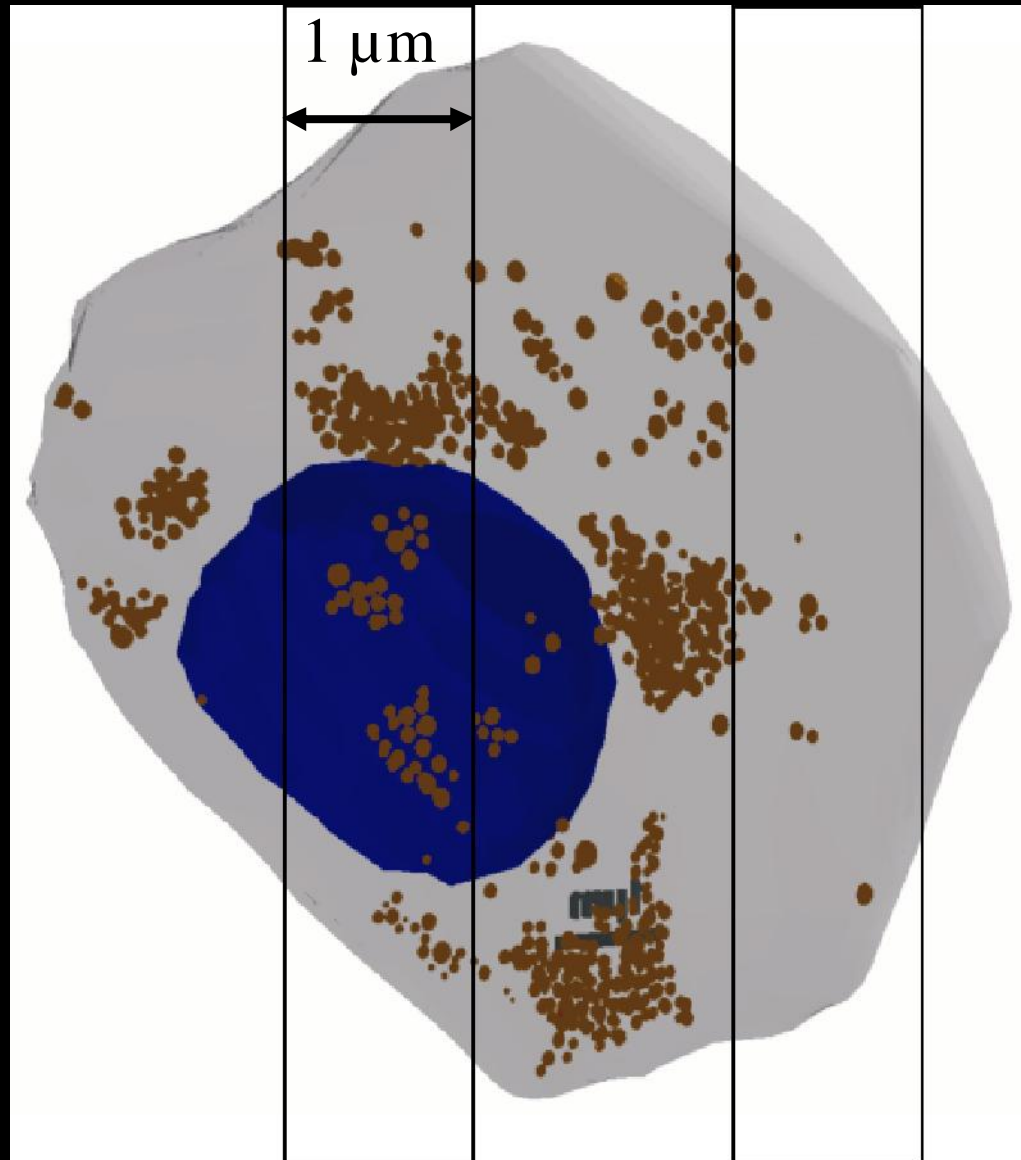


Sectioned parts of chloroplasts

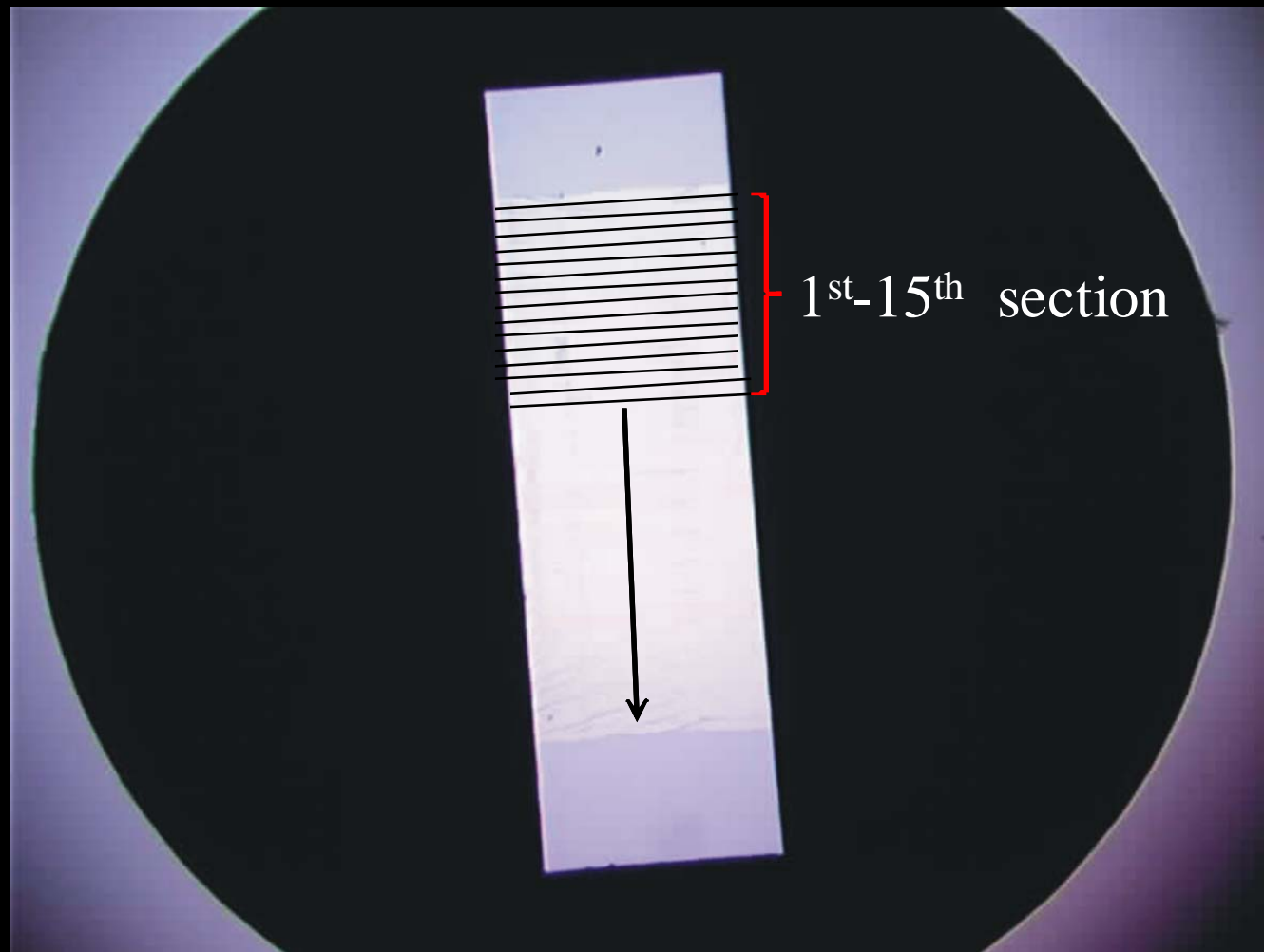


10-15
sections

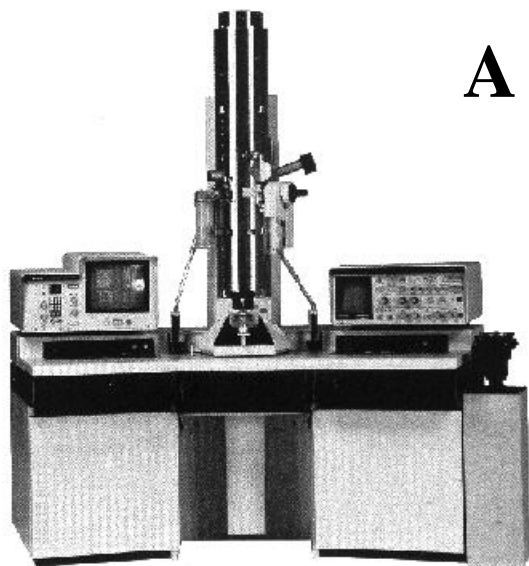
Sectioned parts of chloroplasts



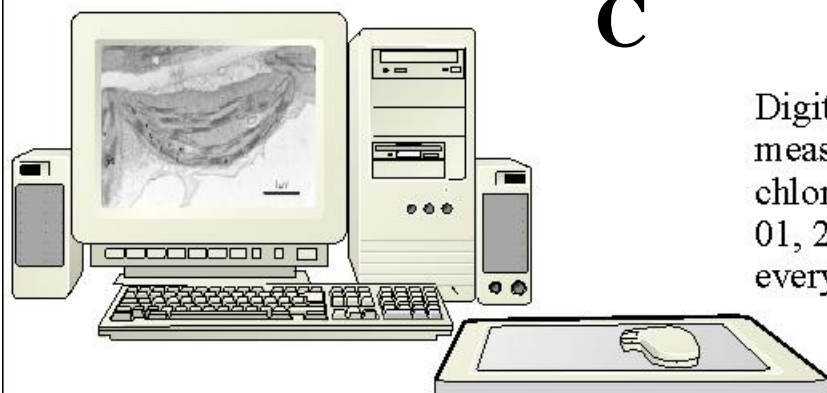
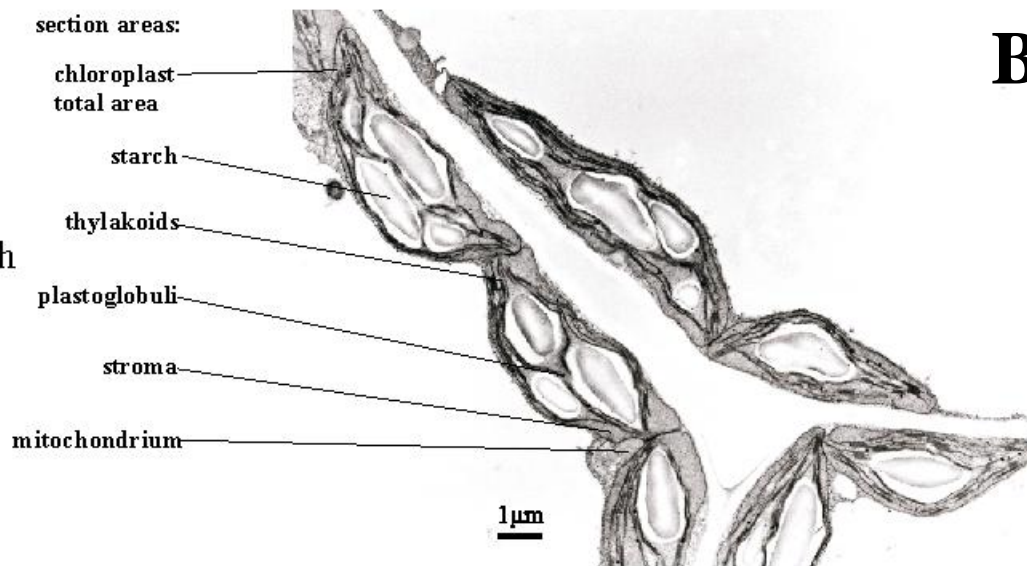
Ultrathin serial sections on a Single-Slot Grid



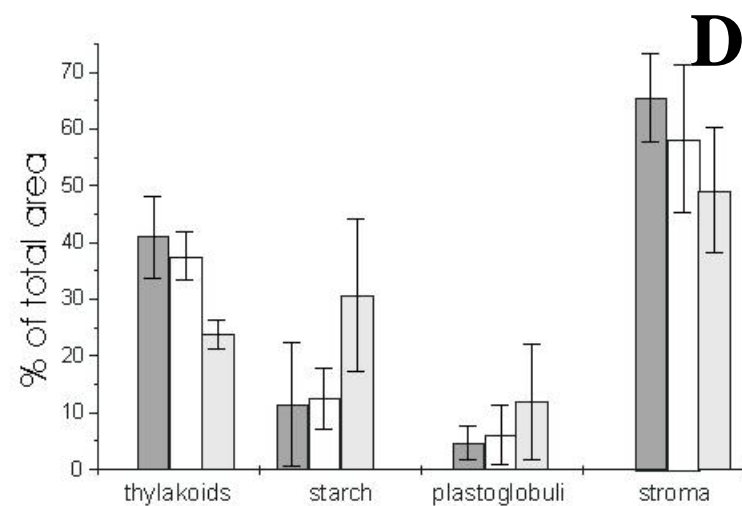
Method



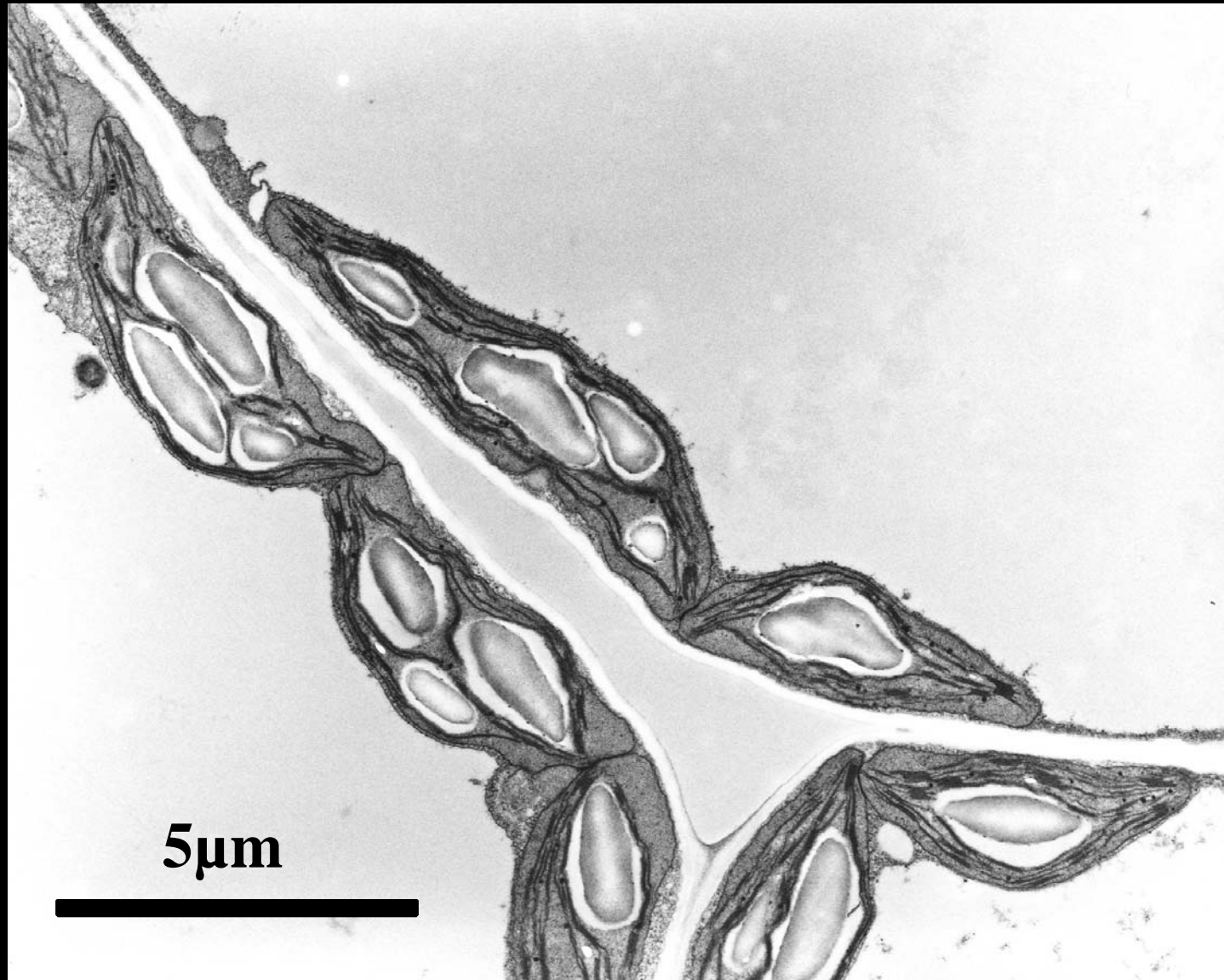
TEM
micrograph
of
01, 20,
40, 60,
80



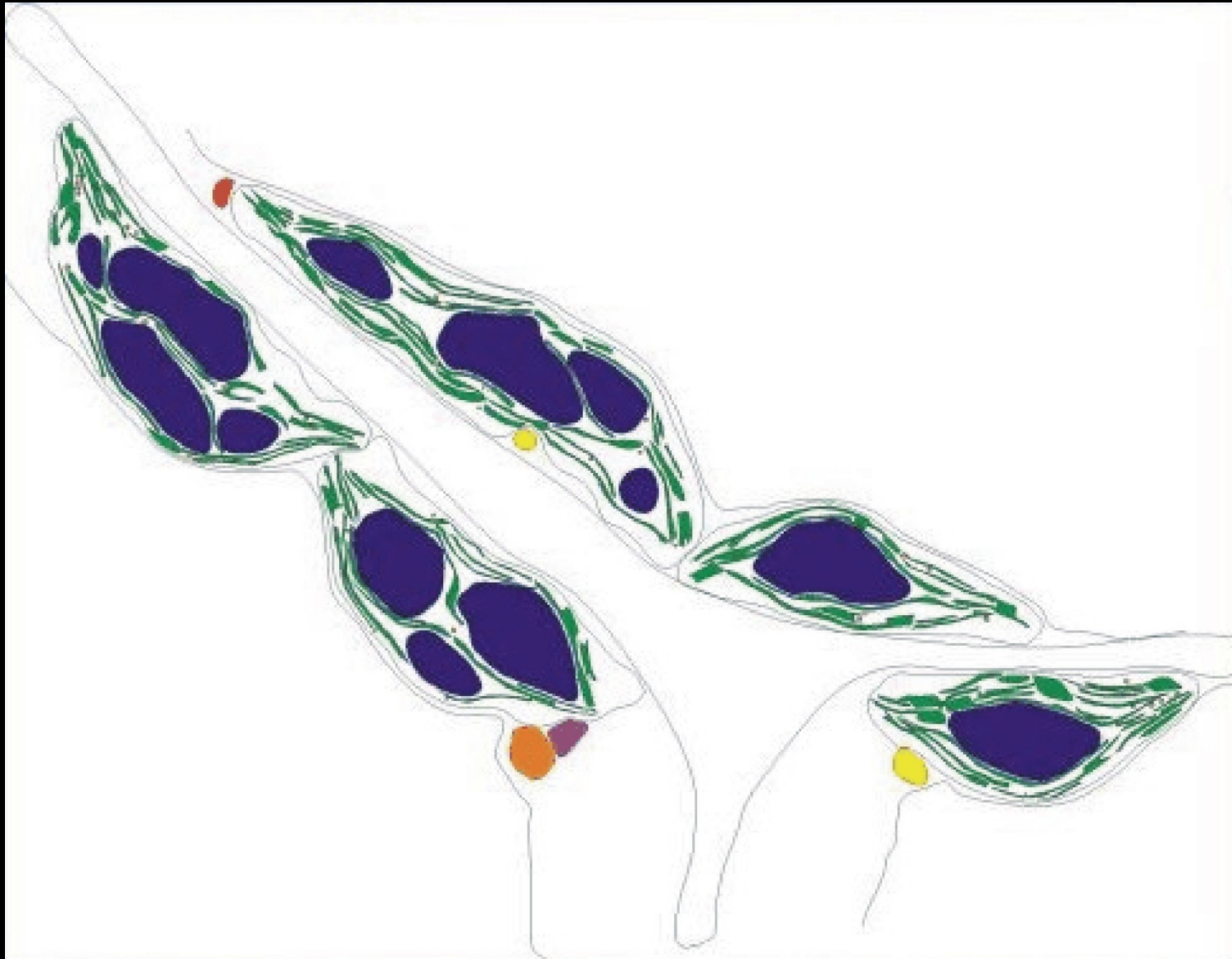
Digital imaging:
measurements of 4
chloroplasts in section
01, 20, 40, 60, 80 of
every needle/leaf



