Plant Development

NST Part 1B Cellular and Developmental Biology Practical

This practical is a student-led exercise in data interpretation, hypothesis generation and experimental design. The practical runs over two weeks in the Teaching Laboratory in the Department of Plant Sciences. You should plan to attend the practical in either the morning session (starting at 11:00) or in the afternoon session (starting at 14:00). Please use the same groups that you have been assigned earlier in the year, as we need to follow Covid rules for spacing and numbers in the laboratory. The practical sessions are theoretical challenges, and lab coats are not required, however we still have a policy of wearing masks in this kind of crowded situation in the laboratory.

WEEK 2 timetable: Data analysis

In the first week of this practical, we examined the effect of exogenous auxin on the growth of *Marchantia polymorpha* gemmae.

(i) We observed that exposure to exogenous auxin triggered formation of rhizoid cells on the entire surface of the gemmae, and appeared to inhibit the growth of the gemmae.

(ii) Study of single-cell transcriptomic data suggested that rhizoids express all the genes required for auxin response, but show very low levels of gene expression for genes associated with auxin production. It looked possible that auxin might be synthesised elsewhere in the plant.

(iii) We explored possible experimental designs for testing whether auxin flow, from source to sink, might play a role in patterning of young Marchantia plants.

This week, you are provided with the results of experiments to further investigate this:

1. **Time course of cell expansion and cell division**. Results of time course observations to look at normal patterns of cell expansion and cell division after gemmae germination. Cells were tracked by quantitative microscopy over the first three days after germination. The areal expansion and patterns of cell division were measured and plotted on maps of the gemma dorsal surface.

   Identify the meristematic notches. Are there distinct regions of cell division and expansion in the young growing gemma?

2. **Gemma surgery.** Demonstration of a surgical procedure to remove both meristematic domains from a gemma at Day 0. A scalpel can be used to slice off the relevant tissues (A). Following this, the central portion of the gemma was allowed to recover and regrow on nutrient agar media. Microscopic images were collected on each subsequent day. The previously quiescent cells of the central region began to proliferate and contribute to regeneration of new meristematic regions.

   Can you see the gradual emergence of small meristematic domains, that continue to grow into major outgrowths? At 7 days, a set of fully differentiated tissues and apical notches are evident.

3. **Tracking cell divisions**. Removal of both meristematic notches triggers immediate cell division in the dissected central region of the gemma. Expanded cells, including heavier-stained rhizoid precursors, are seen at 0h. New cell divisions start at around 12h, and new daughter cells are clearly evident at 16h. A rapid sequence of divisions is seen over subsequent hours, shown up to 27h. Coloured dots are used to represent clonal daughter cells (full colour versions of the diagrams here can be found at: https://haseloff.plantsci.cam.ac.uk/education/CDB_index/CDB_index.html)

   What happens to rhizoid precursors? Are some diverted from their normal fate? What might

trigger these changes in cell state? What might trigger the wide onset of cell division?

4. **Auxin response after surgery.** Continued observation of the dissected central regions shows that the initial burst of cell proliferation (Day 1) is followed by organisation of growing points into more localised meristem-like domains. The use of a marker for auxin response shows that auxin responses change during this process.

   What might account for the localisation of cell divisions seen by Day 2?

