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The TUBUL experiment of the Dutch Soyuz DELTA Mission in April 2004

"Influence of gravity on the microtubule cytoskeleton of plant cells"

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Abstract

• Microtubules are little tubes in all cells of plants, animals and humans. They are essential for cell division and growth.

• In plant cells, microtubules are highly organized and each developmental stage of a cell has a characteristic microtubule organization.

• The question rises, how a plant cell put microtubules in order. There is evidence that gravity could play a role in making ordered microtubule arrays, but results from earlier space experiments contradict each other.

• Our “TUBUL” experiment aims to solve the paradox that microtubules do not organize in vitro under microgravity, but that plants can grow in space.

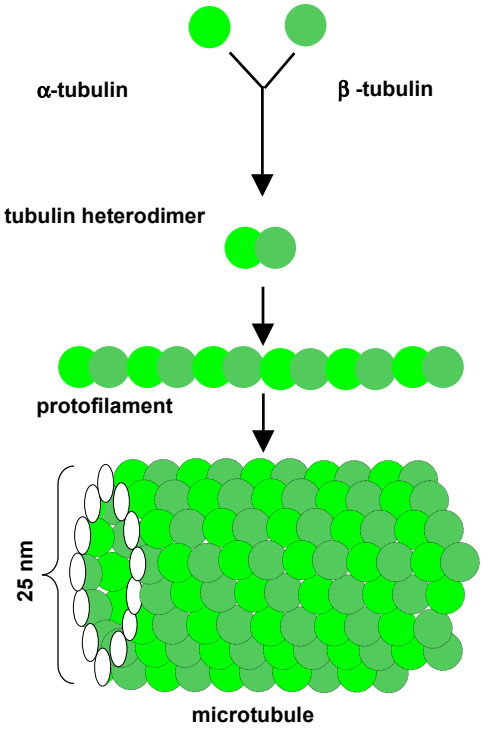
• In our experiment individual plant cells will be in space for up to 10 days on board the International Space Station (ISS) during the Dutch Soyuz Mission DELTA in April 2004. Back on earth, the microtubules will be analyzed.

• What are microtubules?

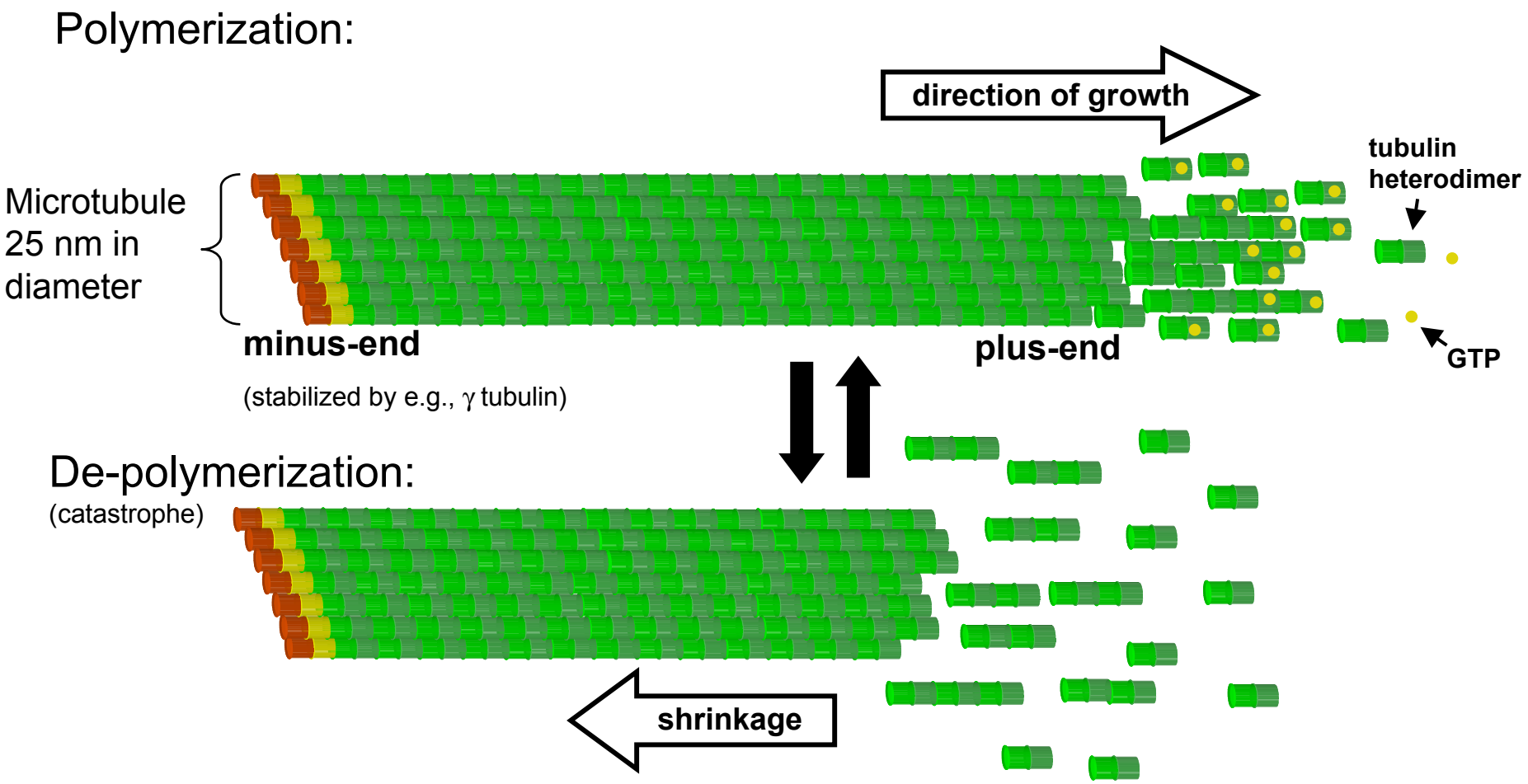
Microtubules are one of the structural compounds of the cell skeleton. They consist of α and β tubulin heterodimer proteins that assemble into protofilaments, which in turn polymerize into long, cylindrical and hollow tubes of 25nm in diameter, the microtubules.

Microtubules can reach a length from up to 25 μm in plant cells.

However, microtubules are not at all static structures. They constantly assemble and disassemble, although the overall appearance of the entire microtubule array gives the impression of a stable structure.



Dynamic instability of microtubules



Simplified scheme of microtubule dynamics; drawings not to scale

- Why are we interested in plant microtubules?

Plant cell microtubules determine the division plane, separate the daughter chromosomes, and determine the direction and amount of cell growth.

Without a functional microtuble cytoskeleton, plant growth is not possible.