TEM: part of a cell



Traced selected cell structures



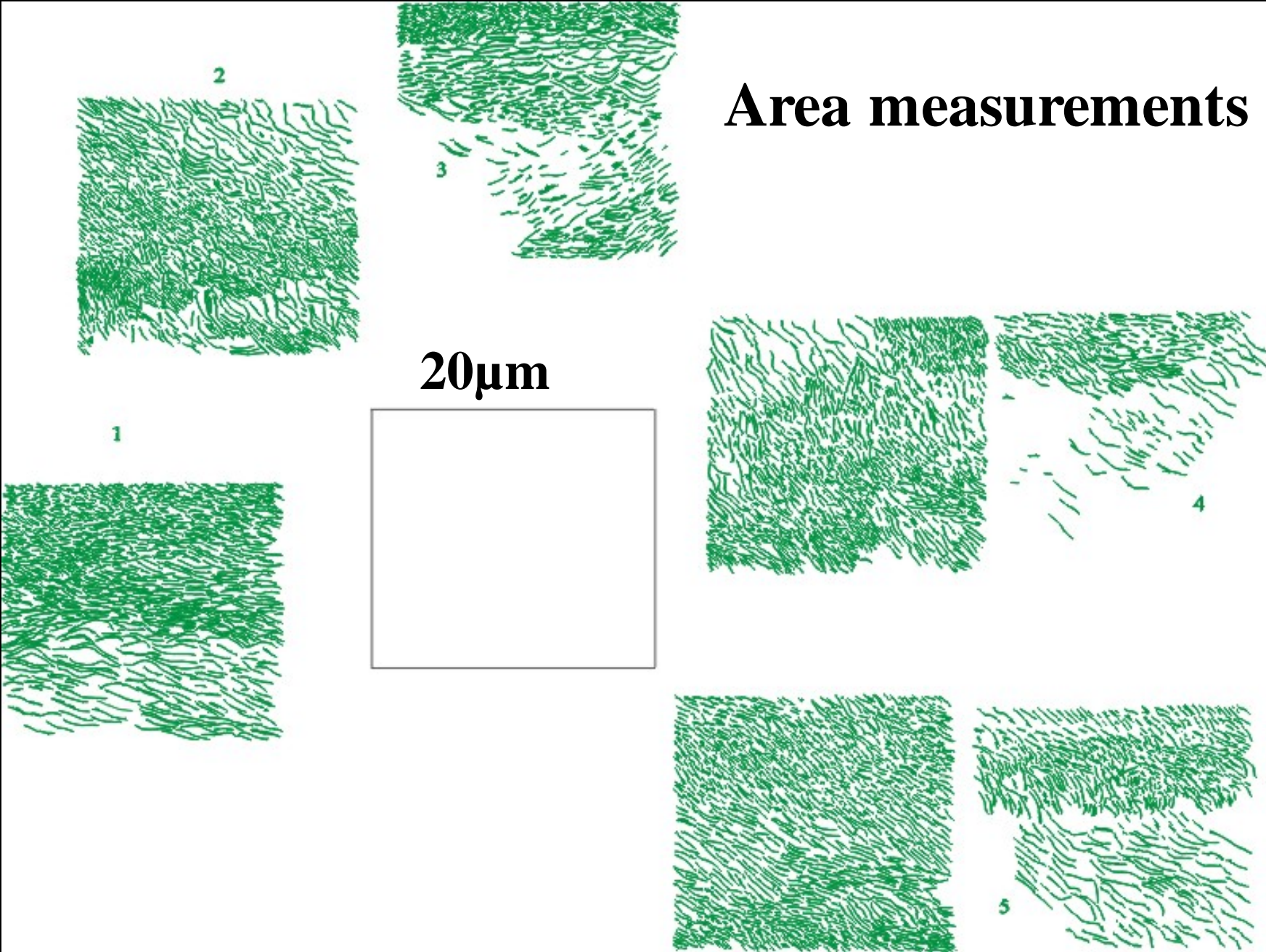
1

Traced cell structures of single sections

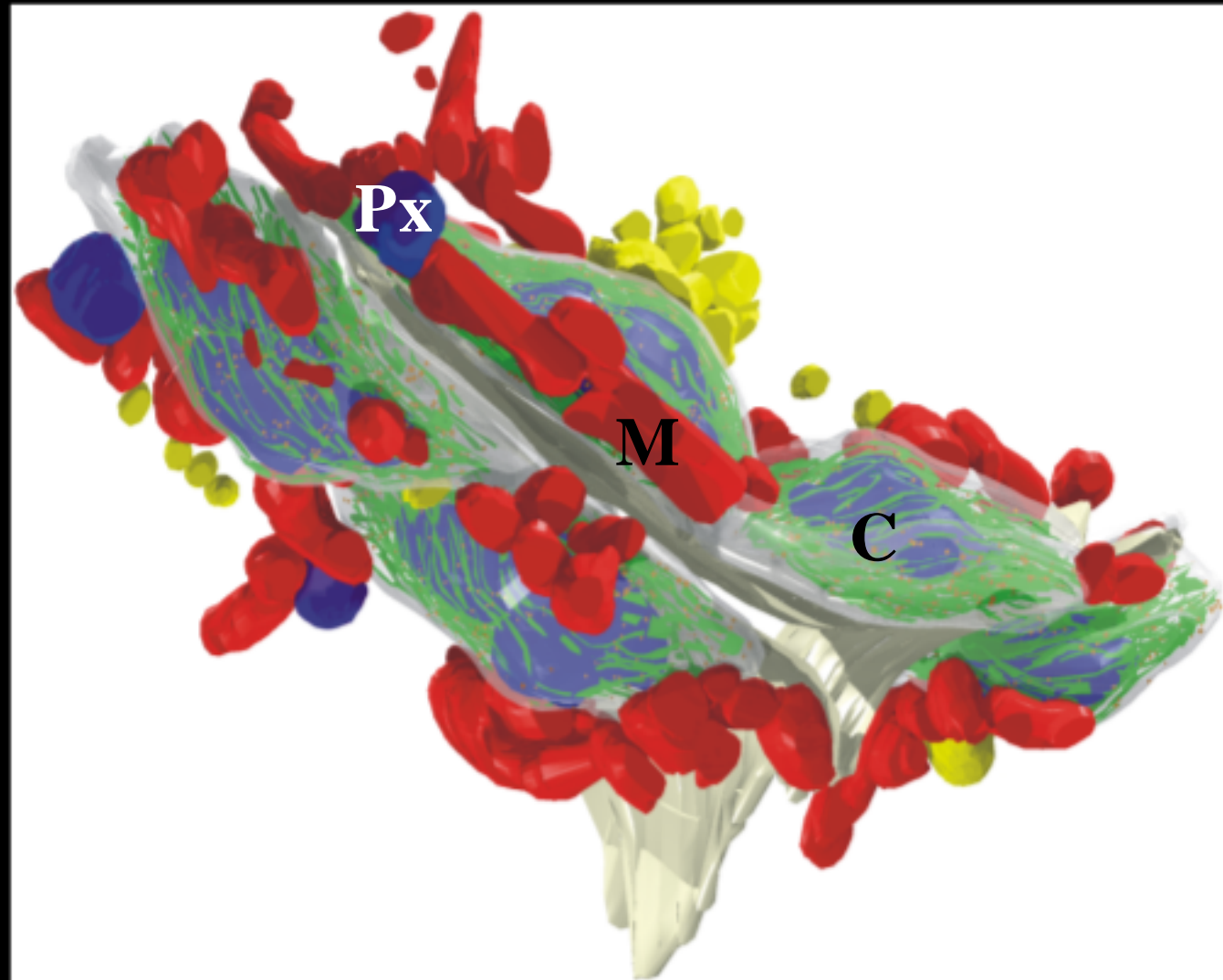
covers a cell volume of about $3000\mu\text{m}^3$

80

Area measurements



3D reconstructed part of the cell



Spinacia oleracea



- diurnal variation of chloroplast structures;
influence of drought stress
- influence of drought stress on mitochondria

Results

Spinach- chloroplasts during the daily course



Spinacia oleracea

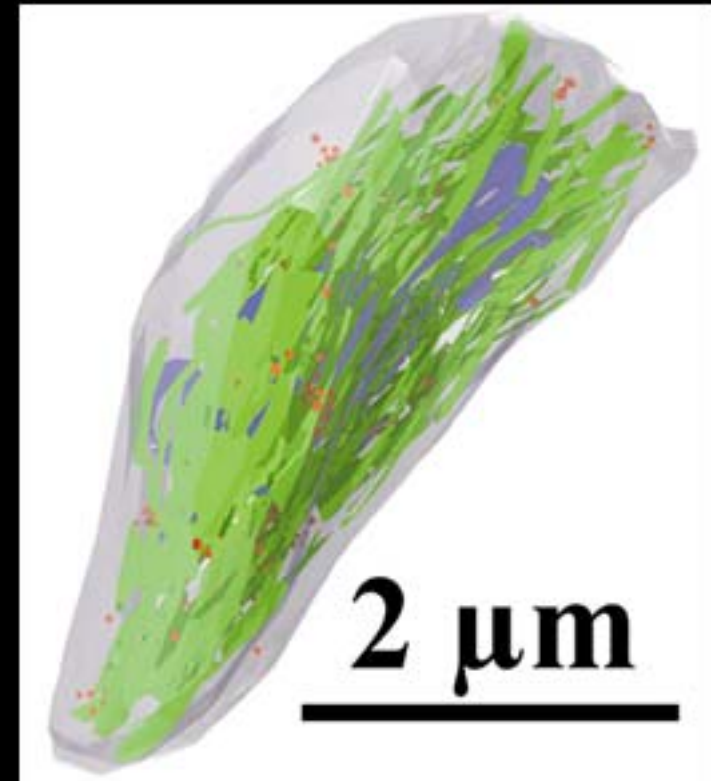
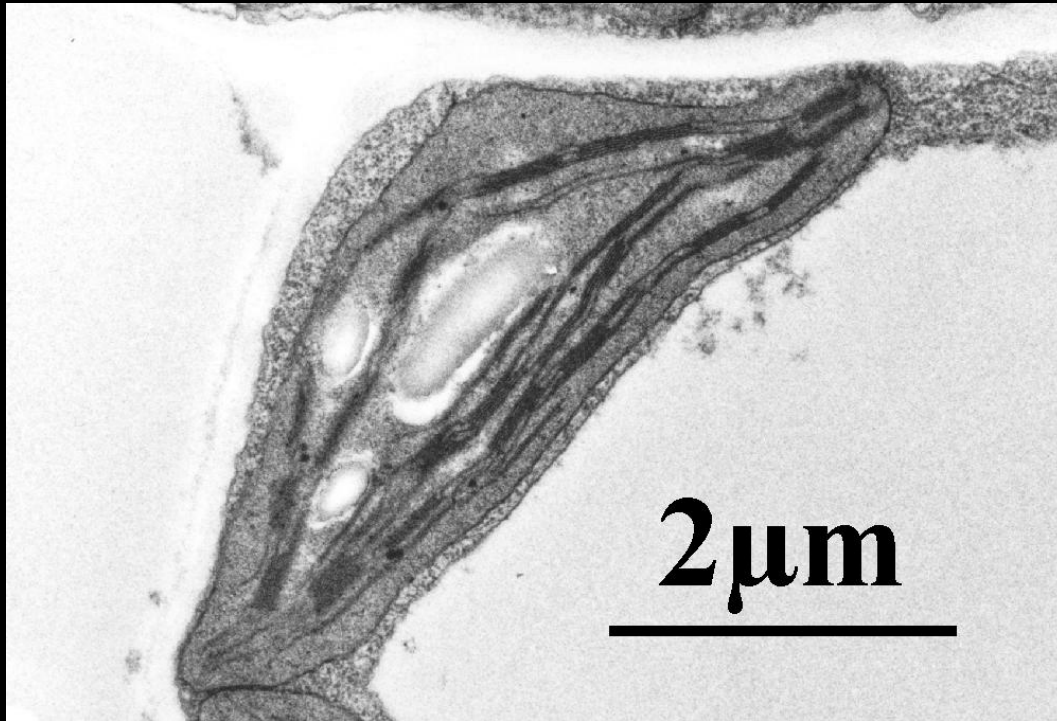
Total number of measured areas (chloroplasts):

52000

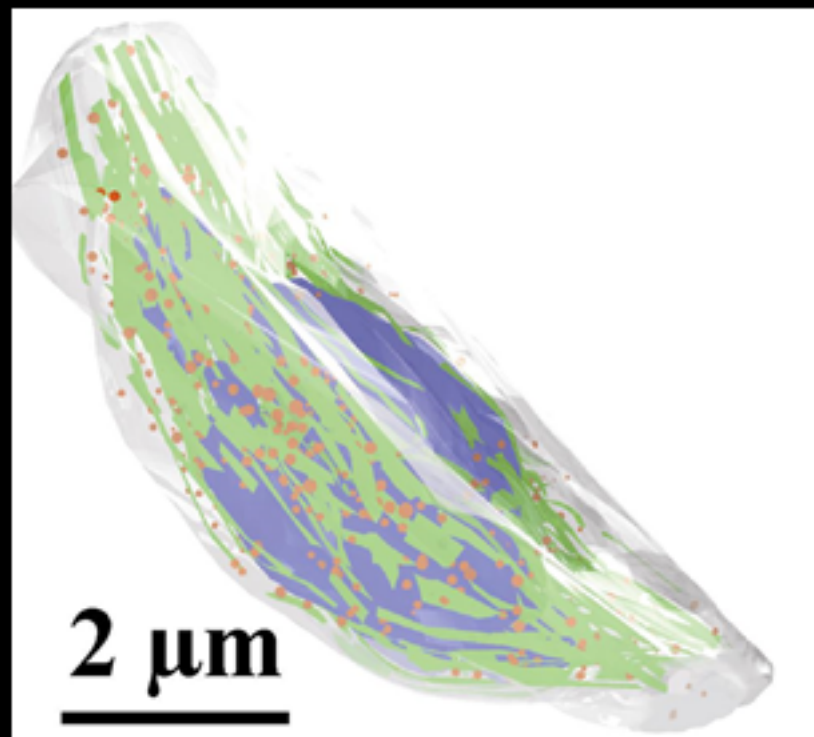
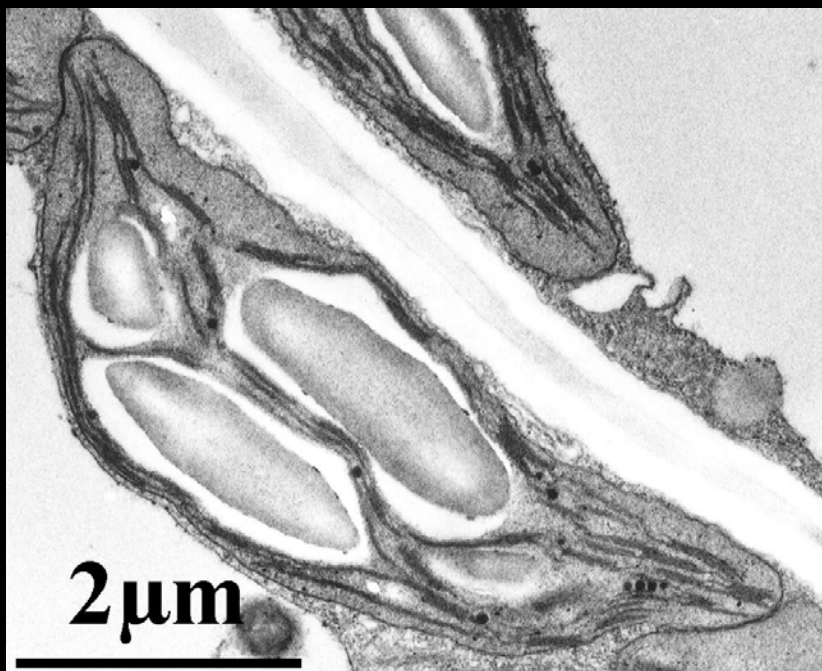
Total number of measured sectioned chloroplasts:

1200

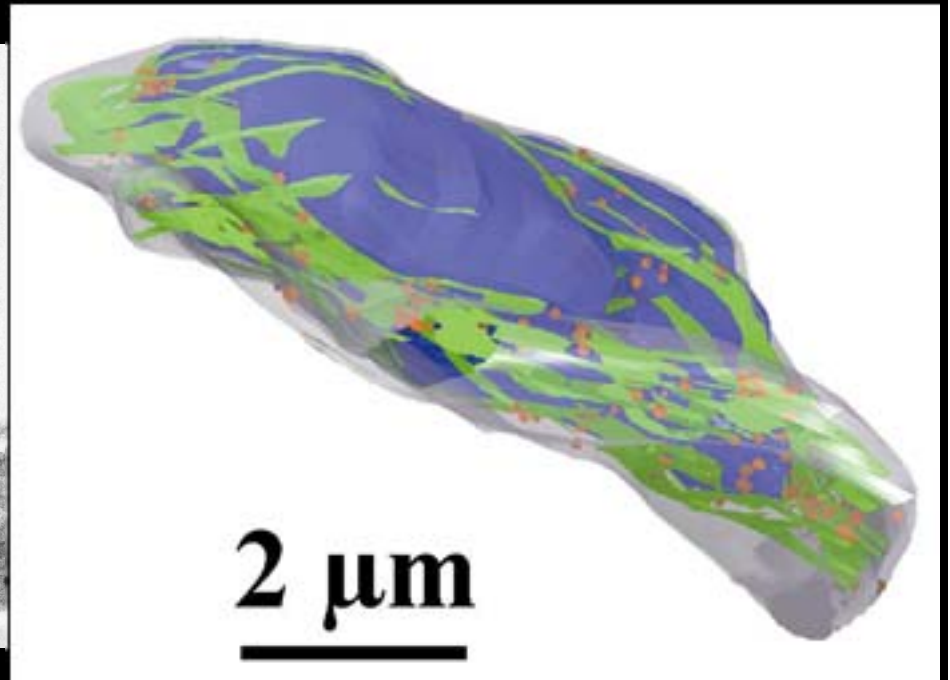
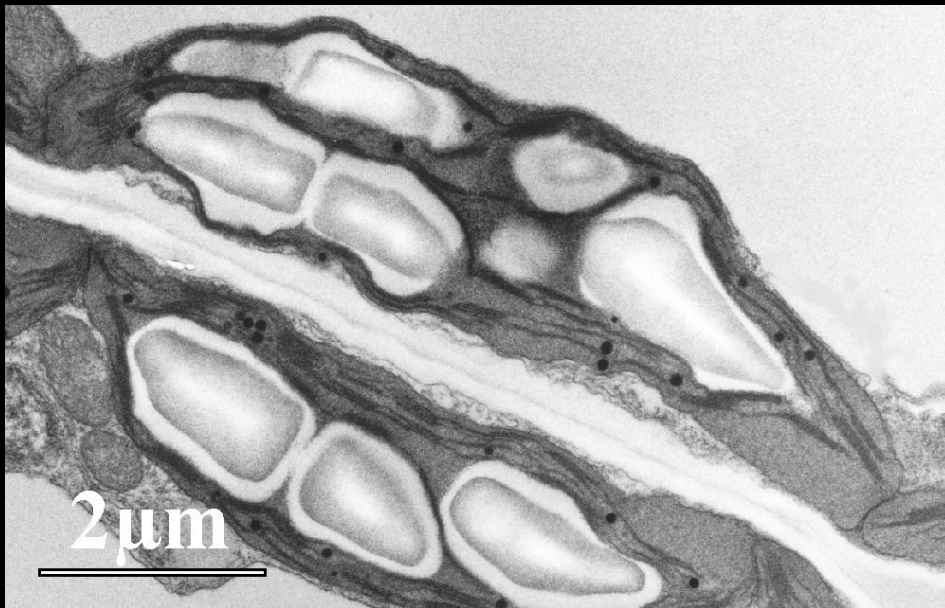
Spinacia 7.00 h