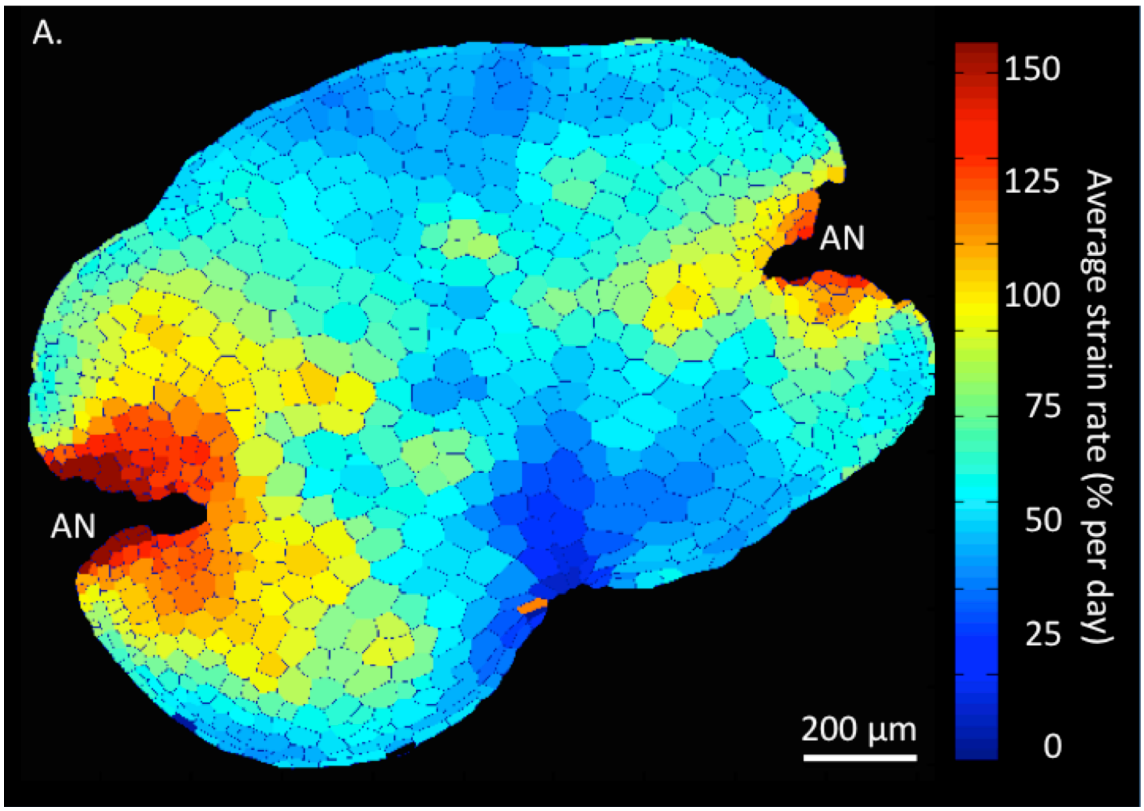
5. **Auxin synthesis in dissected gemmae**. Gemmae were dissected and imaged at 0h, 12h and 24h. Both the central region and one of the notch regions are shown. The Marchantia line was transformed with a nuclear-localised cyan fluorescent gene that was driven by the promoter for the *Yucca2* gene. This was used as a marker to approximate auxin synthesis in the tissues. (A). Untreated Marchantia gemma after dissection. (B). Marchantia gemma grown on 10μM NAA auxin. (C). Marchantia gemma grown on 100μM L-kynurenine (an inhibitor of auxin synthesis). Higher levels of the cyan fluorescent protein marker indicate activity of the *Yucca2* promoter, and likely levels of auxin synthesis. (full colour movies can be found at: https://haseloff.plantsci.cam.ac.uk/education/CDB_index/CDB_index.html)

   Note the changes in cell properties under the influence of auxin and suppressed auxin synthesis. Note what happens to rhizoid precursors under different conditions. Are there different responses in cell proliferation and expansion? Can you see influences on the Yucca2 marker? Can you integrate these observations into a more refined model for the role of auxin in normal gemmae growth, response to surgery, and recovery of meristems?