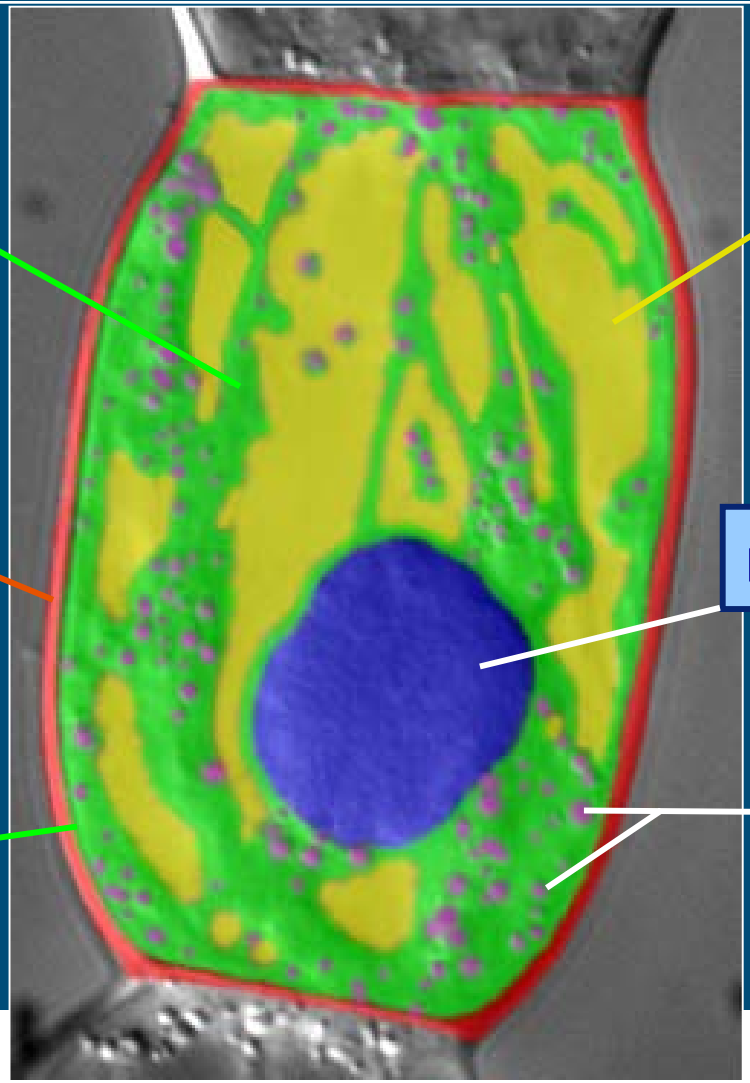
In plant cells, microtubules occur as highly organized arrays and each developmental stage of a cell has a characteristic microtubule configuration.

- What is a plant cell?

Bright field microscopy image (Differential Interference contrast) of a Tradescantia stamen hair cell

For explanation, see next slide.

- What is a plant cell?



• The different plant microtubule cytoskeleton arrays

(normal gravity conditions)

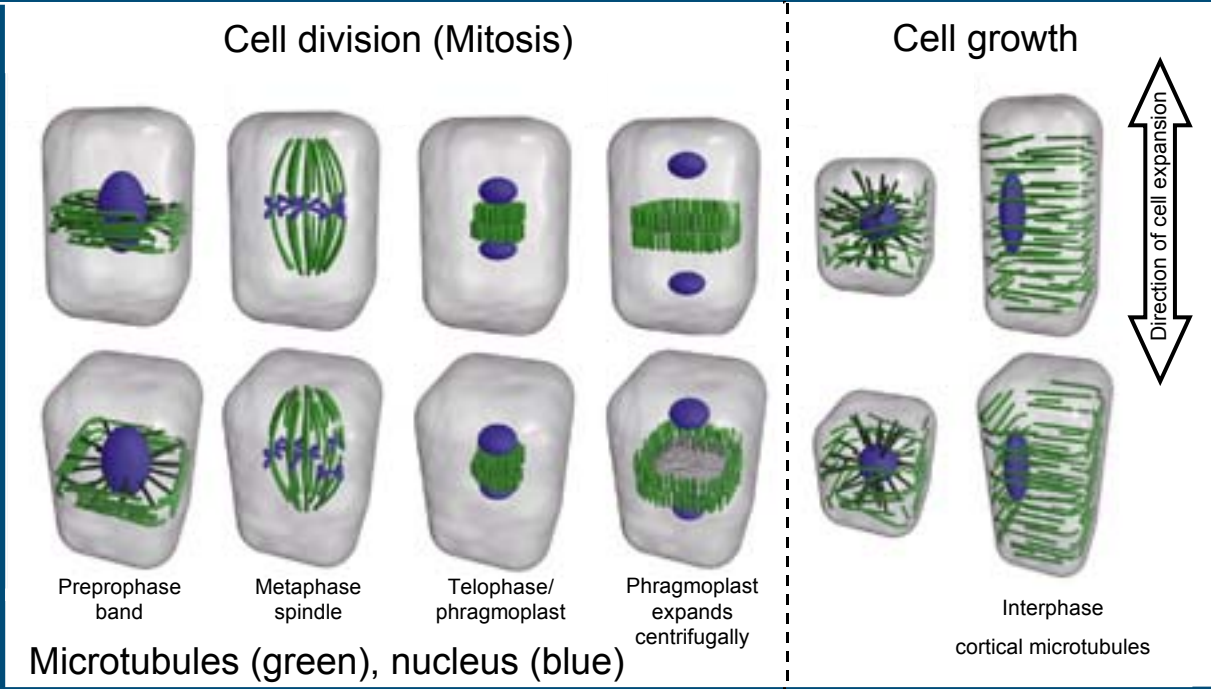


Figure adapted from: **Wasteneys G.O.**, *Journal of Cell Science* 115: 1345 - 1354 (2002); review on the plant microtubule cytoskeleton

Microtubule arrays consist of many microtubules that are organized with a certain patterning.

In plant cells, microtubules appear as highly organized arrays and each cell stage has a characteristic microtubule organization.

• Without microtubules properly organized and functioning, cell division and cell growth are heavily disturbed – normal plant development is not possible.

• The question raises, **how does a plant cell put microtubules in order**?

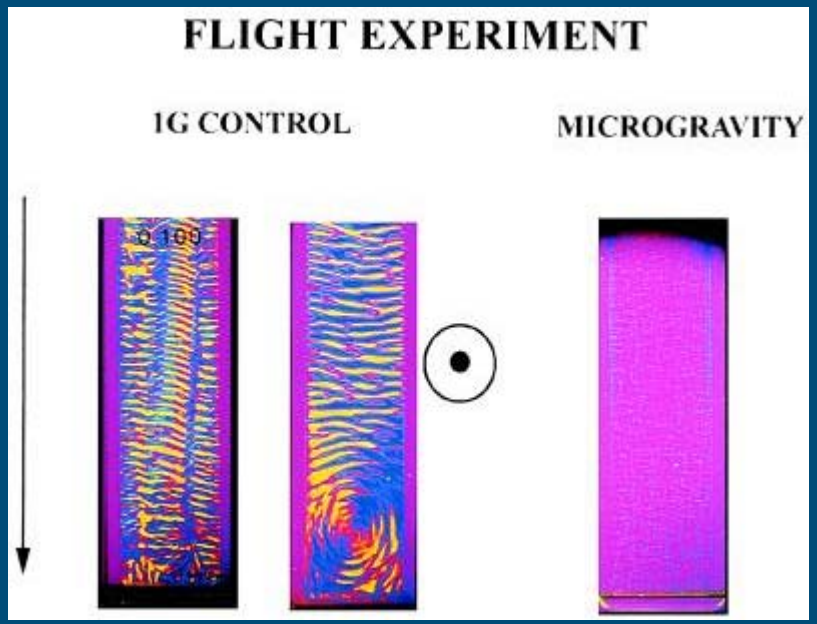
From earlier work there comes evidence that GRAVITY could play a role in this.