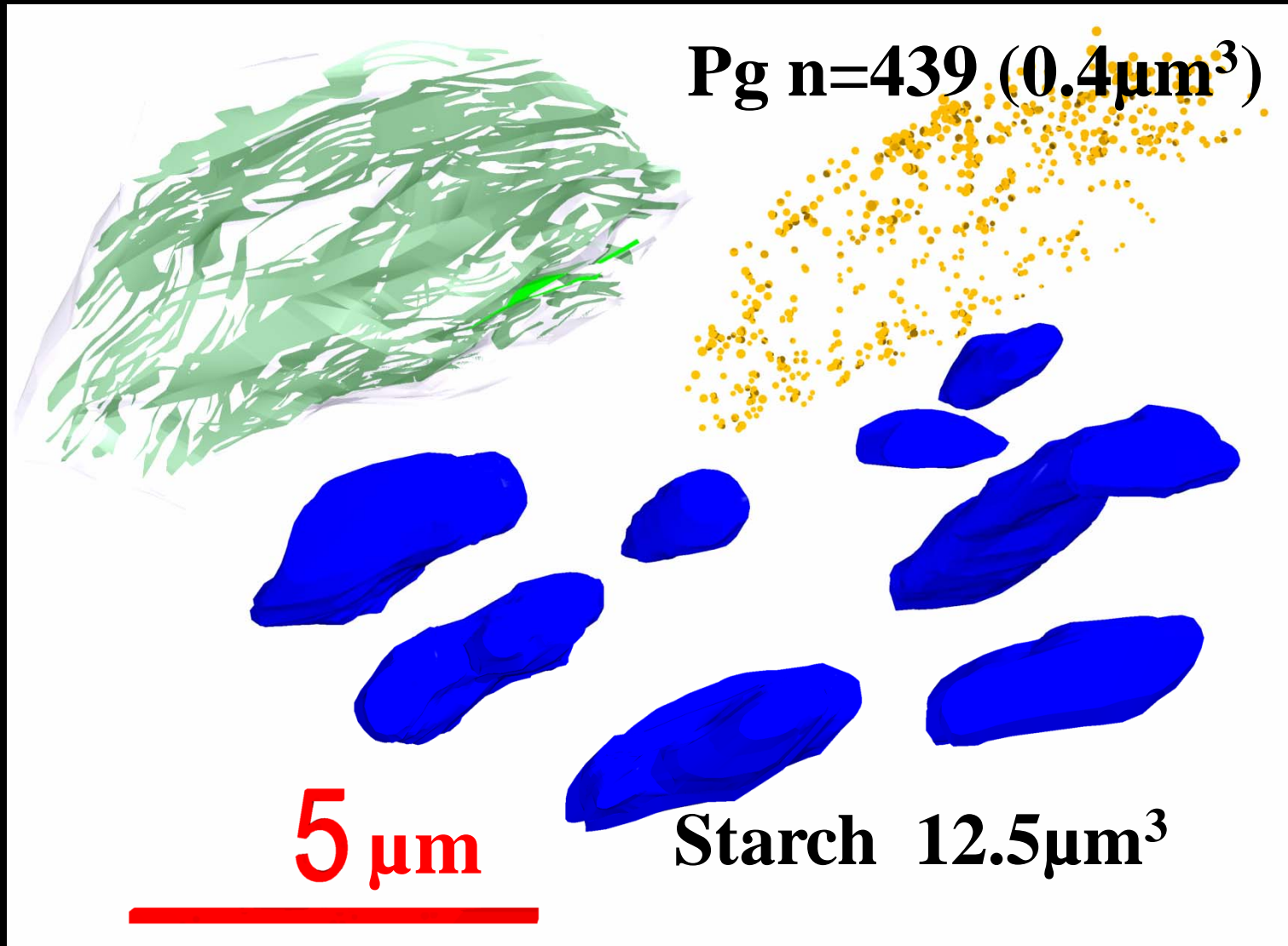
Spinacia 13.00 h



Spinacia 19.00 h

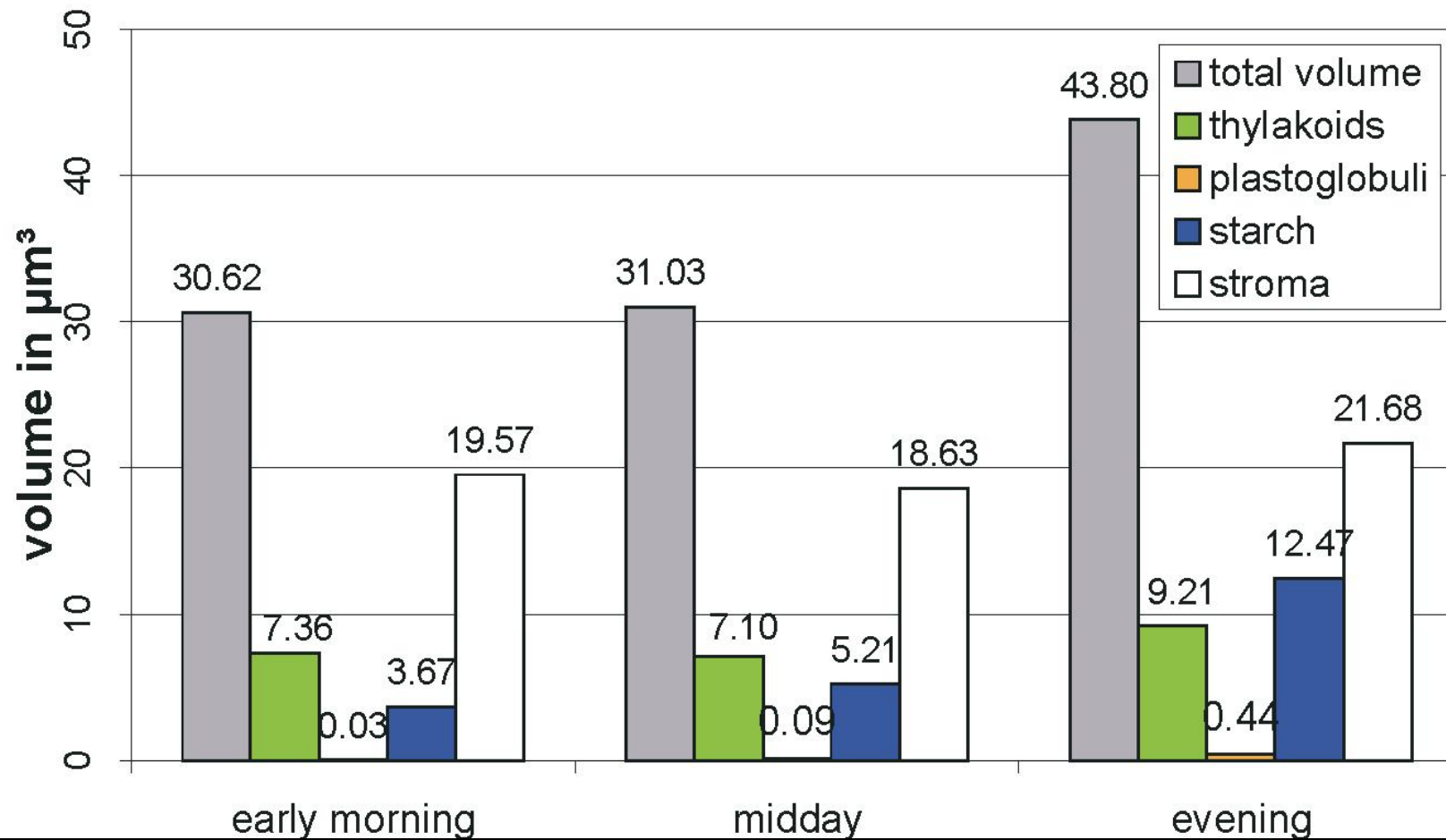


Spinach single chloroplast

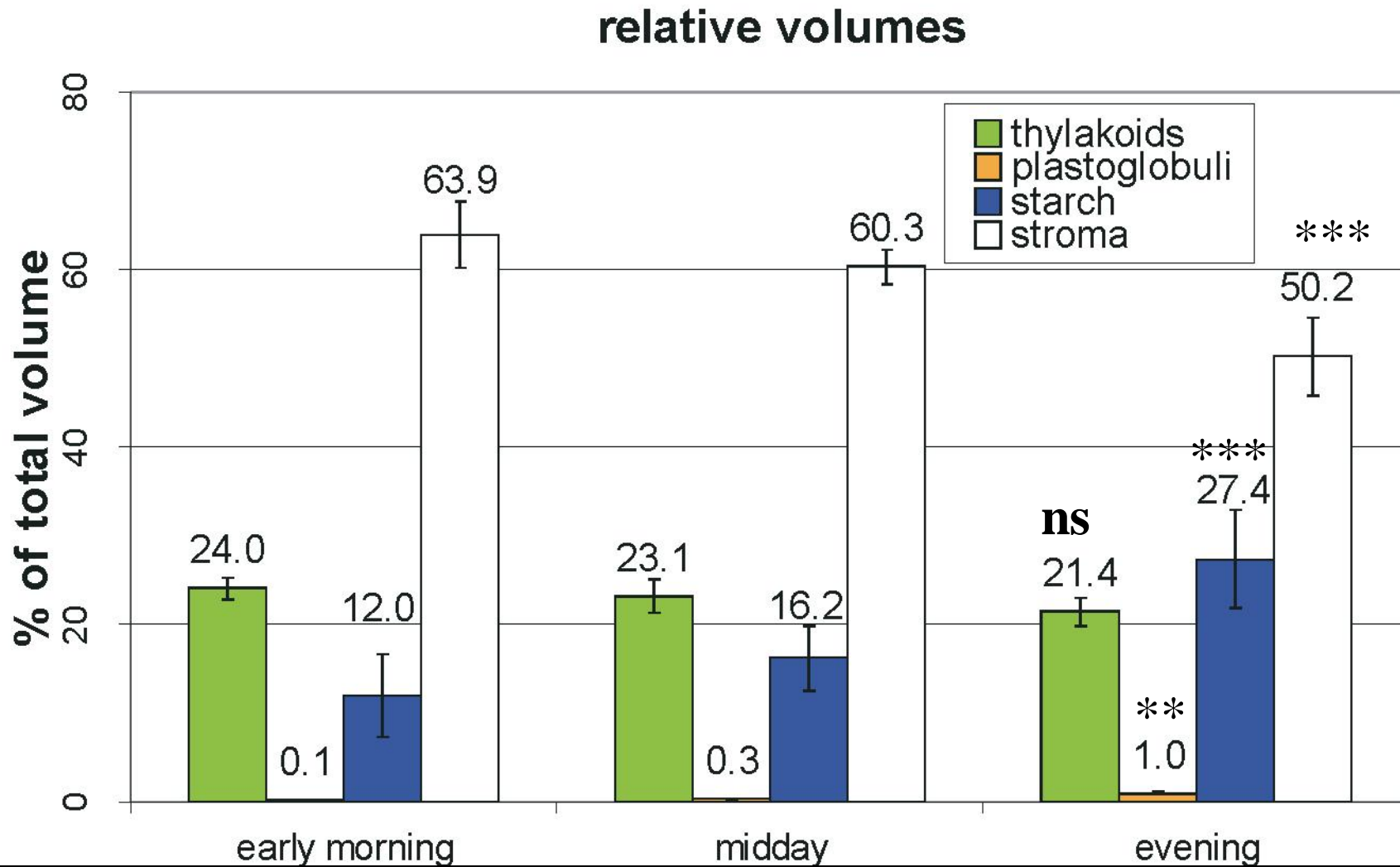


Spinacia chloroplasts

mean values of total volumes



Spinacia chloroplasts



starch, plastoglobuli: increase
stroma: decrease

Spinacia oleracea

Effect of drought stress

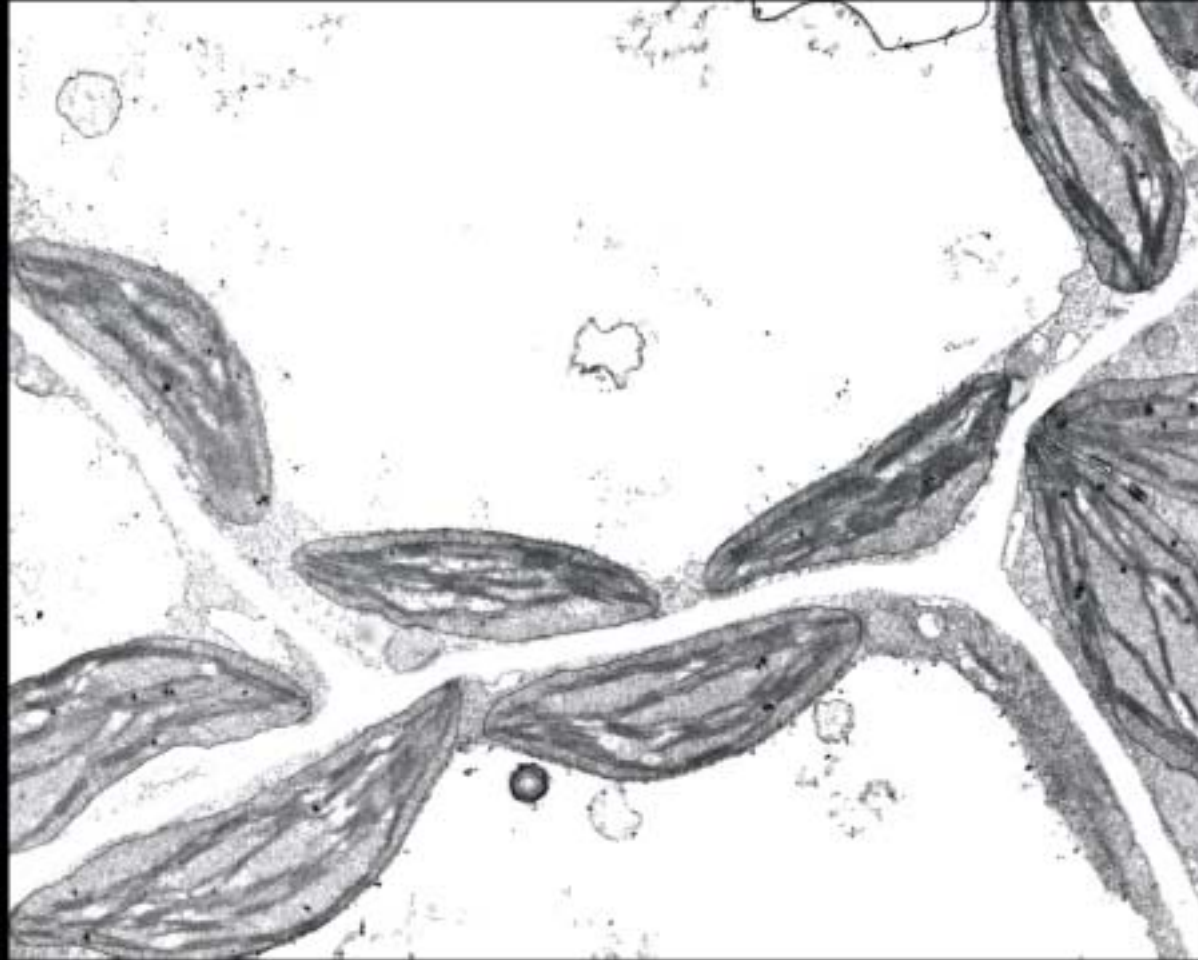
Total number of measured areas (chloroplasts):

45 000

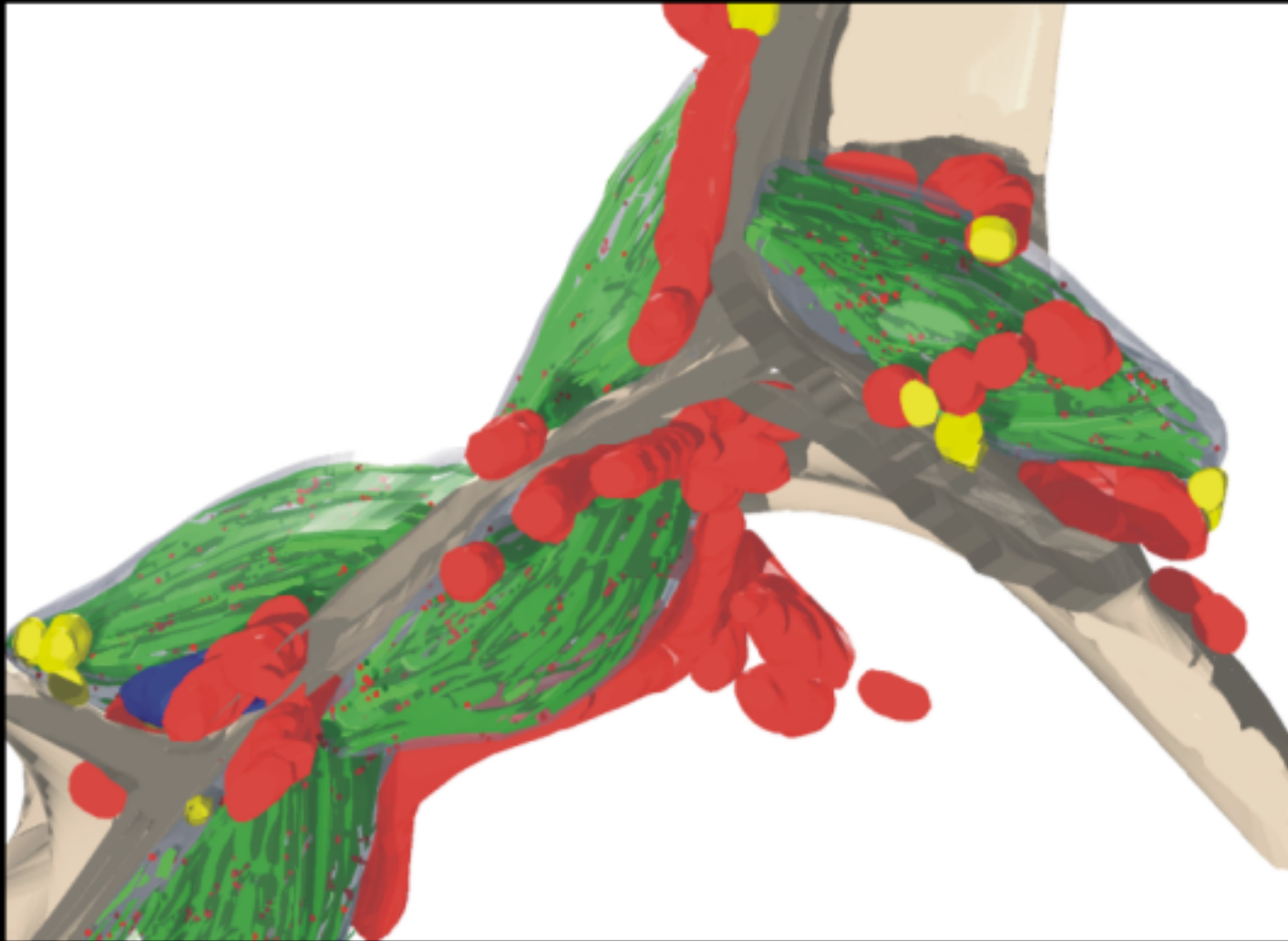
Total number of measured sectioned chloroplasts:

722

Spinacia drought stress

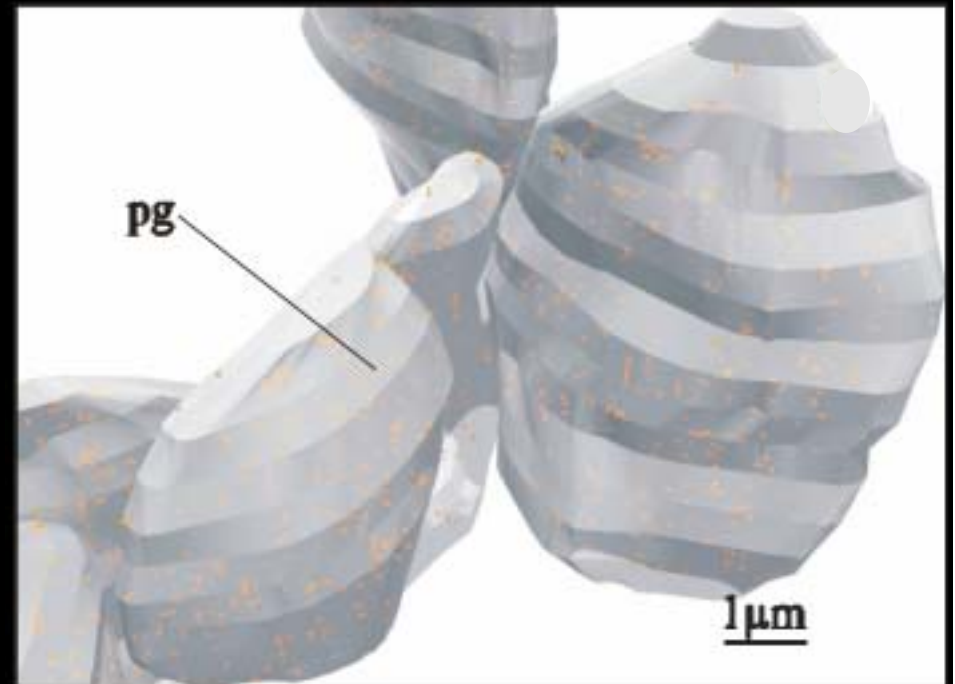
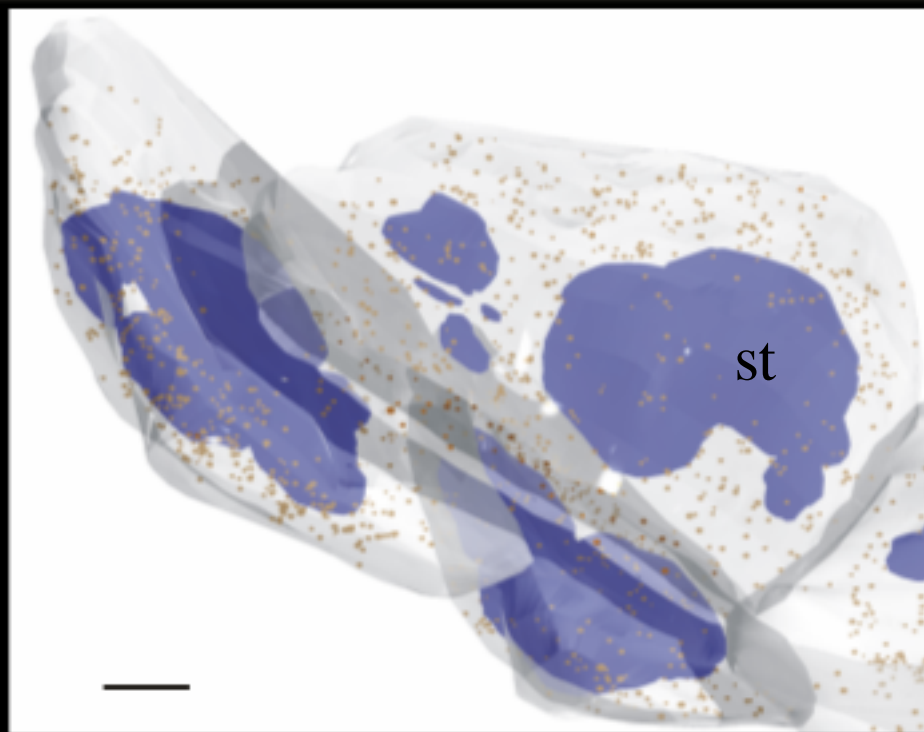


Spinacia drought stress

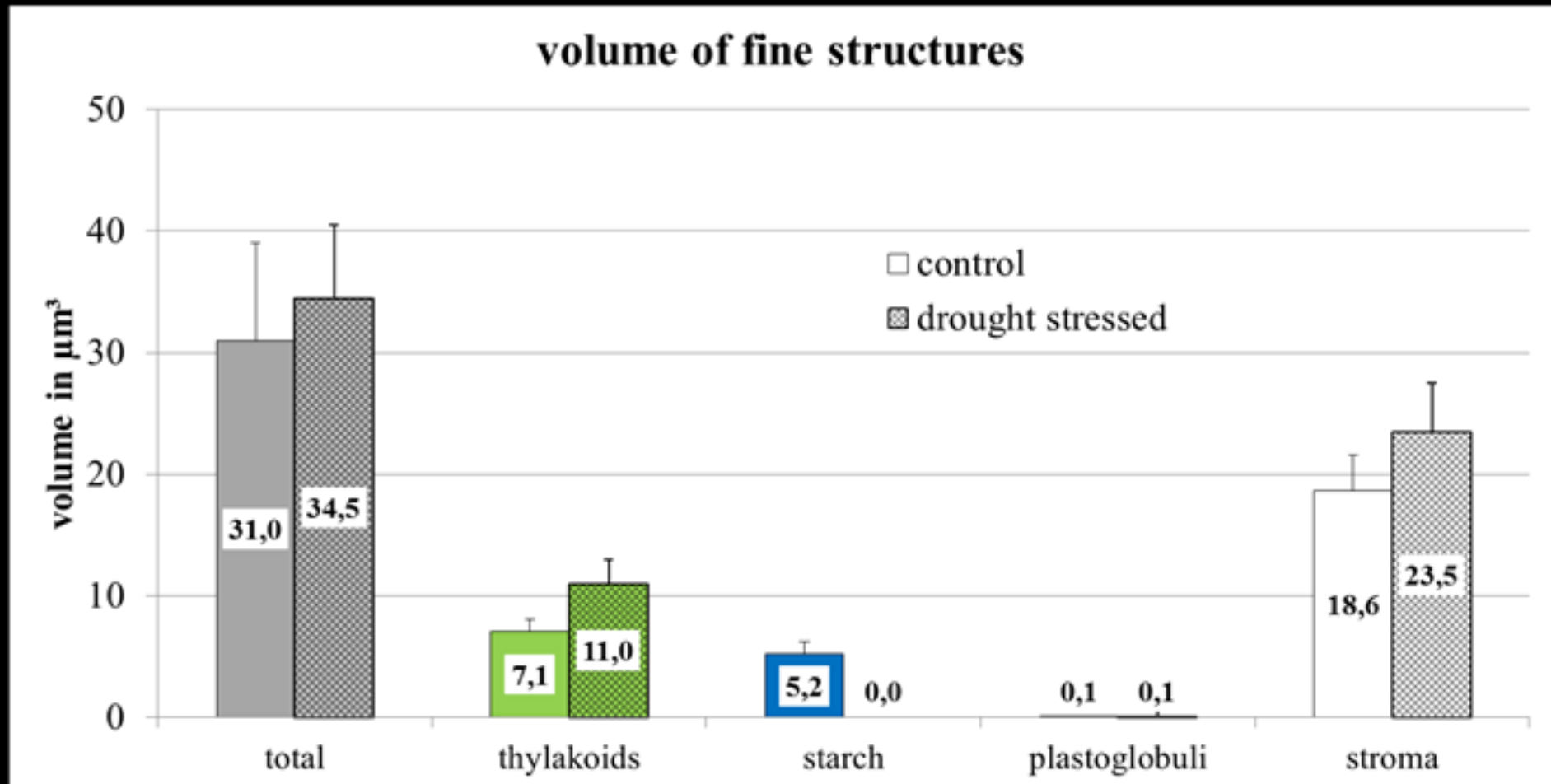


Spinacia drought stress

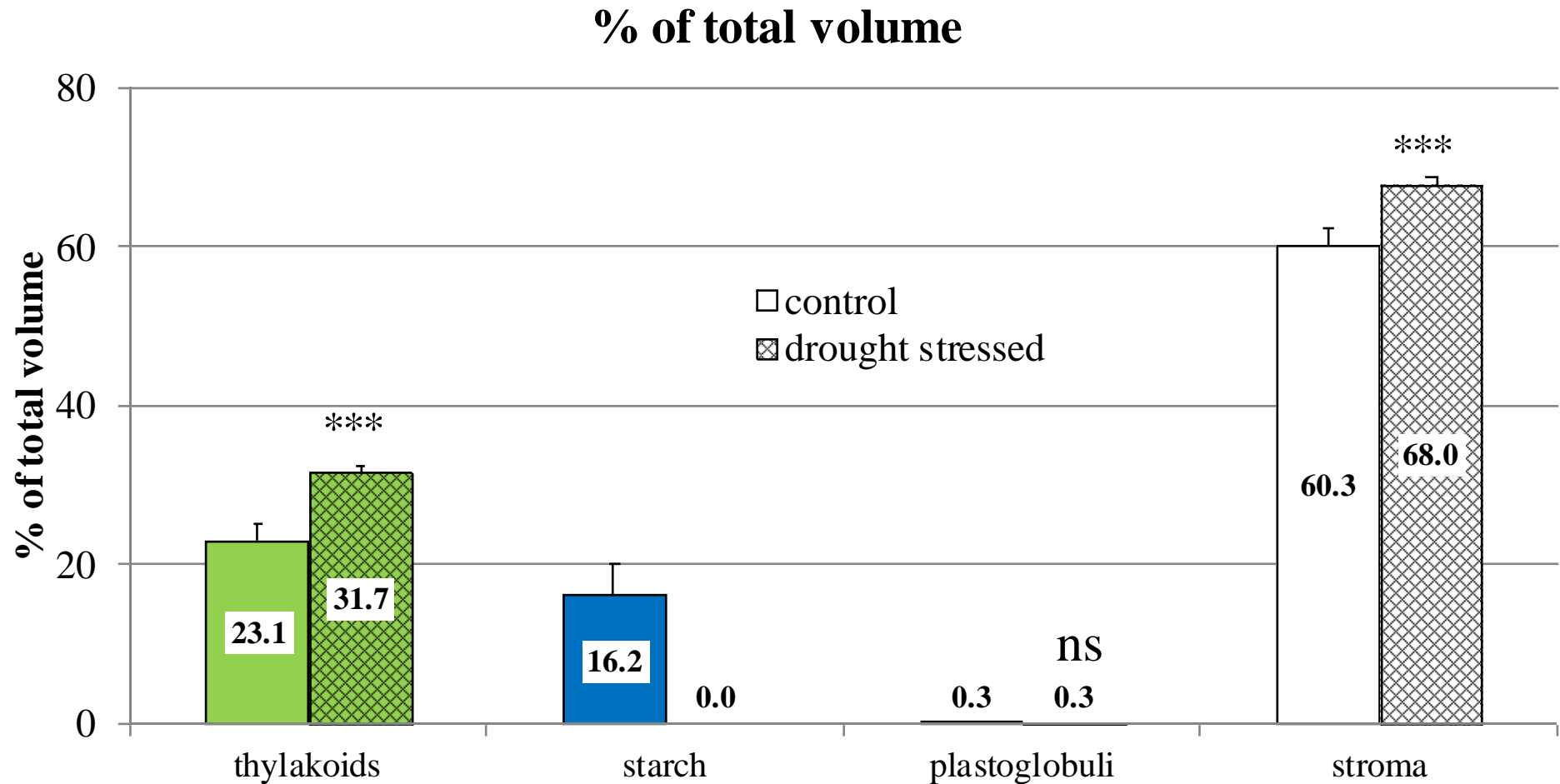
control



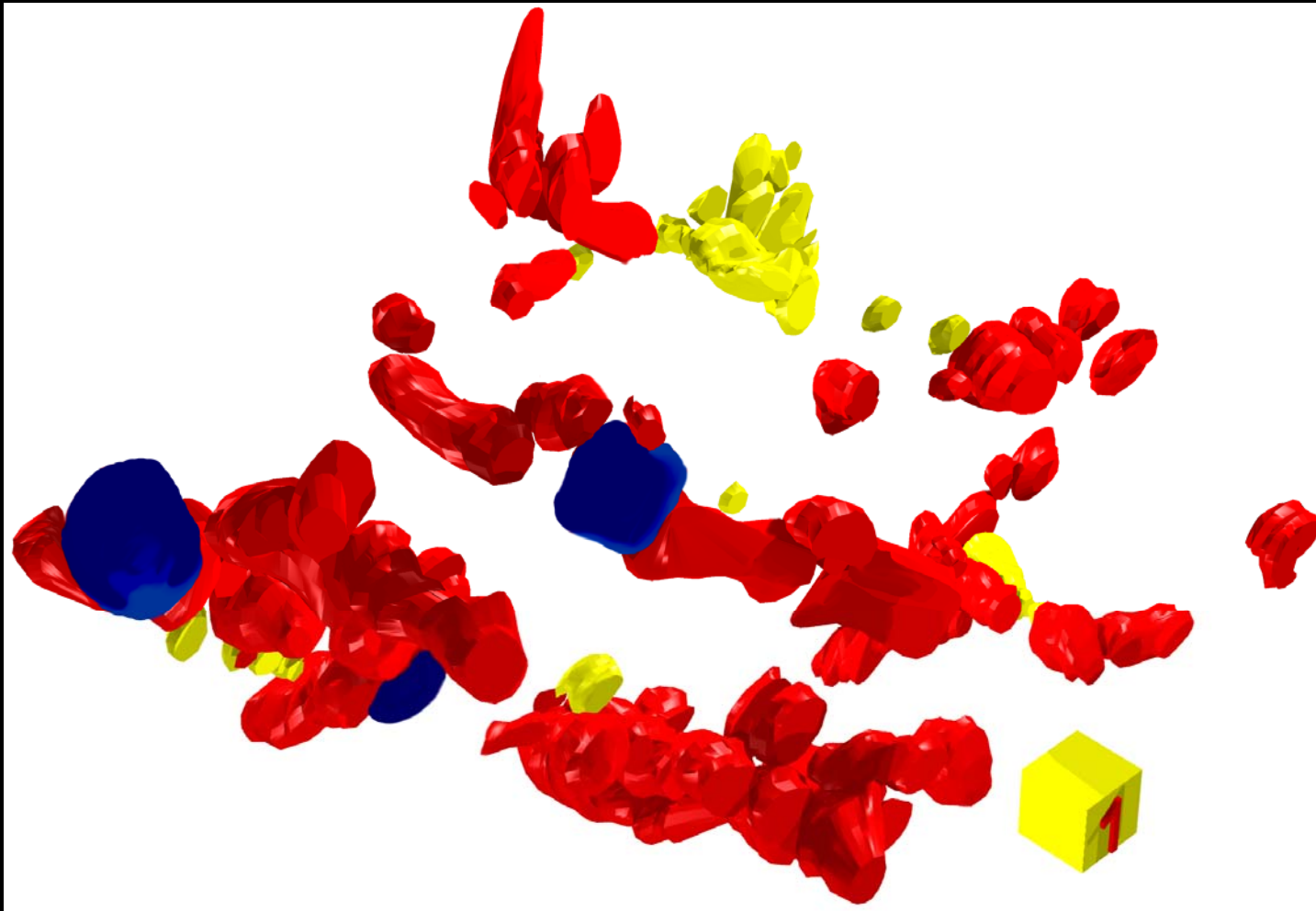
Spinacia chloroplast structures - volumes



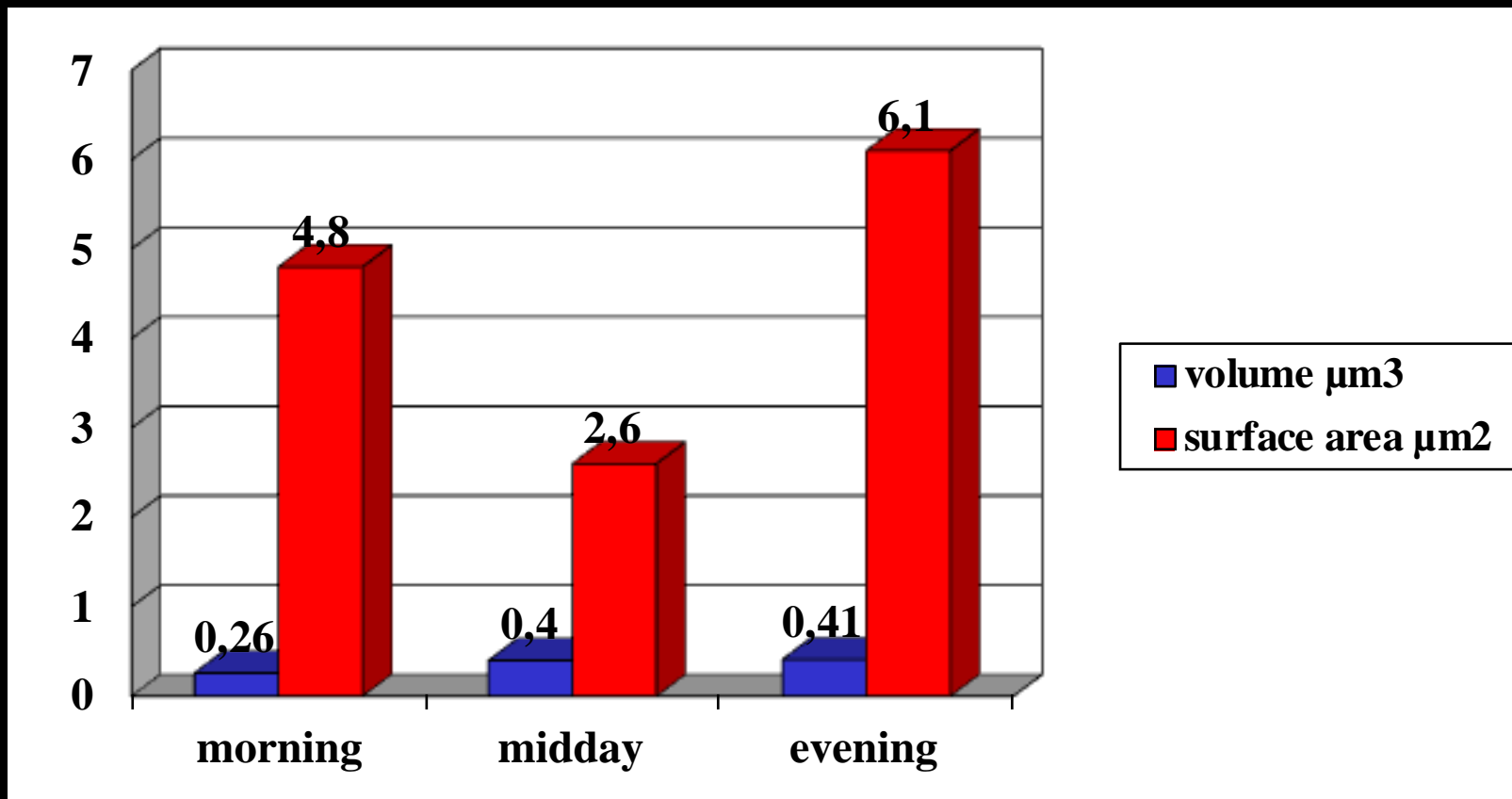
Spinacia chloroplasts – relative volume values