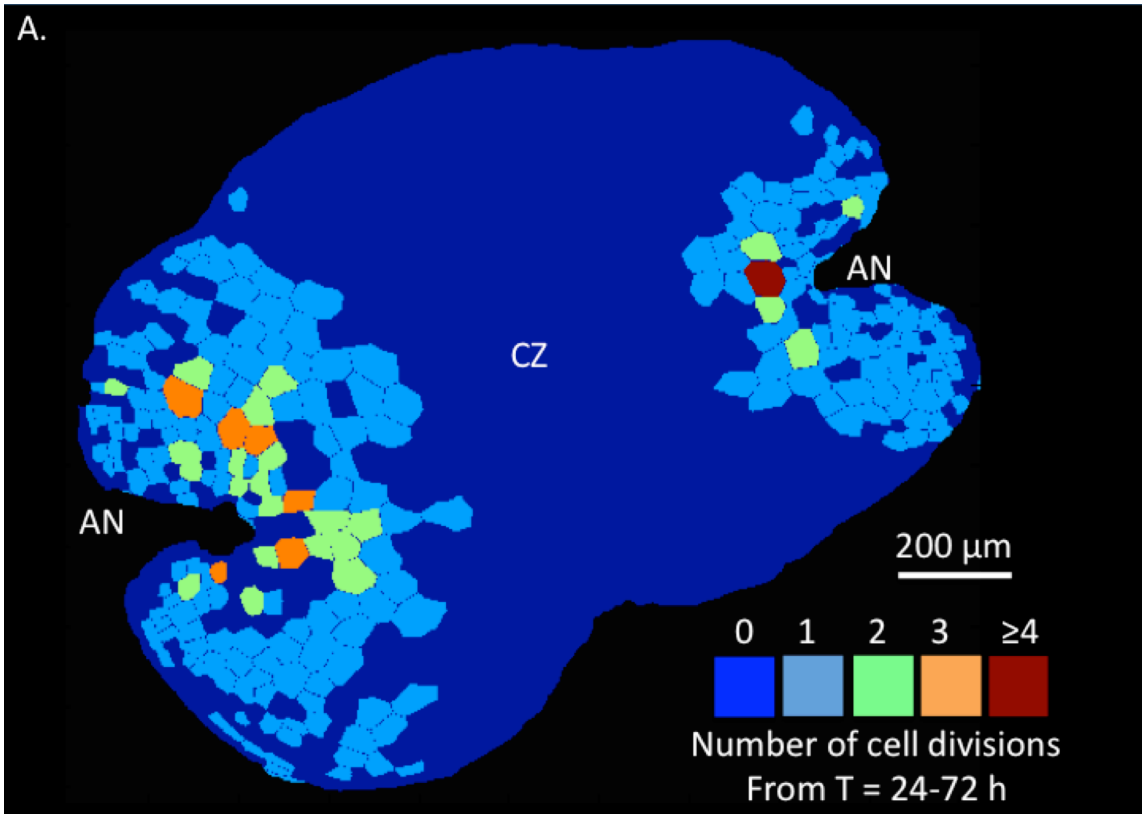
If possible draw diagrams for the different stages of gemma growth, dissection and recovery. We will provide coloured pens and paper. Your models should indicate (where you can) possible signals and responses. Try to identify the main set of predicted interactions and assemble the evidence for these. Does your model help explain self-organised growth of the gemmae, as well as disruption and recovery?

Experiment 1:

Patterns of cell division and cell elongation during normal gemmae development.

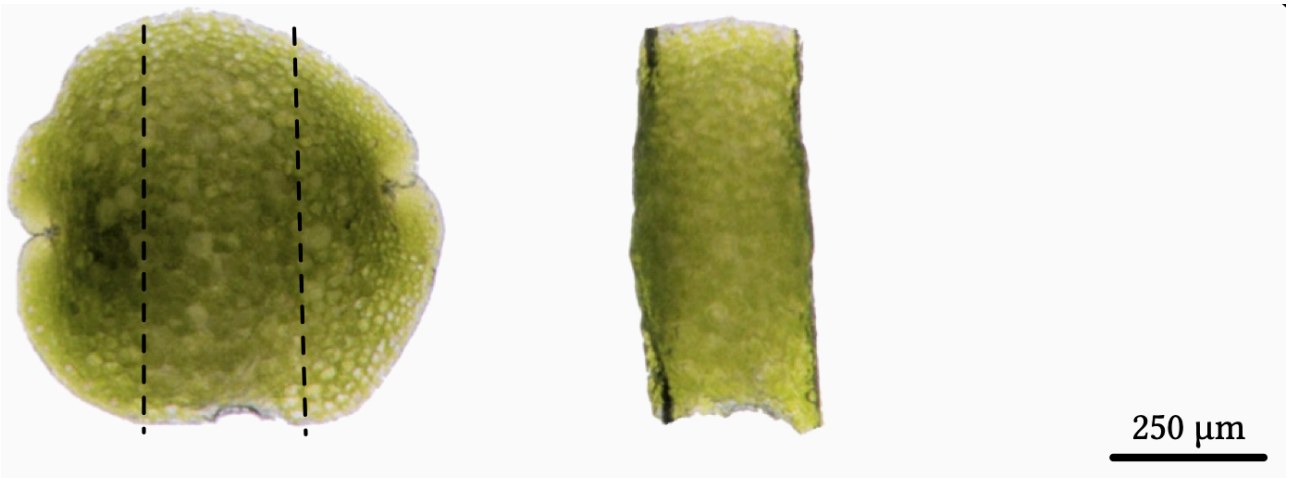


Average strain rates for cells 24-72h after germination.