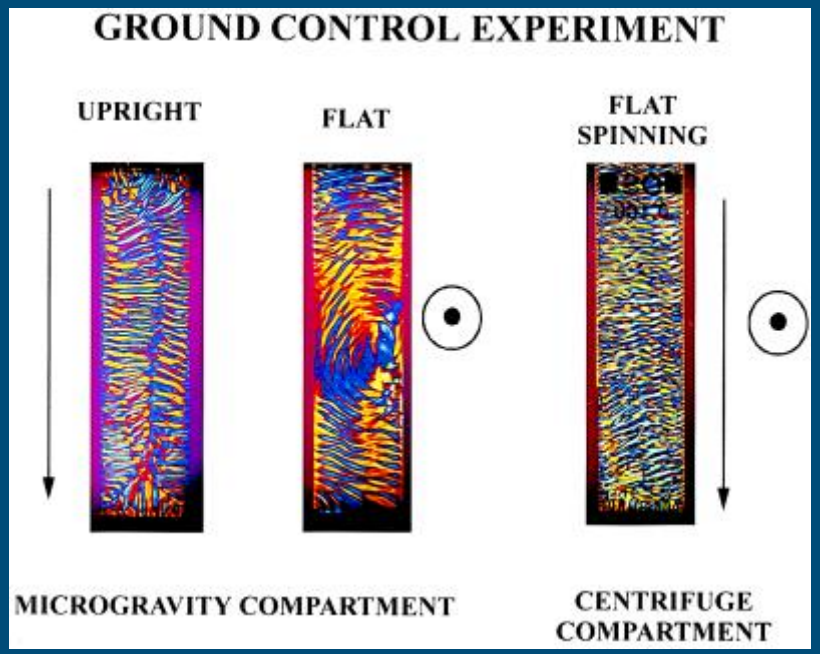
Gravity seems to have some ordering effect on microtubules

Tabony and co-workers:

In artificial micro-chambers, microtubules do not align in parallel arrays under microgravity conditions (experiment performed during free-fall phase of a sounding rocket).

Papaseit C., Pochon N., Tabony J. (2000): Microtubule self-organization is gravity-dependent. PNAS 97: 8364-8368

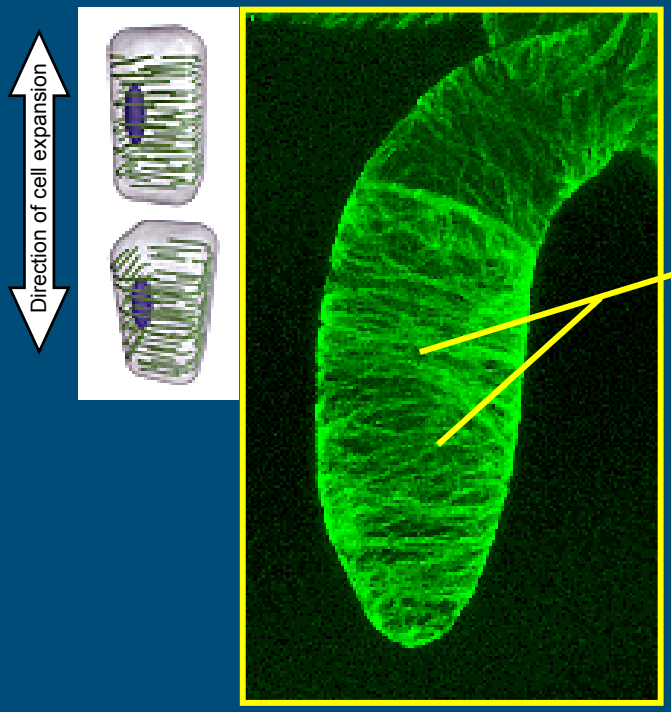




Both images: Proc Natl Acad Sci U S A. 2000 Jul 18; 97(15): 8364-8.

Why microgravity?

- Cortical Microtubules are organized in parallel arrays in growing plant cells:



In growing plant cells, microtubules are organized in parallel arrays that lay perpendicular to the growth direction of the cell ("hoops of a barrel").

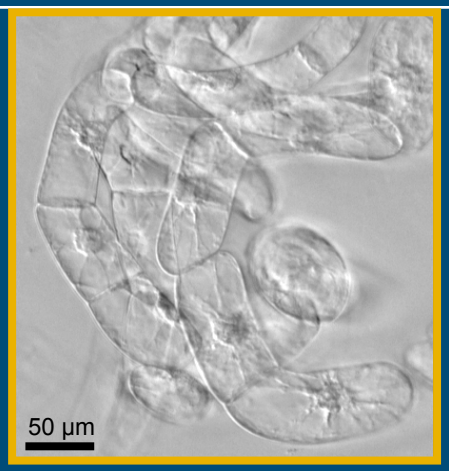
- Is this patterning in plant cells gravity dependent?

Why microgravity?

Normal gravity conditions: protoplasts regenerate into plants.

Protoplasts

1. Plant cells with cell wall artificially removed



2. regeneration into intact cells with cell wall

3. Callus formation

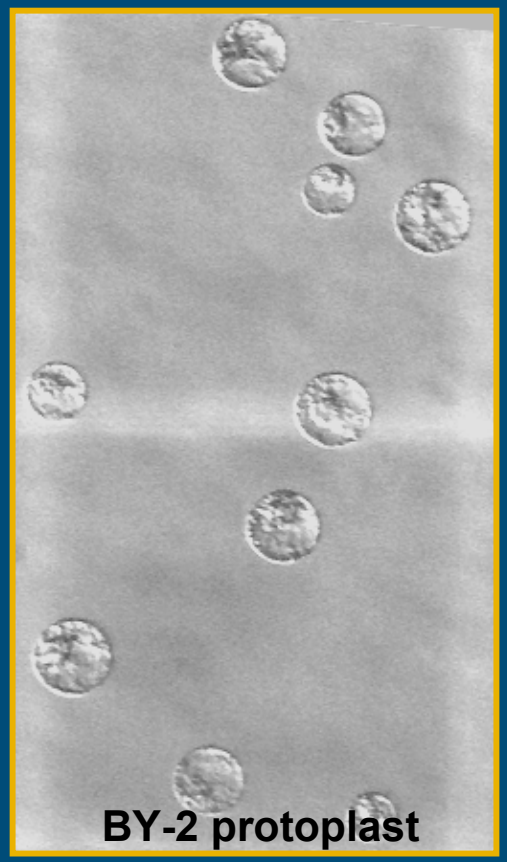
4. Plant regeneration starts in tissue culture from callus

5. Plant from tissue culture

6. New tobacco plant

Why microgravity?