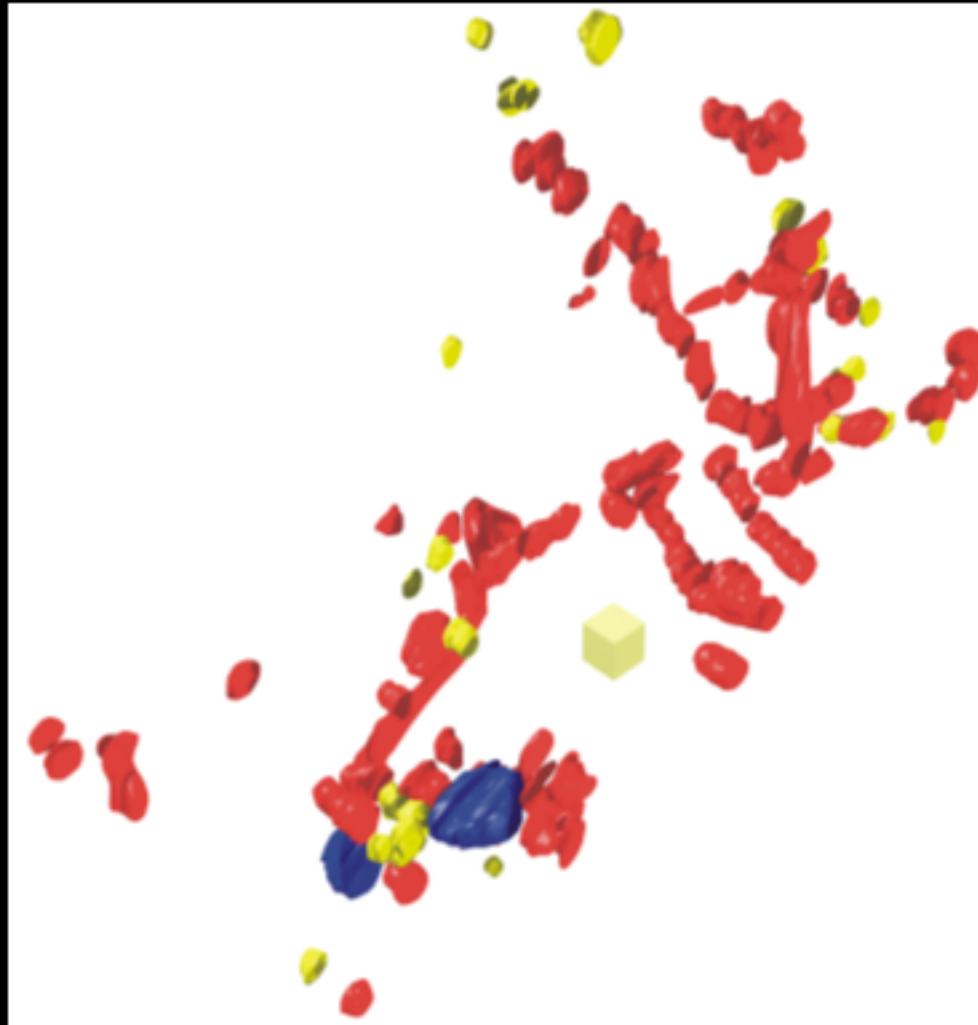
Spinacia control – Mitochondria (red)



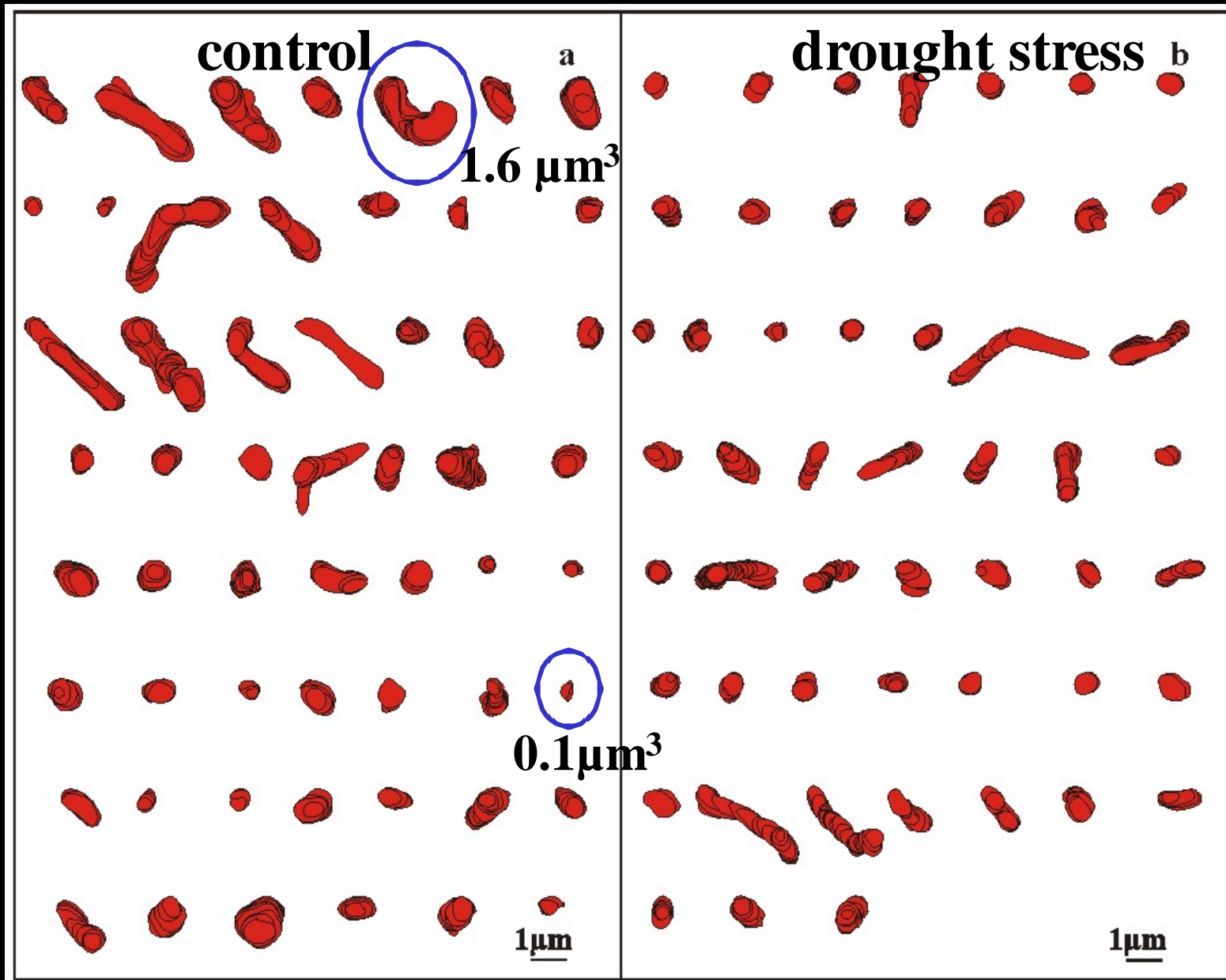
Spinacia – Mitochondria: daily course



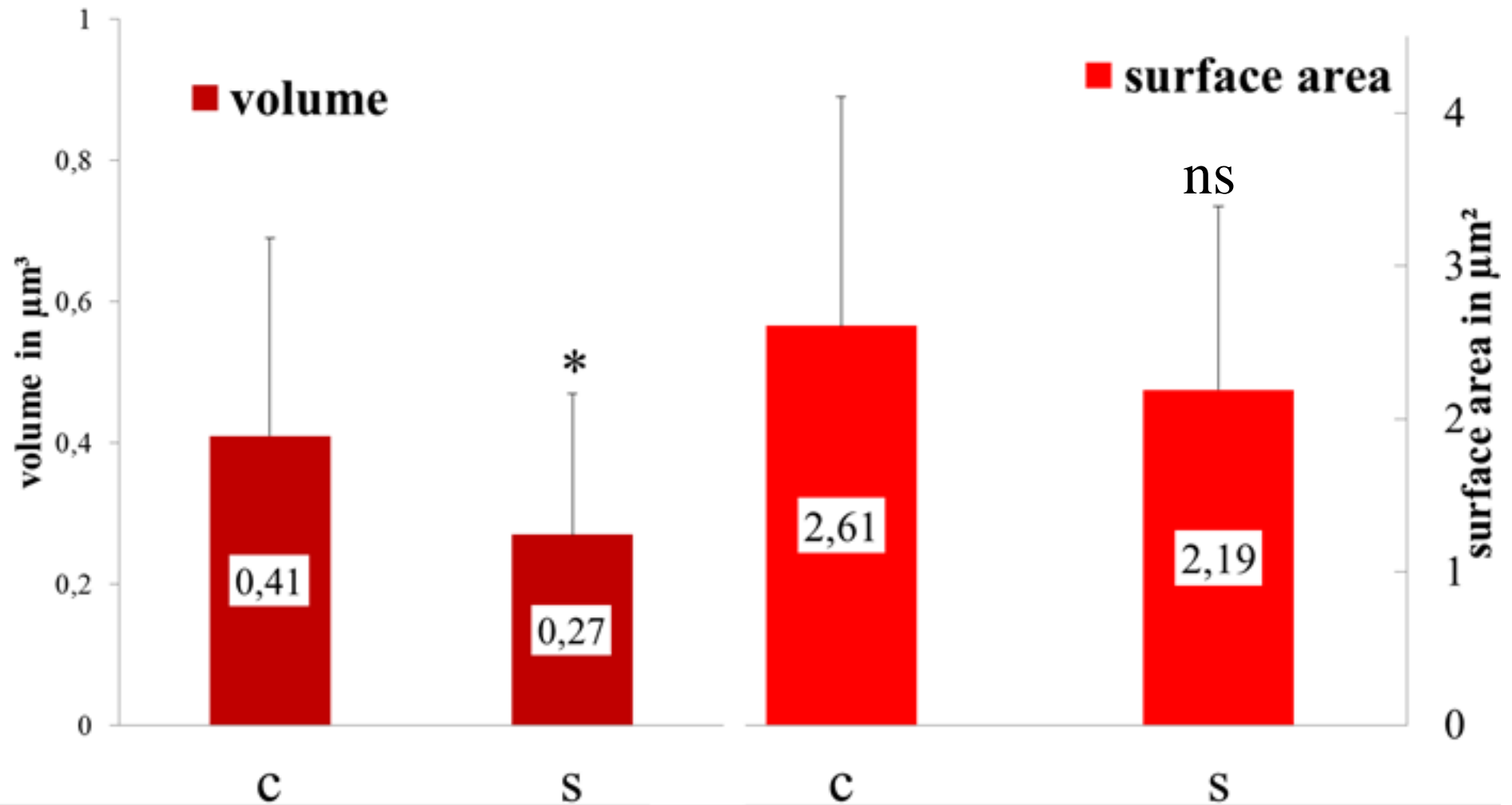
Spinacia drought stress – Mitochondria (red)

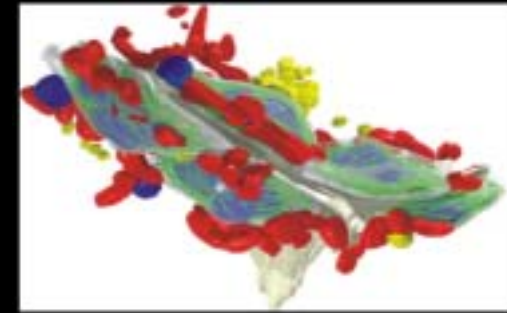
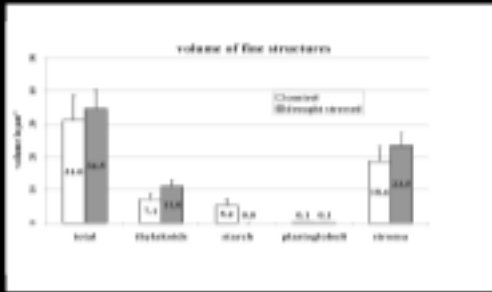


Spinacia - Mitochondria



Spinacia – mitochondria: drought stress





Summary

- 3D measurements of **spinach** chloroplasts show a characteristic internal organization with a good developed thylakoid system, plastoglobuli occurring as single particles and the presence of starch grains
- Chloroplast structures and mitochondria show an adaptation during the daily course

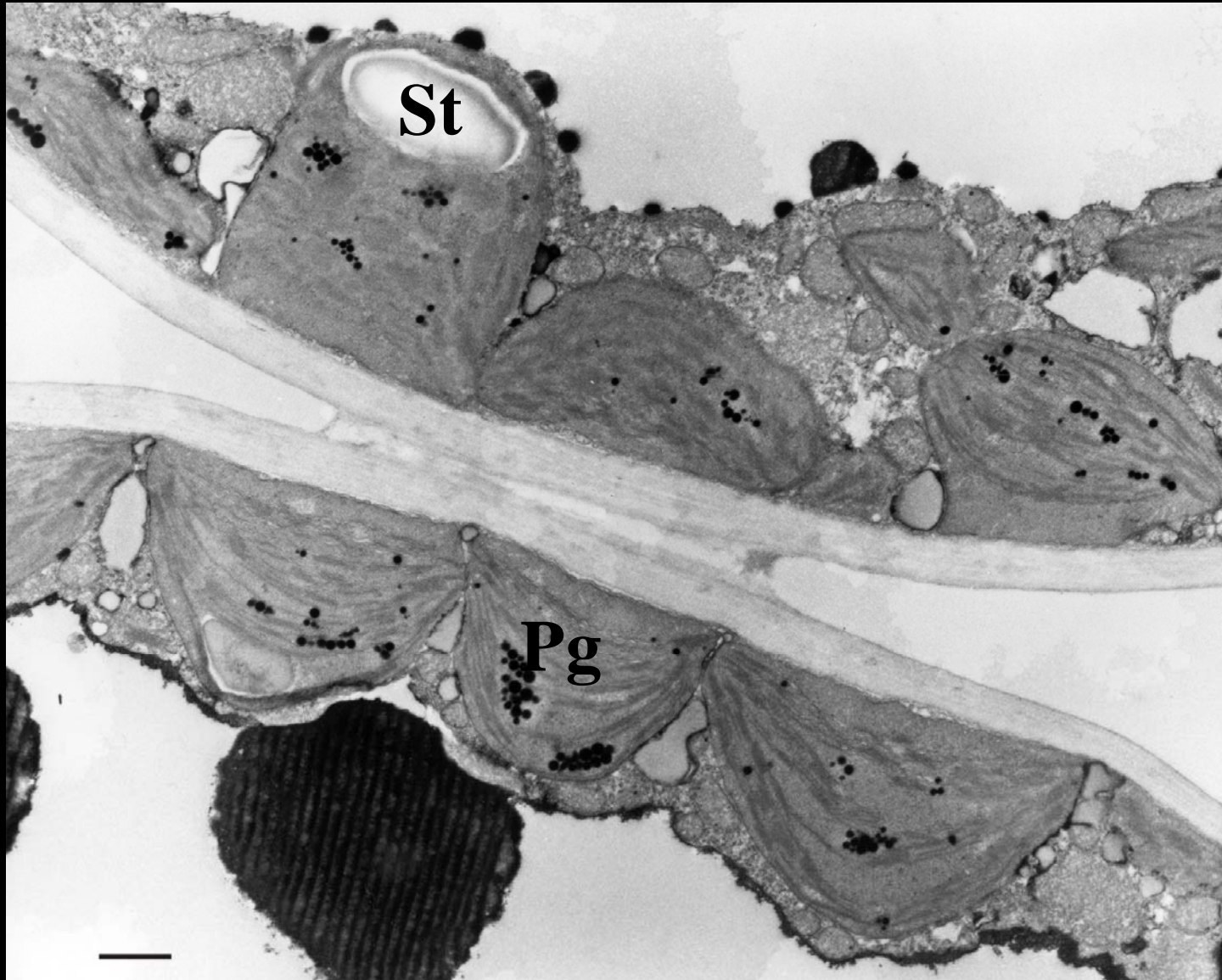
drought stress induces:

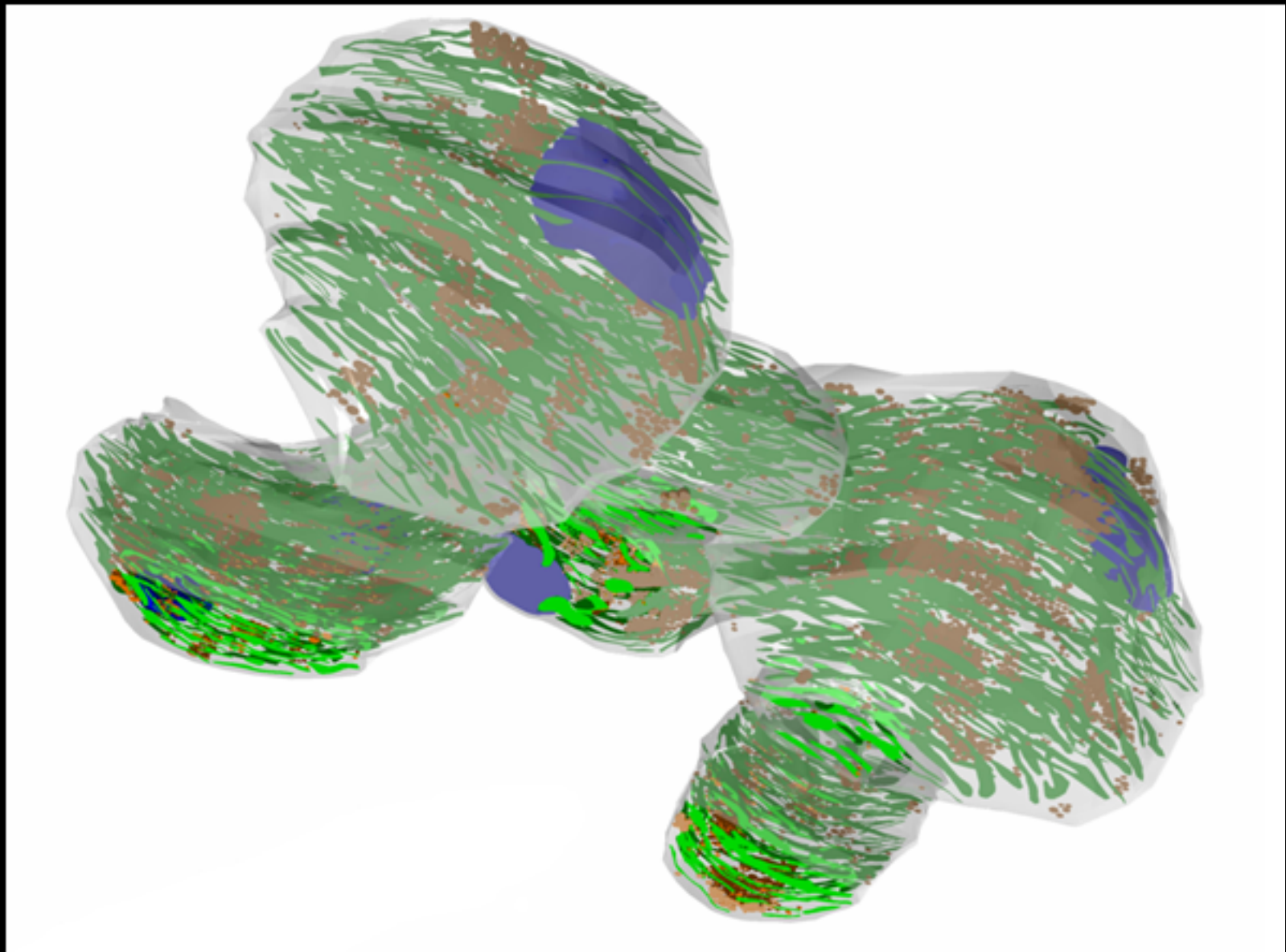
- significant increase of thylakoids and stroma
- absence of starch
- decrease in the mitochondrial volume