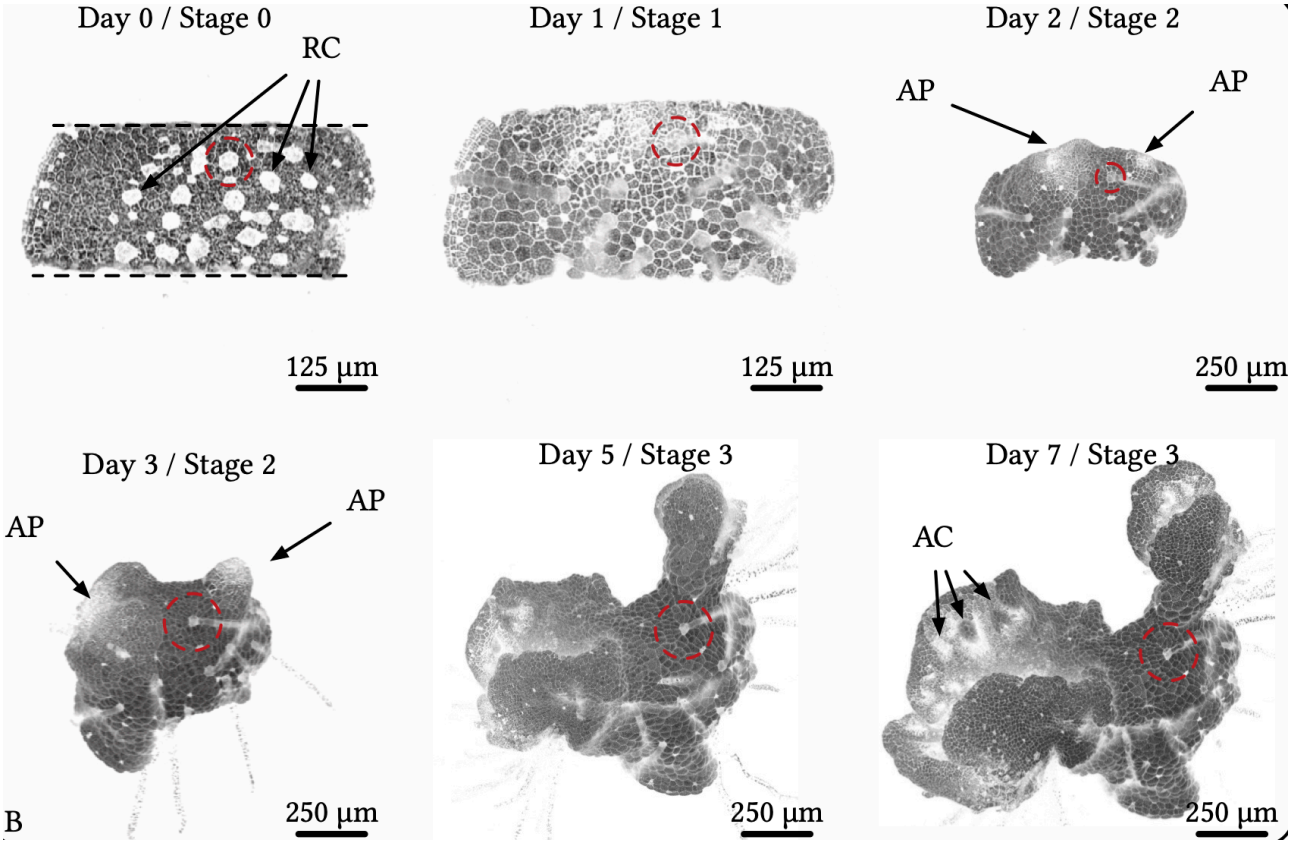


Patterns of cell division 24-72h after germination (data from Nuri Purswani).