Protoplasts do not regenerate into plants under microgravity



BY-2 protoplast

Skagen EB, Iversen TH. (2000)

Effect of simulated and real weightlessness on early regeneration stages of *Brassica napus* protoplasts. In Vitro Cell Dev Biol Plant. 36(5): 312-318

under microgravity conditions

source: www.rgs.uky.edu

new tobacco plant

Data collected during:
- Biokosmos 9 satellite
- Space Shuttle IML-1
- S/MM-03-mission

- The microtubule cytoskeleton array in protoplasts exposed to microgravity is disturbed.

- Cell and tissue culture in space: Future need for clonal plant propagation during long lasting space missions?

For note: the experiment discussed here, was done with Brassica napus, not with tobacco

…but: Plants can be grown from seeds in space!

Wheat plants grown for 10 days in space show no significant difference in growth performance compared to plants grown on earth.

Levine et al. (2001):
Cell-wall architecture and lignin composition of wheat developed in a microgravity environment. Phytochemistry 57: 835 –846
[Shuttle mission STS-51]

- We presume that the microtubule cytoskeleton in these plants must be functioning properly.

Paradox:

Protoplasts, plant cells without cell walls, do not regenerate into plants in space (microtubule cytoskeleton disturbed).

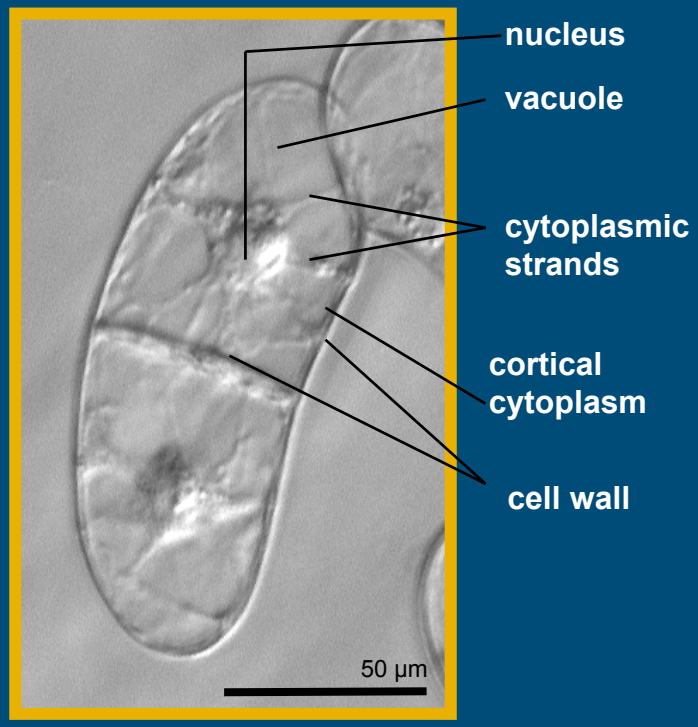
Our TUBUL experiment aims to solve the paradox by exposing the "missing link" - **single plant cells** with cell walls - to microgravity.

- Will they develop normally in space?
- Organization of microtubule cytoskeleton?

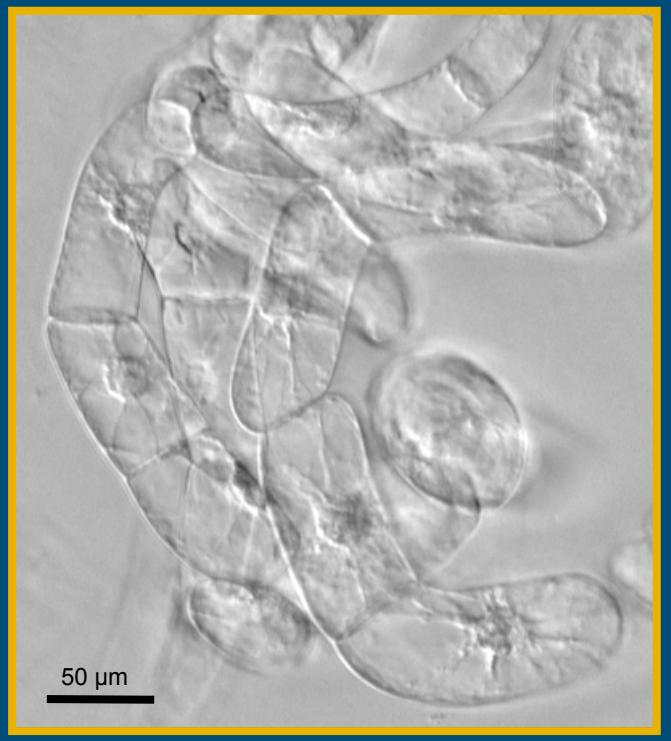
Plants can be grown from seeds in space
(normal microtubule cytoskeleton).

TUBUL experiment: Biological object

Wild-type Nicotiana tabaccum (tobacco) Bright Yellow-2 suspension cells (**BY-2 cells**) embedded in low gelling agarose