Picea abies

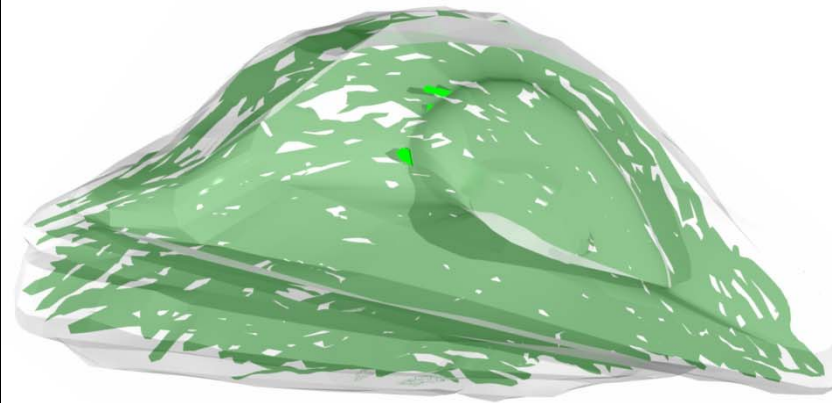


Spruce chloroplasts





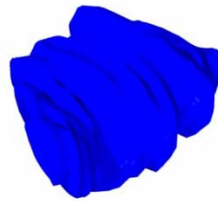
Spruce single chloroplast



Pg $n=1556$ $(2.7\mu\text{m})^3$

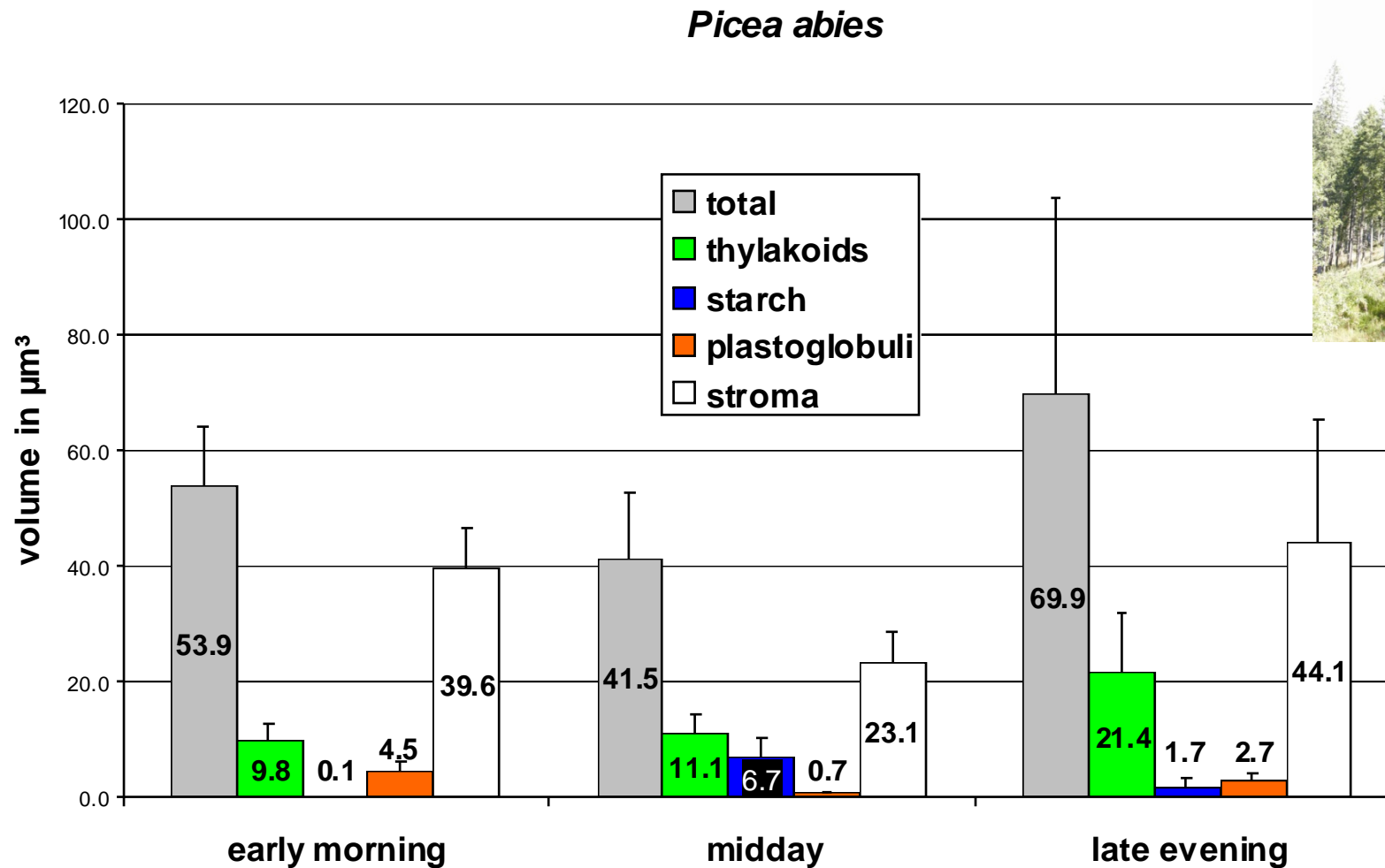


1 μm



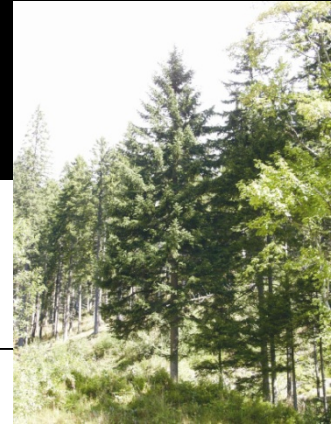
Starch $1.7\mu\text{m}^3$

Absolute volumes of chloroplast structures

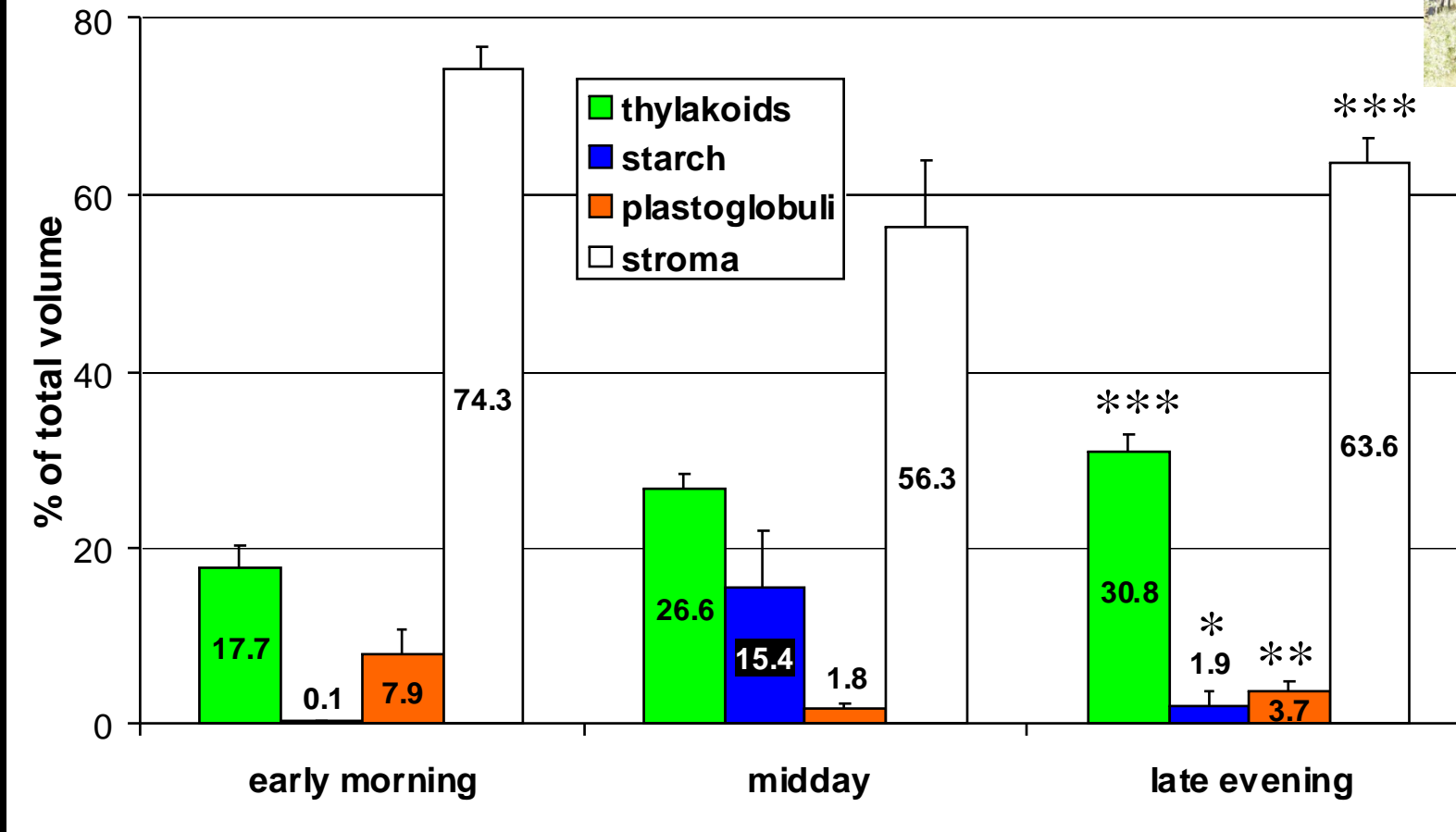


thylakoids , starch : increase
plastoglobuli : decrease

Relative values for chloroplast structures

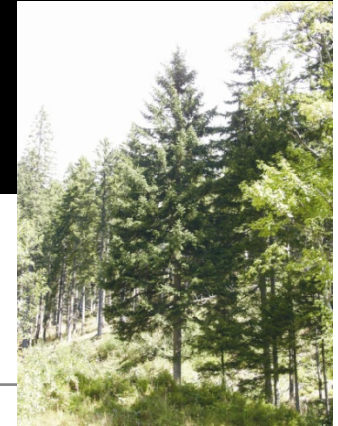


Picea abies relative data

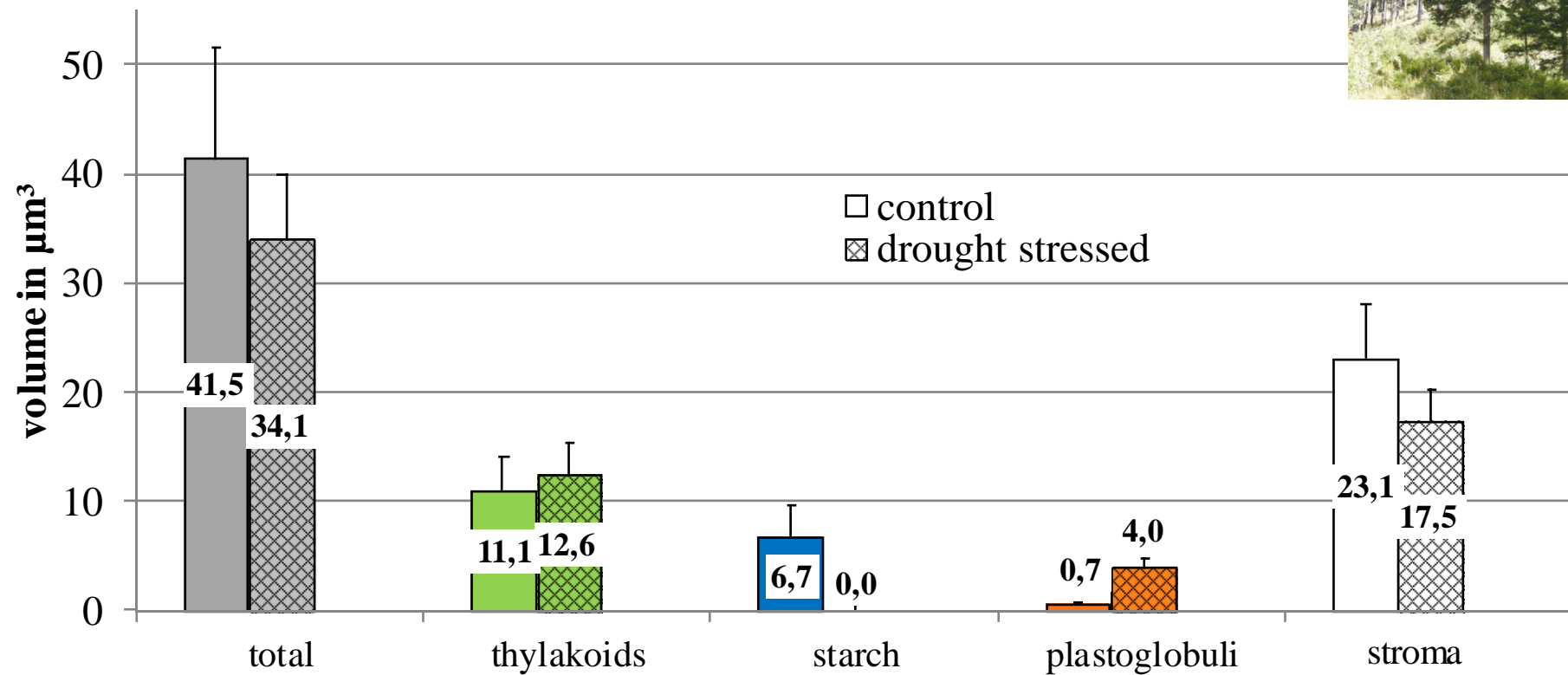


thylakoids, starch: increase
stroma, plastoglobules: decrease

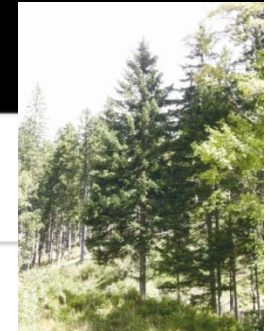
Spruce: drought stress



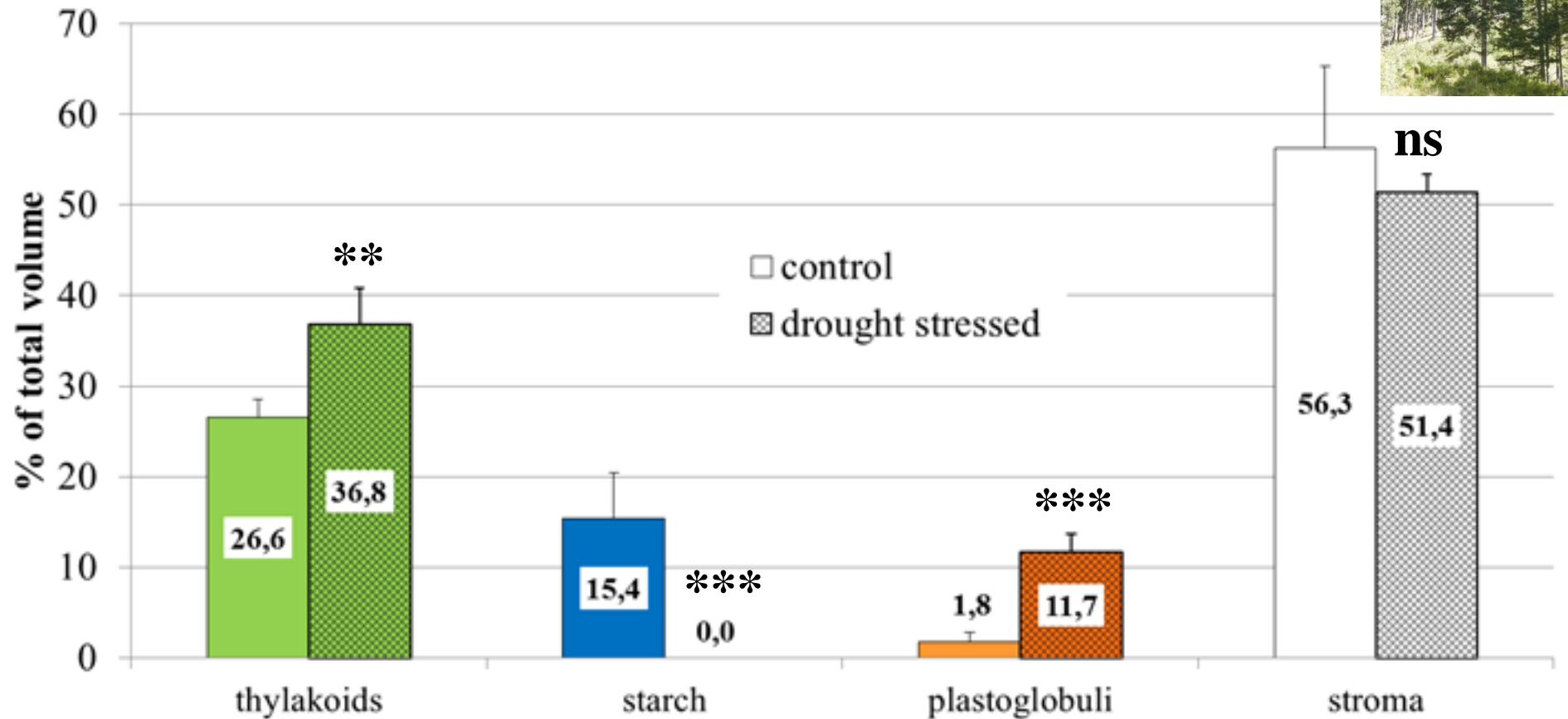
Pices abies – volume of fine structure



Relative values for stressed chloroplast structures



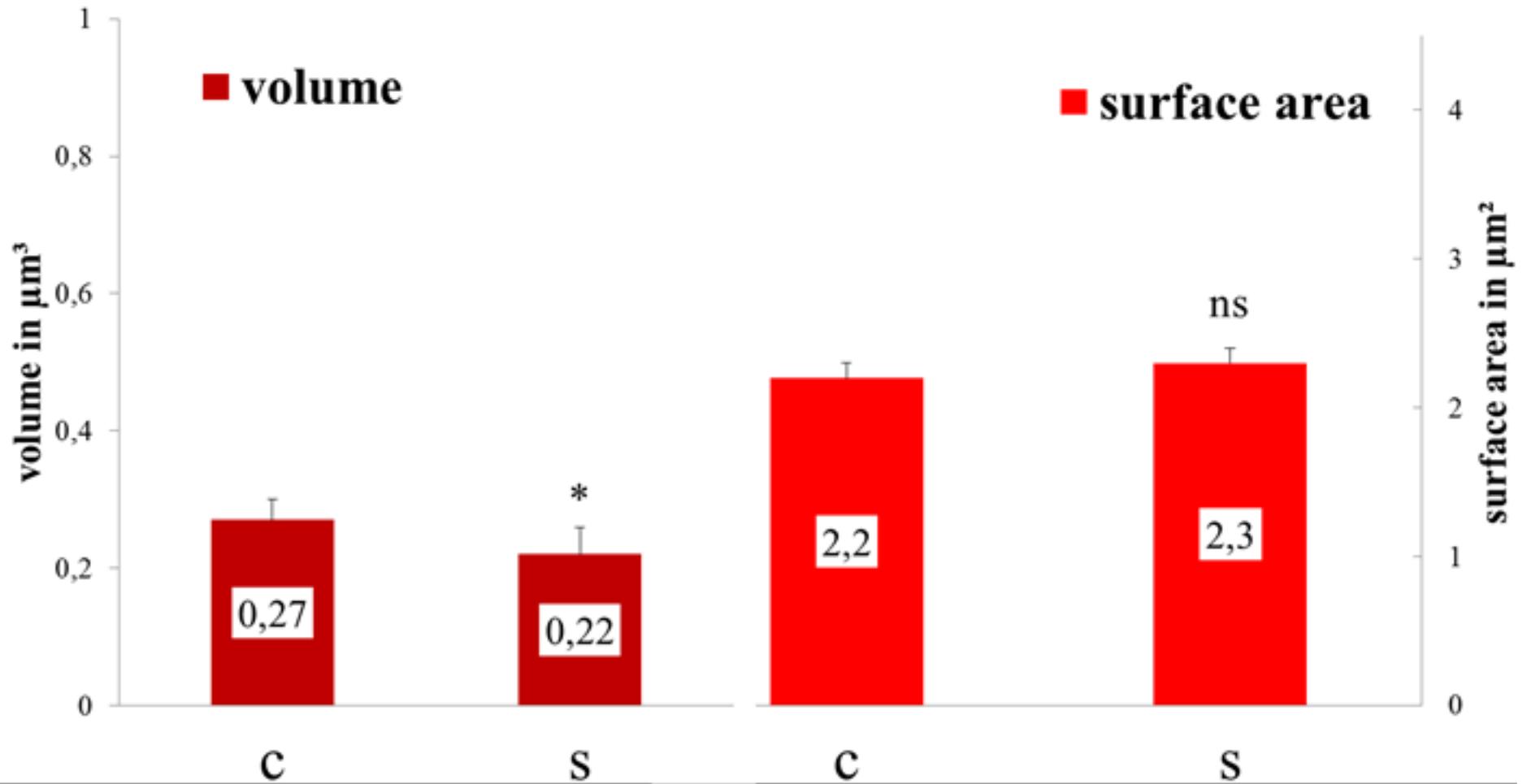
Picea abies - % of total volume



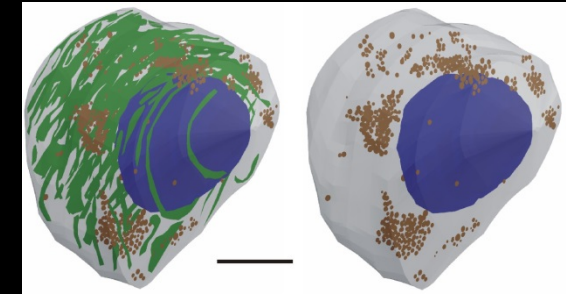
thylakoids, plastoglobuli: increase

starch: strong decrease

Spruce – mitochondria: drought stress



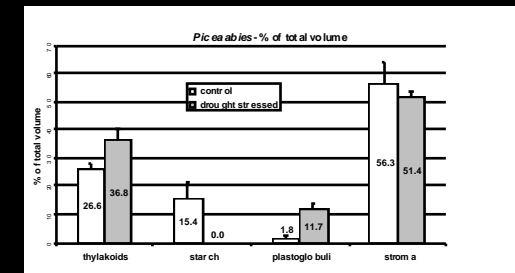
Summary



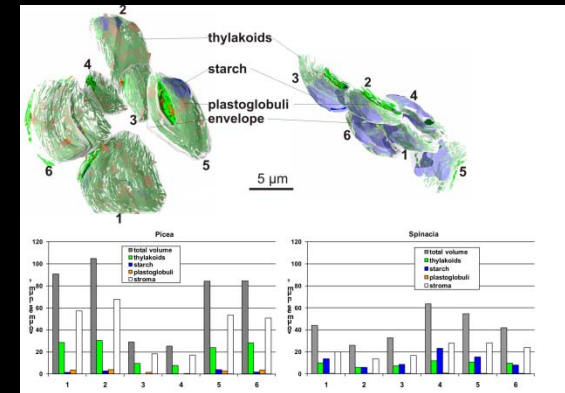
- 3D measurements of **spruce** chloroplasts show a characteristic internal organization with a good developed thylakoid system, plastoglobuli occurring in groups and the presence of starch grains