Experiment 2:
Gemma surgery

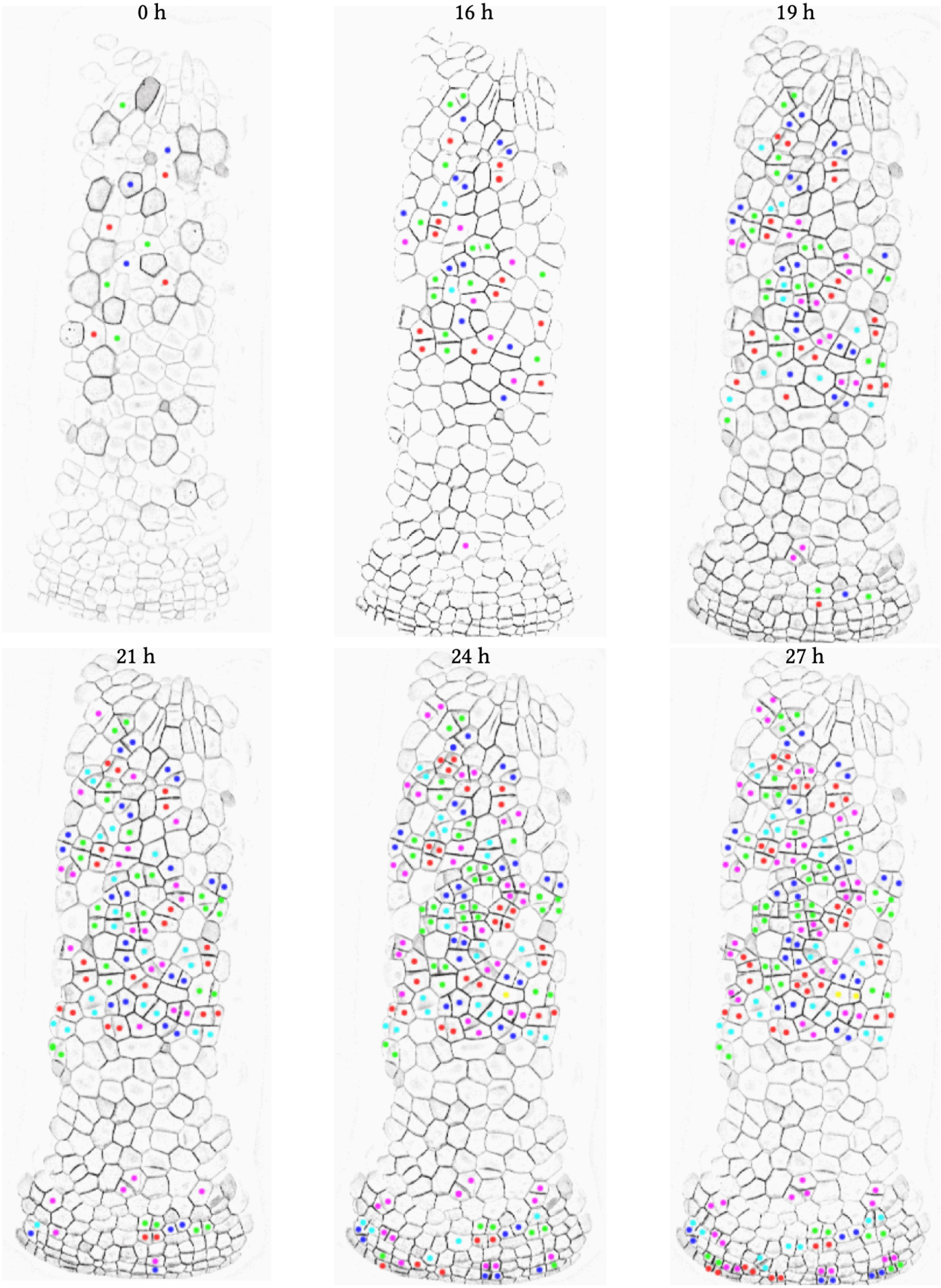


A. Gemma before and after surgery to remove meristematic domains from the central portion.



B. An example of the regeneration of the central region after excision of the meristems. AP denotes apical precursor regions, AC denotes air chambers, RC denotes examples of rhizoid cells, dashed lines denote the cut sites. (data from Mihails Delmans).