2 cells shortly after cell division

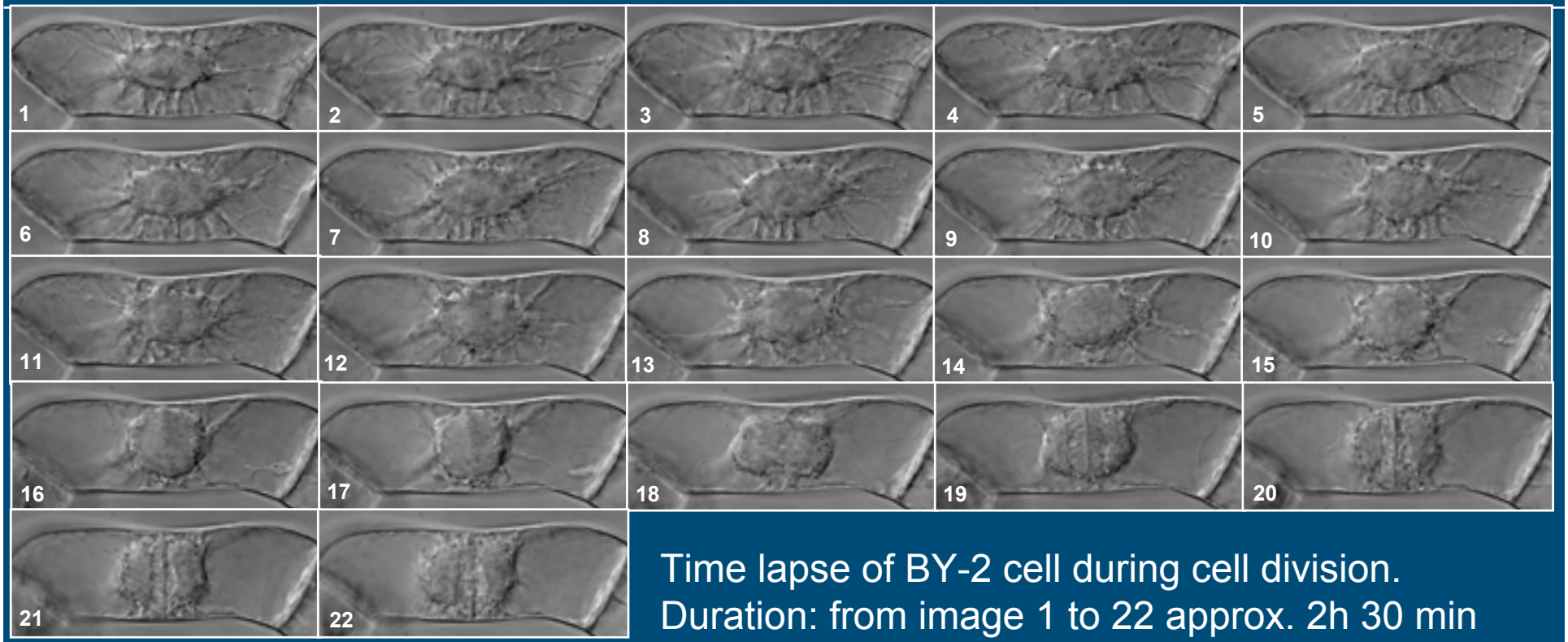


BY-2 cell cluster suspended in low-gelling agarose

Cortical Microtubules in BY-2 cells (during cell growth), labeled with anti-tubulin antibodies

TUBUL experiment: Biological object

Cell division (BY-2 cell)

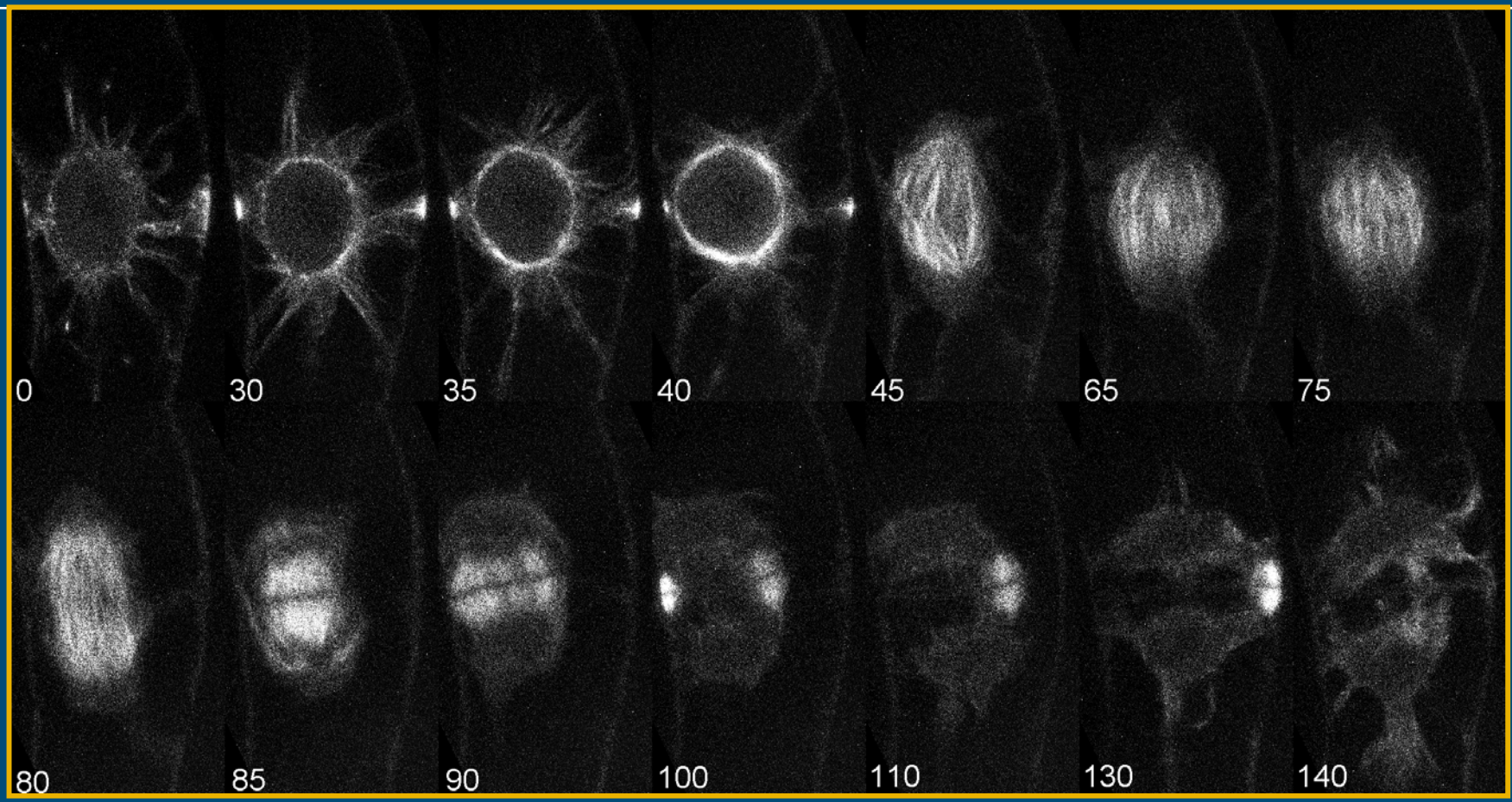


Time lapse of BY-2 cell during cell division.
Duration: from image 1 to 22 approx. 2h 30 min

Courtesy Jan Vos

TUBUL experiment: Biological object

The microtubule cytoskeleton changes during cell division



Time lapse record of microtubules during mitosis in a GFP-MBD expressing BY-2 cell; time in min.

Courtesy Jan Vos

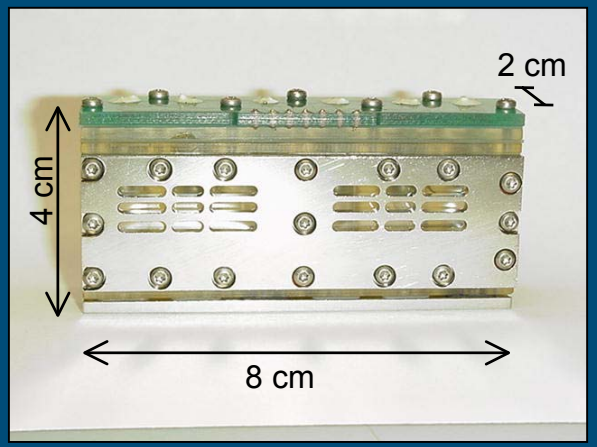
TUBUL experiment: Experiment hard-ware

• BY-2 plant suspension cells will be cultivated for up to 5 days in space. For this purpose, medium refreshment is necessary.

• The cells will be chemically fixed at different time points after reaching orbit. The chemical fixative preserves the cells and their ultra-structure, including the microtubules in their actual state.