Drought induces:

- reduction in the volume and surface area
- increase in the thylakoid volume and surface area
- absence of starch grains
- increase in the plastoglobuli content

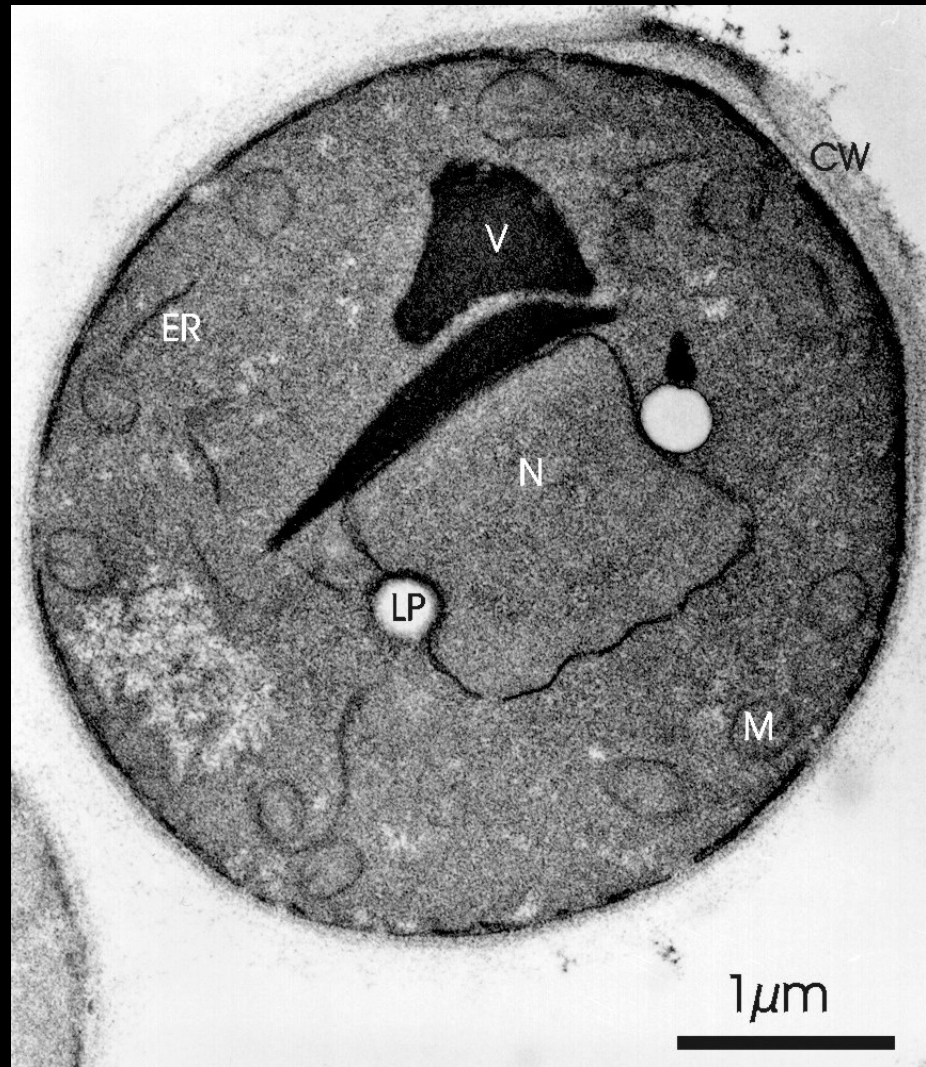


Summary



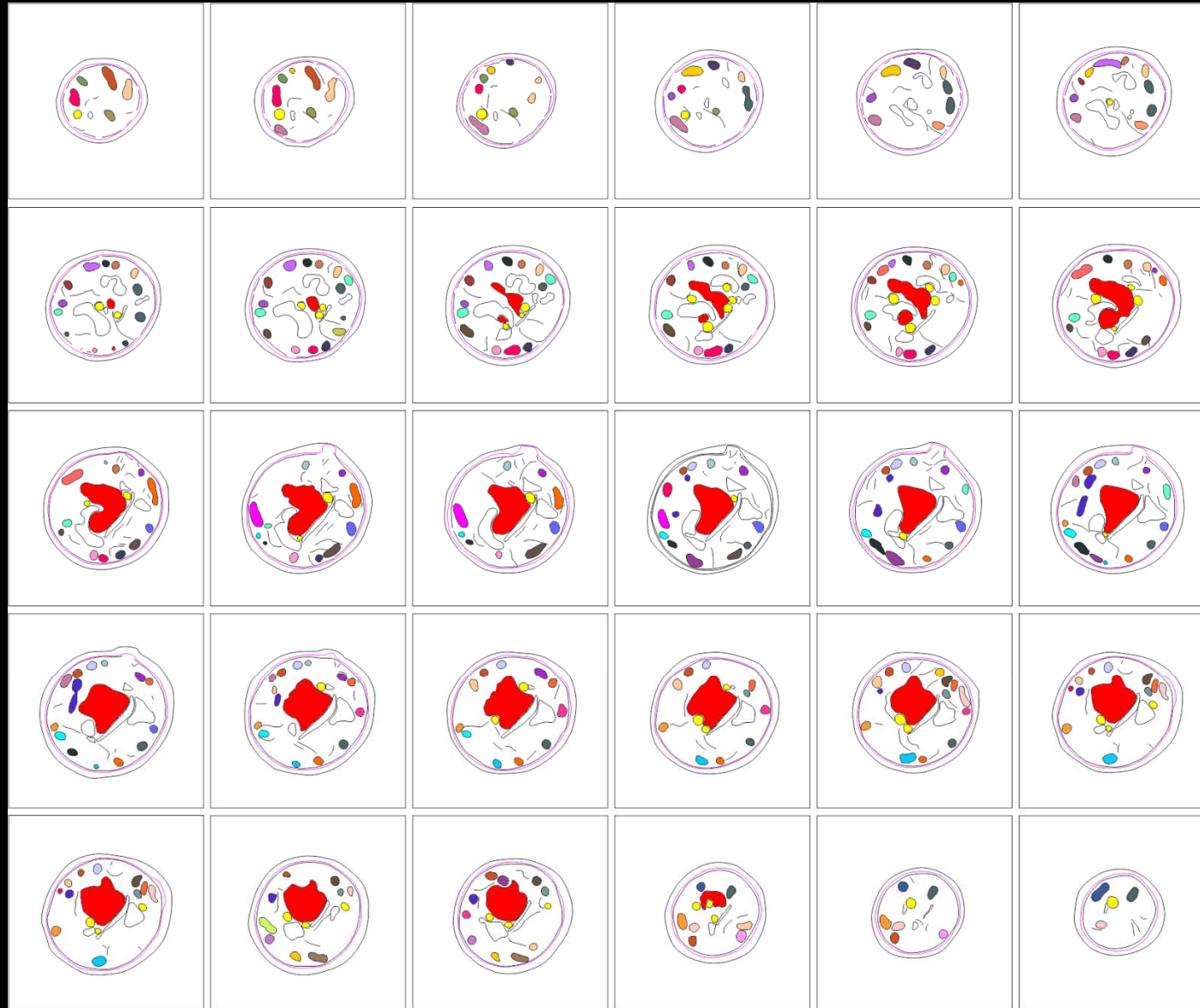
- **Spinach** and **spruce** chloroplasts are different regarding their internal fine structures showing **dynamic adaptations** to diurnal changes or stress factors

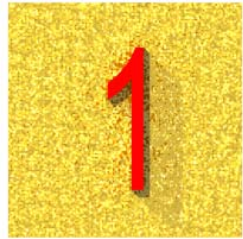
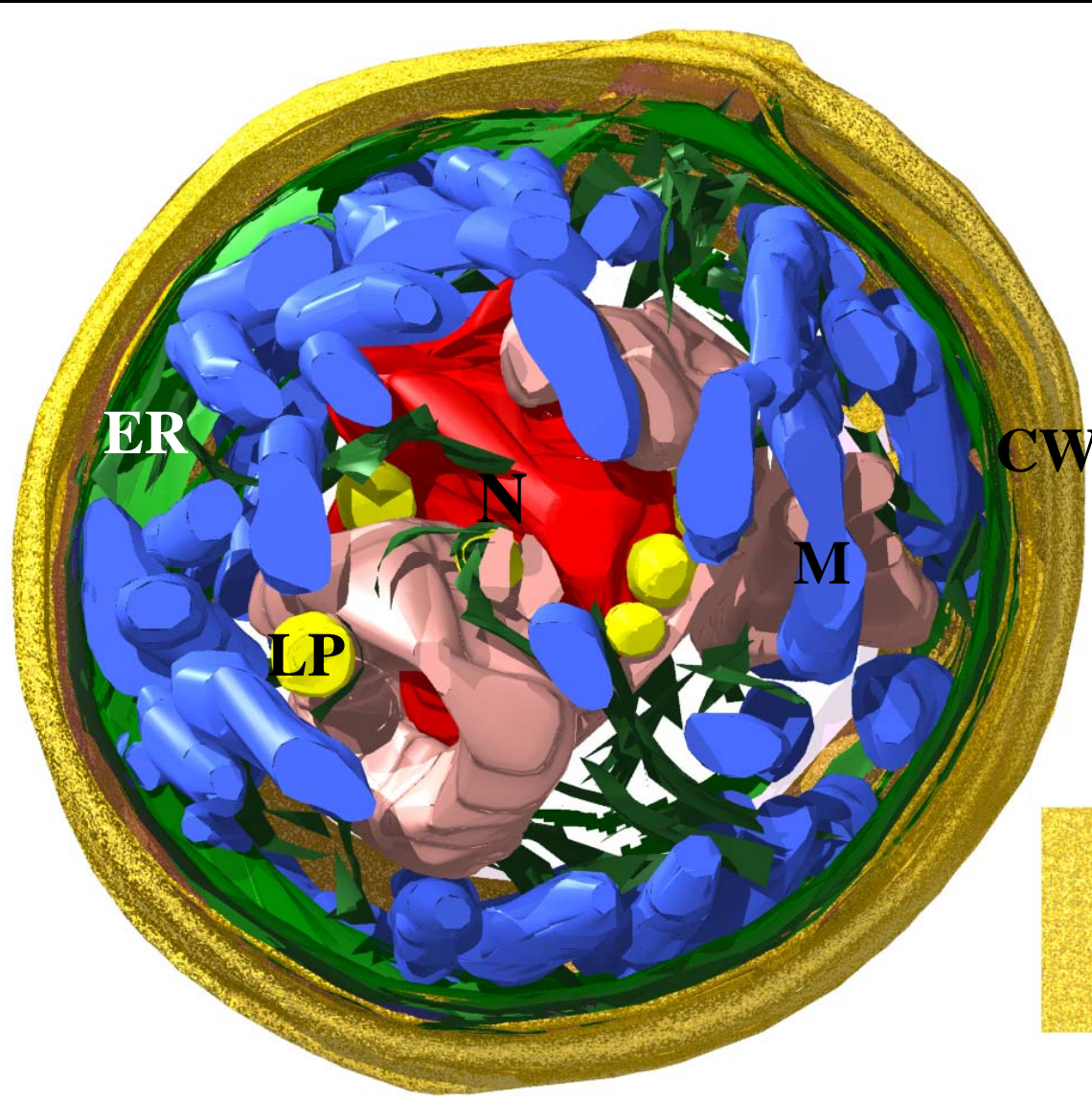
Yeast cell (*Saccharomyces cerevisiae*)



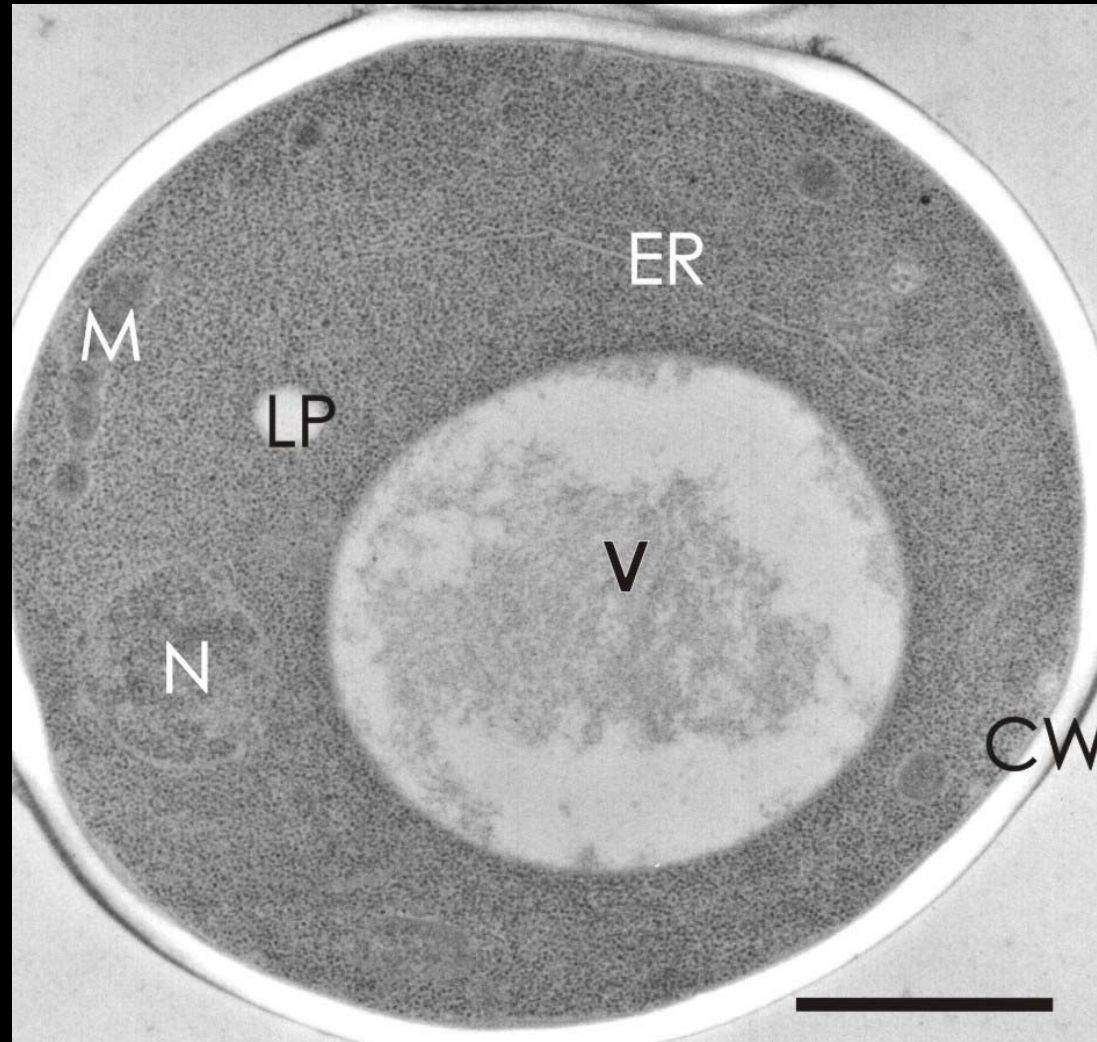
Chemical fixation

Traced serial sections

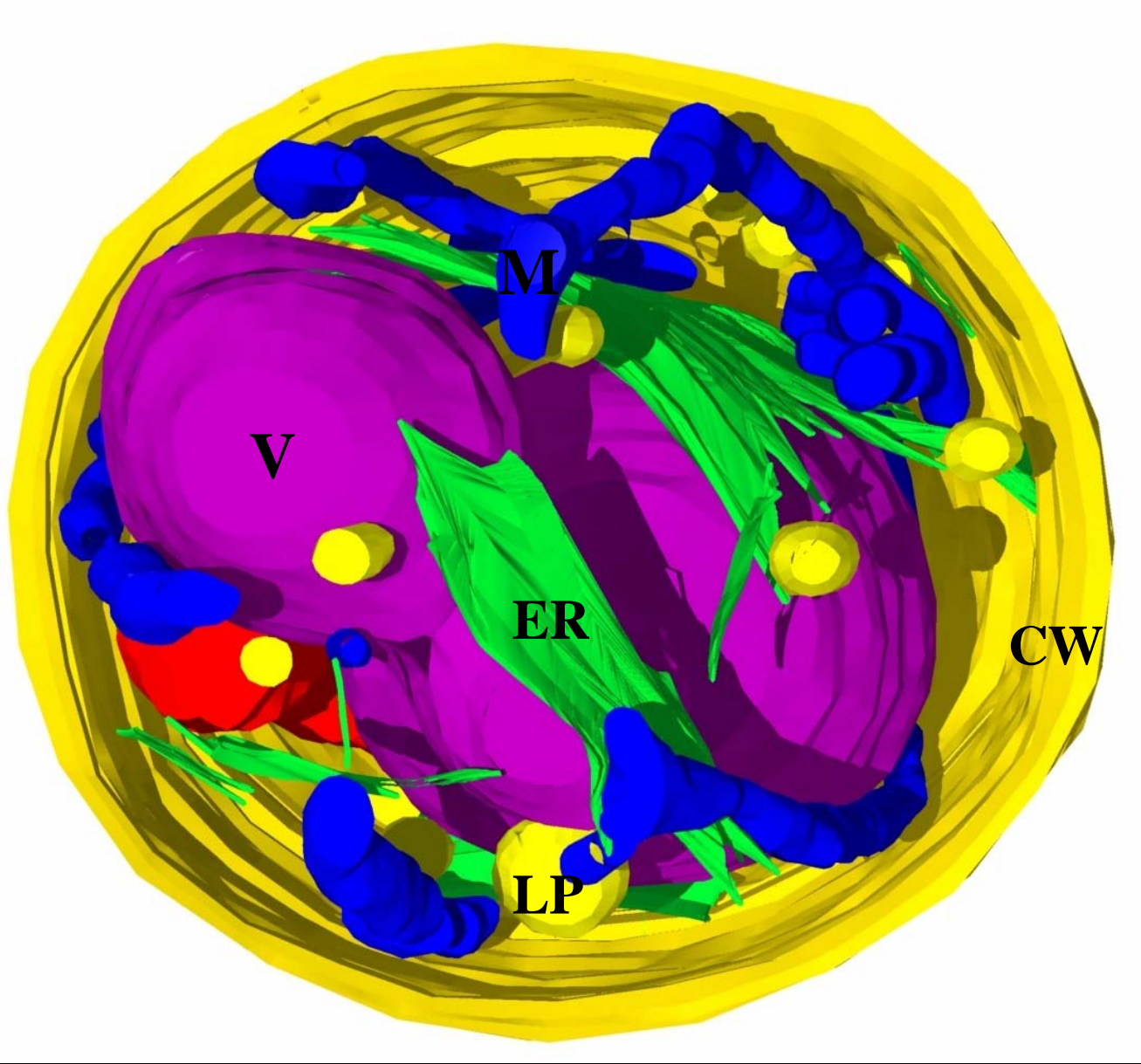




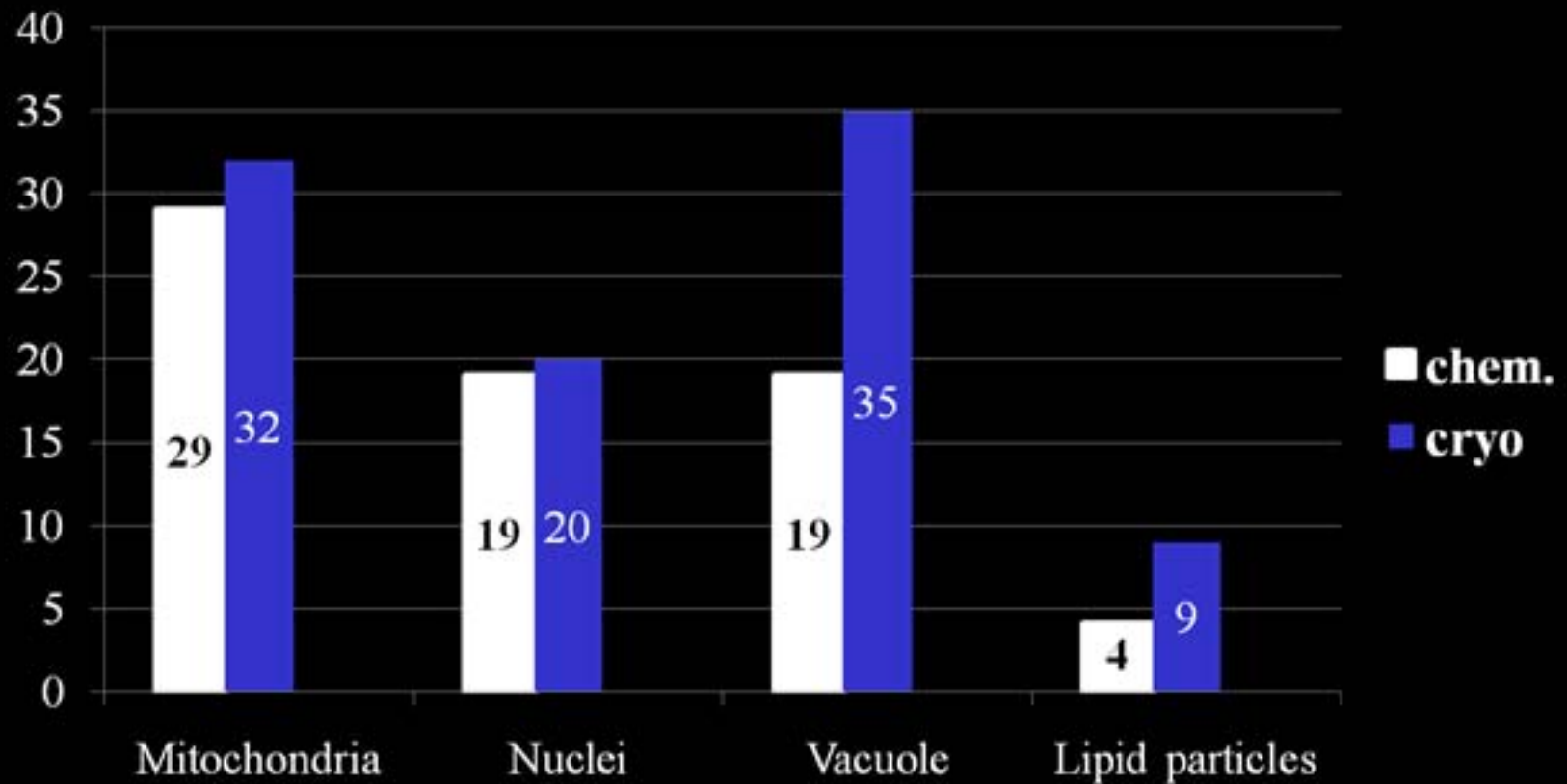
Yeast cell



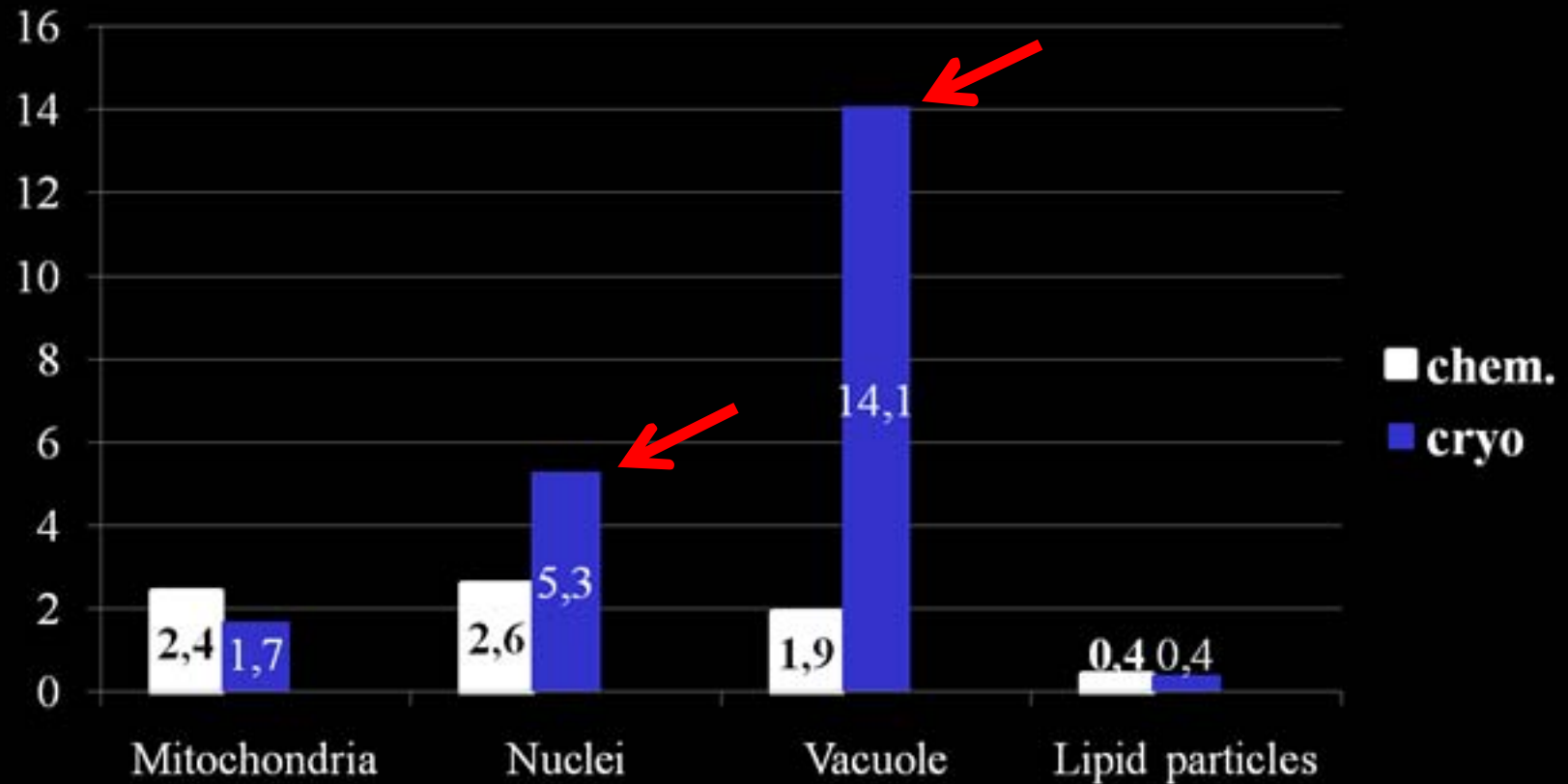
Cryofixation



Surface areas in μm^2



Volumes in μm^3



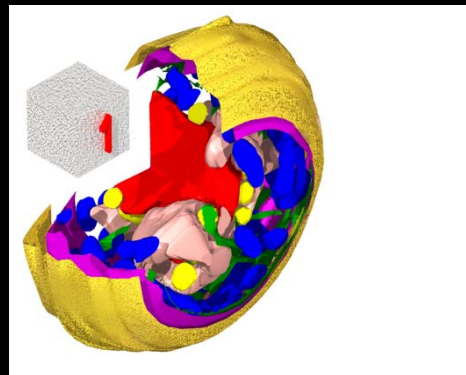
Number of associations

chemically/cryofixed

	ER	M	N	V	LP	PM
ER	18/0	79/11	1/0	2/4	14/1	1139/10
M	79/11	4/7	0/4	0/2	0/4	0/1
N	1/0	0/4	0/0	22/3	35/2	0/0
V	2/4	0/2	22/3	15/0	32/1	0/0
LP	14/1	0/4	35/2	32/1	2/3	0/0
PM	1139/10	0/1	0/0	0/0	0/0	0/0

Summary

- 3D measurements of differently fixed yeast cells clearly show **differences in the volumes** of certain cell organelles thereby also affecting the association rate between organelles.



Zucchini Yellow Mosaic Virus (ZYMV) Infection

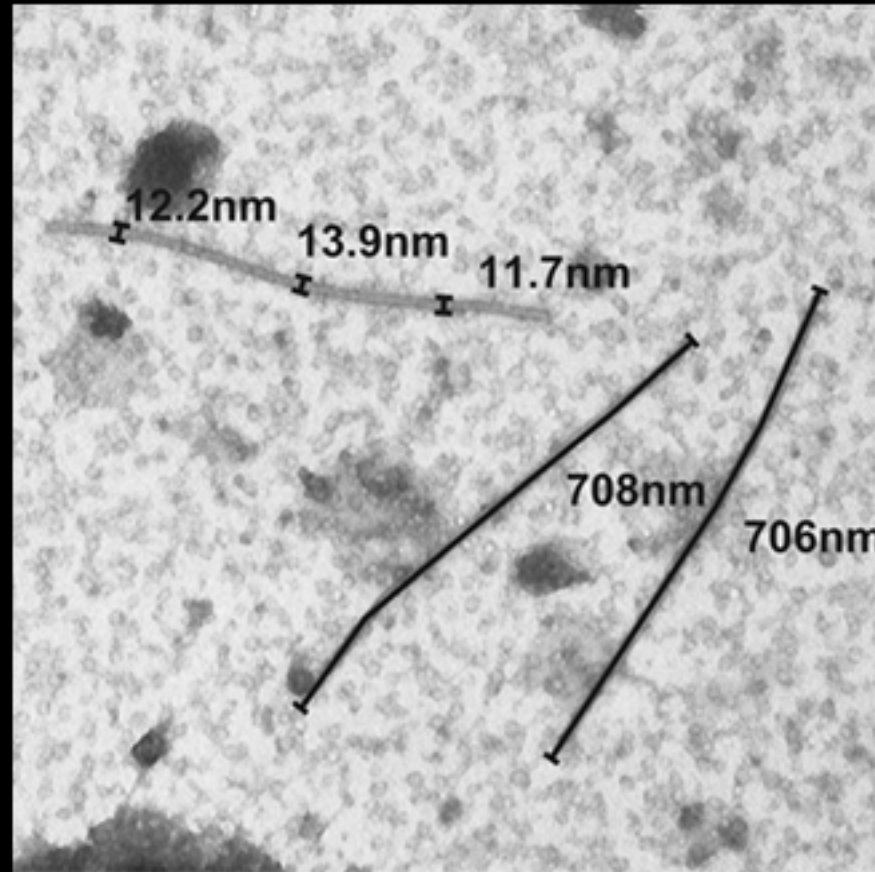


Older leaves

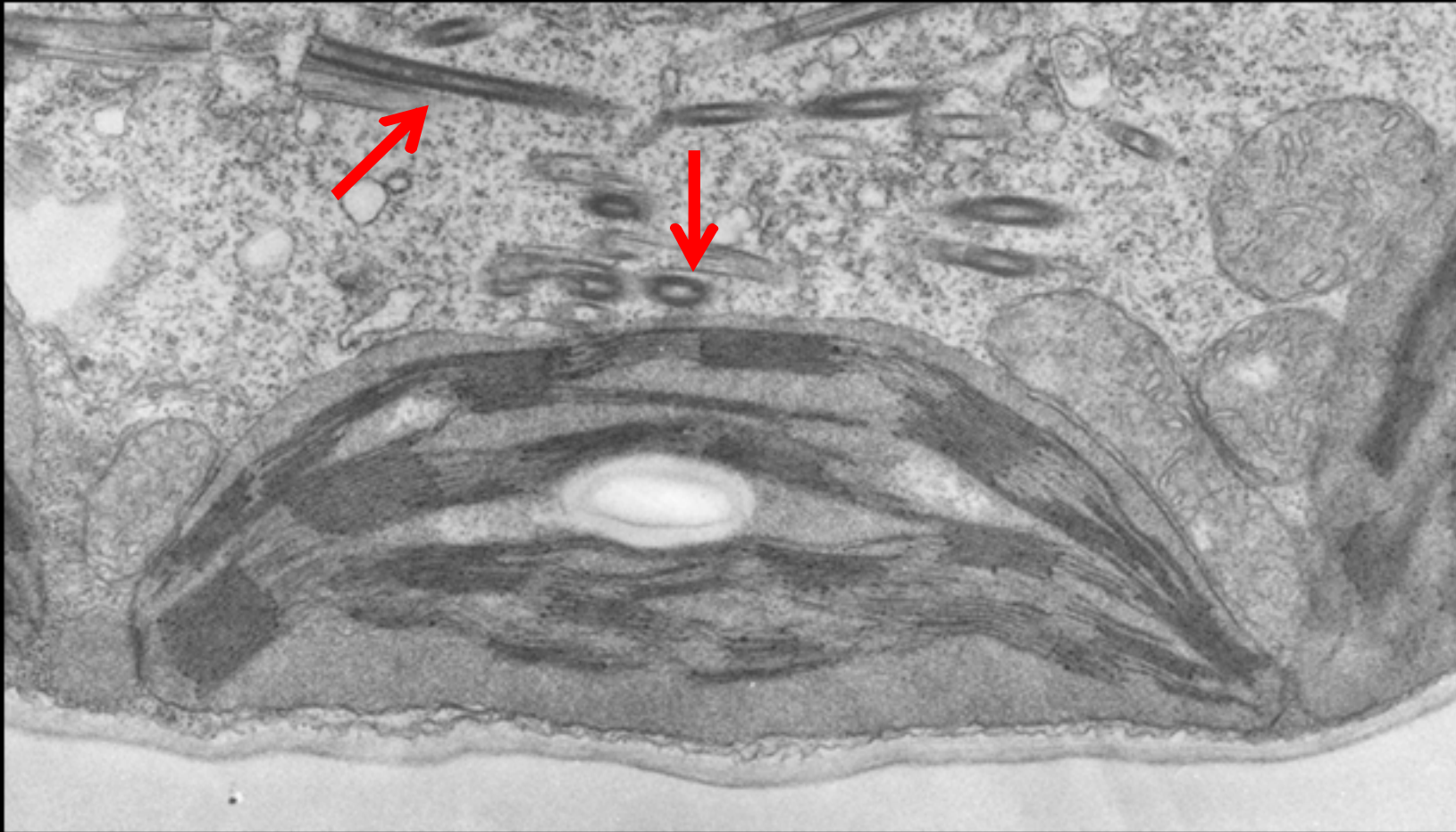


Younger leaves

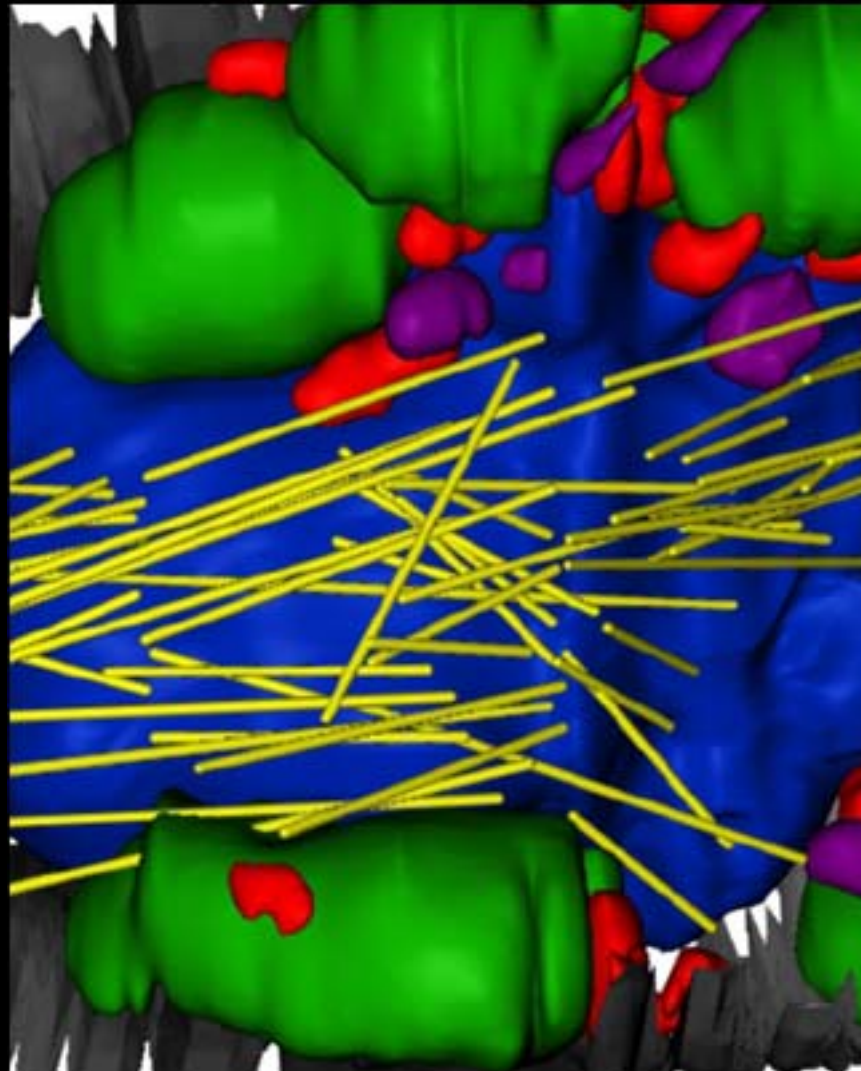
ZYMV – Negative staining



Cylindrical inclusions



3D reconstructed ZYMV cylindrical inclusions



Cylindrical inclusions



Length: $3\mu\text{m}$
Width: 120nm



Thanks to:

B. Zechmann

G. Daum

A. Perktold

G. Graggaber

S. Möstl

This work was supported by the