• The post-fixative treatment, applied 45 min after release of the chem. fixative avoids post-fixative artifacts and preserves the fixed cells until we receive them in our laboratory at Wageningen University, Netherlands.

• All three different treatments are possible to do within a Plunger-box Unit (experiment unit). They will run fully automated.



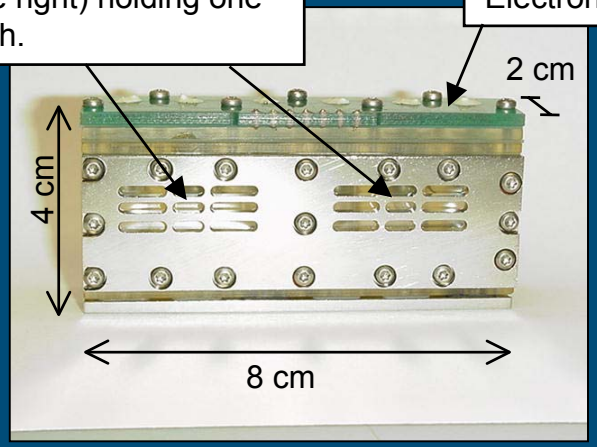
Plunger-box Unit (PBU; experiment unit) flight model manufactured by the Dutch company Centre for Concepts in Mechatronics (CCM)

TUBUL experiment: Experiment hard-ware

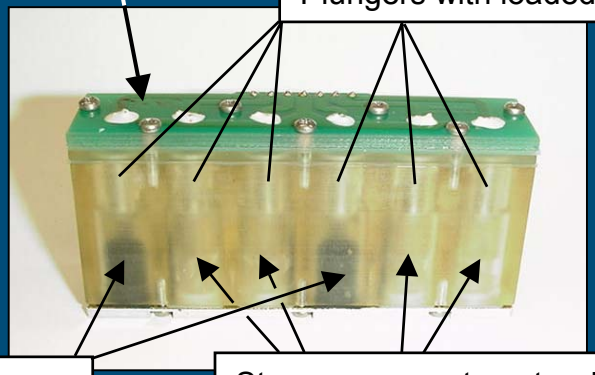
Plunger-box Unit (PBU; experiment unit) flight model manufactured by CCM
(Centre for Concepts in Mechatronics, Dutch pay-load developer)

Two independent growth compartments (A to the left, B to the right) holding one BY-2 cell culture each.

Electronic plunger activation mechanism



Plungers with loaded springs



Storage compartment with Viton storage membrane (1.3 ml each; for chemical fixative) for culture compartment B (to the left) and A (to the right).

Storage compartments with silicon storage membranes (1.3 ml each; for BY-2 growth medium) for culture compartment B (to the left) and A (to the right).

8 Plungerbox Units for TUBUL flight experiment

- **Establishing a self-sustaining growth system for cell suspension cultures for experiments (10 days) in space.**

TUBUL experiment: Experiment hard-ware

Plunger-box Unit (PBU; experiment unit) flight model manufactured by CCM

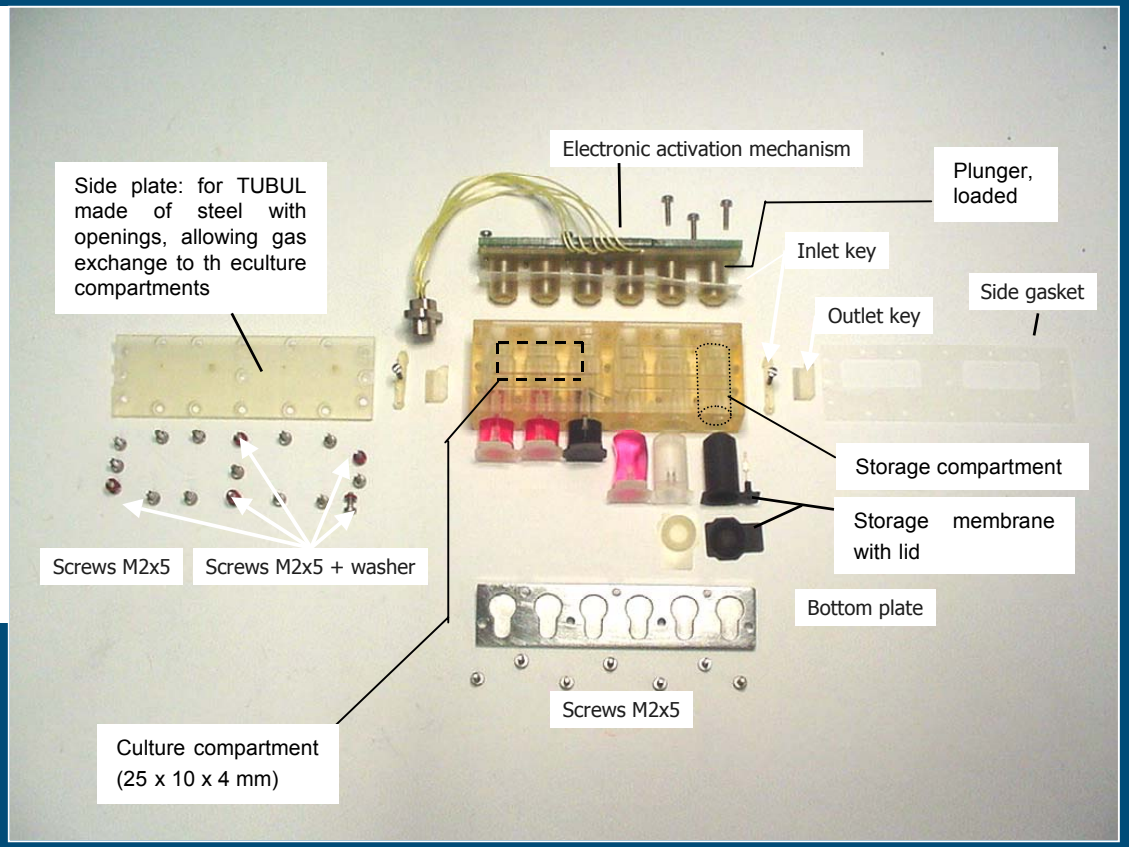
Disassembled Plungerbox-unit.

Edges of one culture compart-ment and one storage compart-ment have been indicated with dotted lines

Dimensions:

80 x 40 x 20 mm

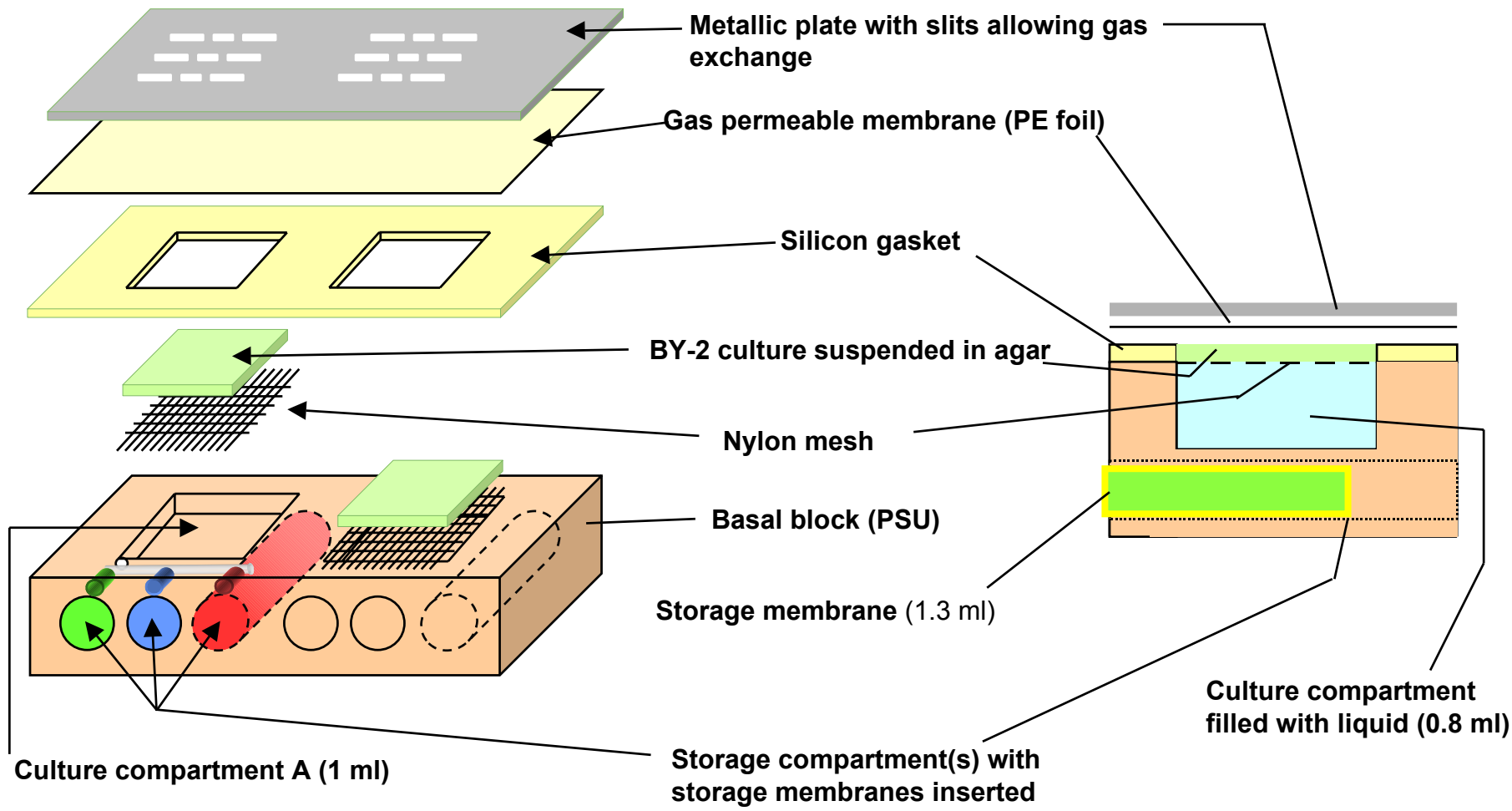
Weight: approx. 0.125 kg



TUBUL experiment: Experiment hard-ware

Plungerbox-Unit (experiment unit)

Drawings not to scale



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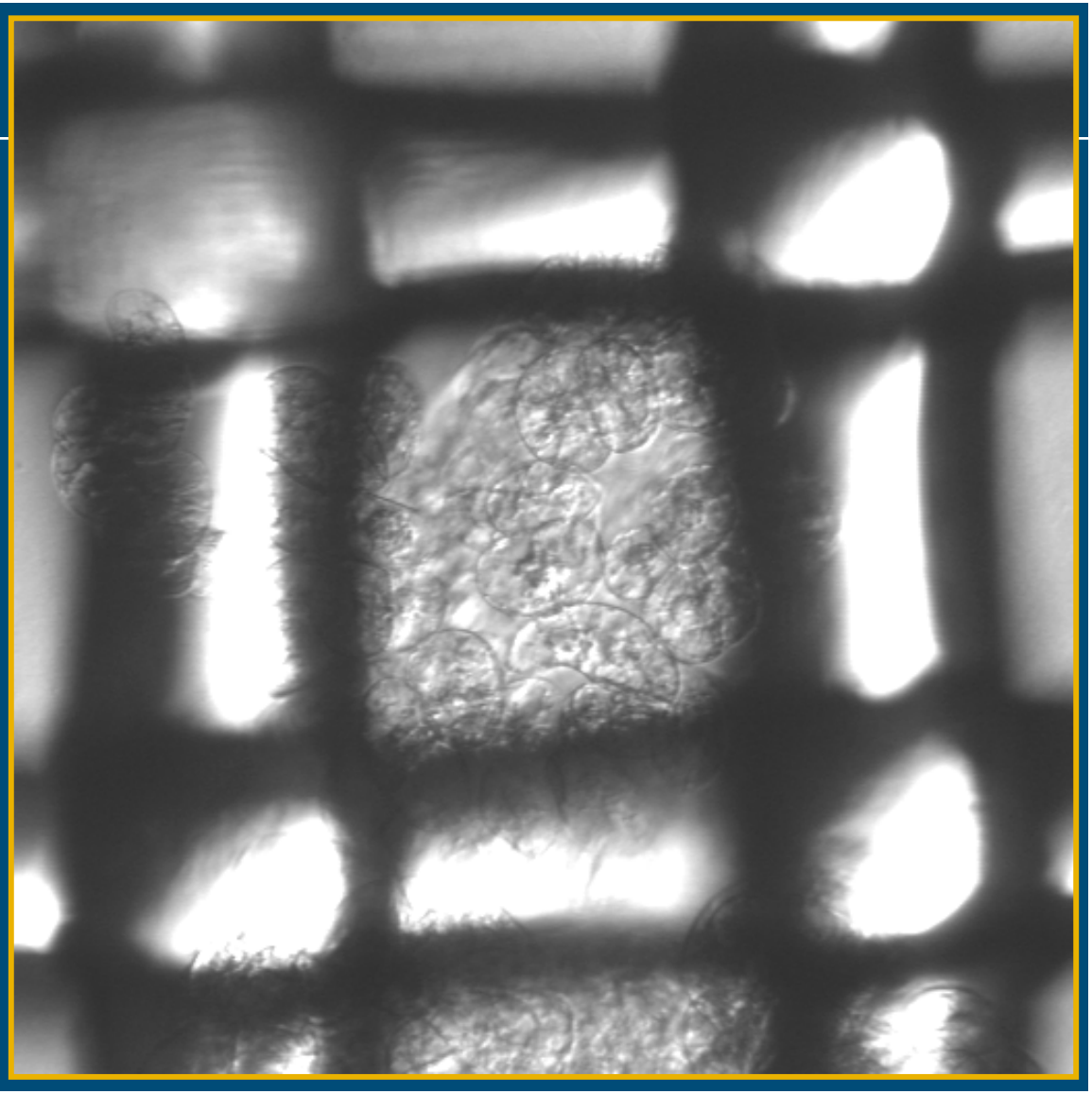
TUBUL experiment: Experiment hard-ware

BY-2 cells grown in culture compartment of Plunger-box Unit (2% agarose, ‘reinforced’ by a nylon-mesh)



Ground experiments

Hand operated Plunger-Box Units will be used for **ground reference experiments** during the space flight.

Hand operated Plunger-Box Units also will be used by us for **random positioning machine** experiments at the Dutch Experiment Support Center for gravity research (DESC) in Amsterdam (short time effects of changes in gravity directionality on the microtubule cytoskeleton of BY-2 cells; SRON microgravity research program).

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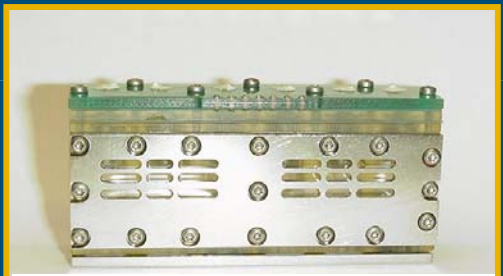
TUBUL experiment: Experiment hard-ware



Assembled Plunger-Box Unit (PBU)

PBU are to be inserted in hermetically sealed **experiment containers** with extra gas bag (volume of gas bag ca. 25 ml; passive oxygen supply for plant cell cultures) and electronic interfaces for connecting PBU with incubator

Integration of TUBUL experiment containers in **KUBIK incubator**

- during rocket launch, stay on board the Soyuz, and transfer to ISS: **KUBIK TOPAZ**
- during TUBUL experiment conduct on board the ISS: **KUBIK AMBER**

AMBER TOPAZ

Incubator (ca. 40 x 40 x 40 cm):

Maintains stable temperature during experiment (temperature settings can be changed during flight).

Powers containers/units for automated release of growth medium, chem. fixative, and post-fixative buffer solution

Once powered by the incubator, the TUBUL experiment will run fully automated accordingly to a pre-programmed time line

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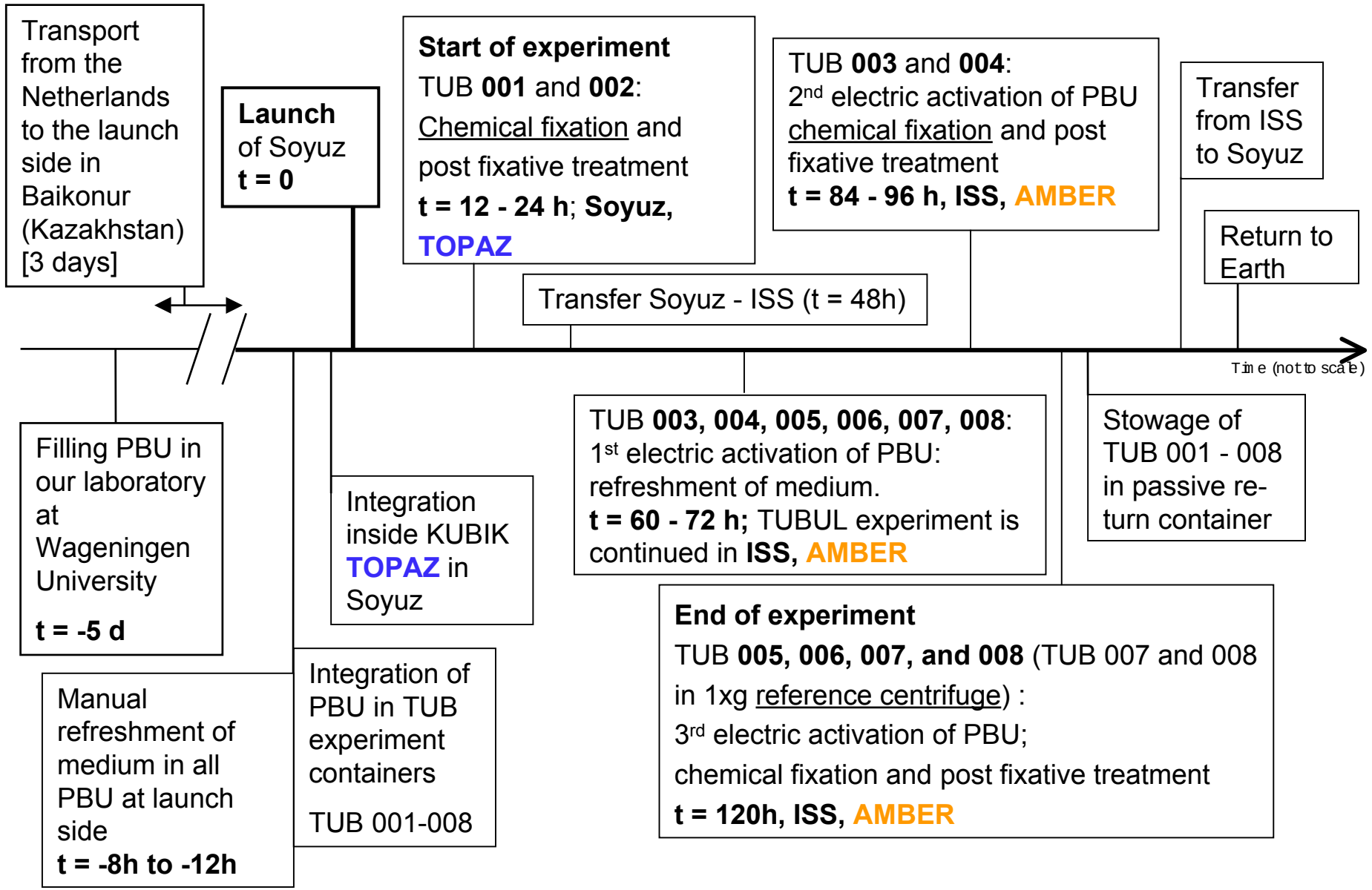
TUBUL experiment time line



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TUBUL experiment: post flight processing

- Early retrieval of chemically fixed samples required after return to Earth.
- Transport to the Netherlands as quickly as possible.

Analysis of fixed cells upon retrieval in our laboratory (i.e., data collection on ground only):

- Microtubule alignment (order)
- Microtubule arrays
- Transition from one array to another
- Microtubule density

…will we observe similar microtubule organization as in cells grown under normal gravity condition?

Further cell biological analyses:

- Mitotic index
- Cell shape
- Cell length
- Density of cells in culture

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TUBUL experiment: What do we expect?

The results and interpretation of the TUBUL experiment will shed light on microtubule behavior in living plant cells under microgravity conditions.

From the differences between this behavior in space, and on earth, we will learn new aspects of microtubule organization and the role of gravity in this process.

In addition, we will learn whether production of whole plants (food) from suspension culture is possible for plant production during long flights.