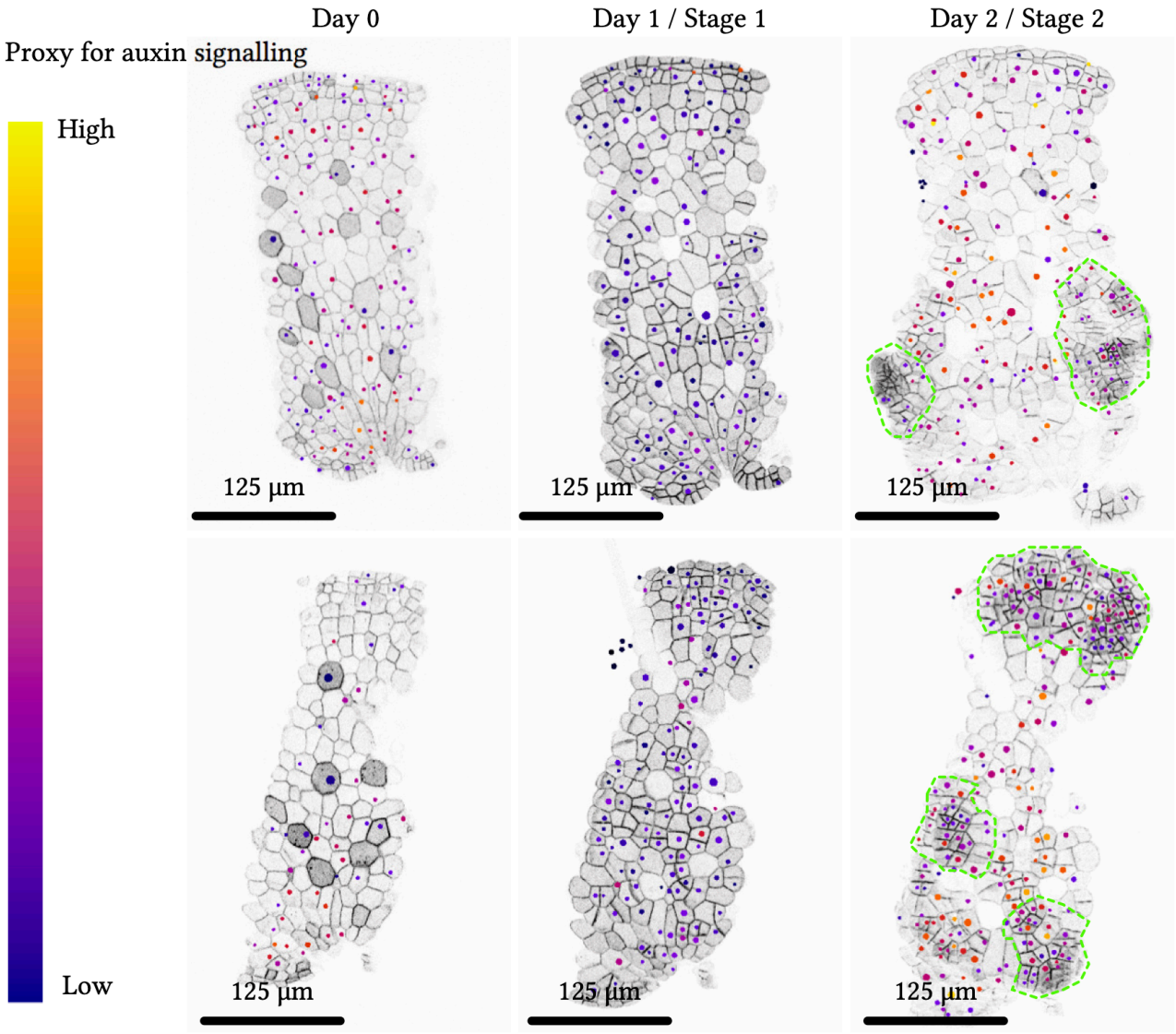
Experiment 3: Tracking cell divisions in the middle region of cut gemmae.



Recovery of the central region of gemmae after removal of both meristem domains in the first 27h after surgery. New cell division events are marked by coloured dots. (data from Mihails Delmans).

Experiment 4:

Auxin response during re-establishment of meristems after surgery.

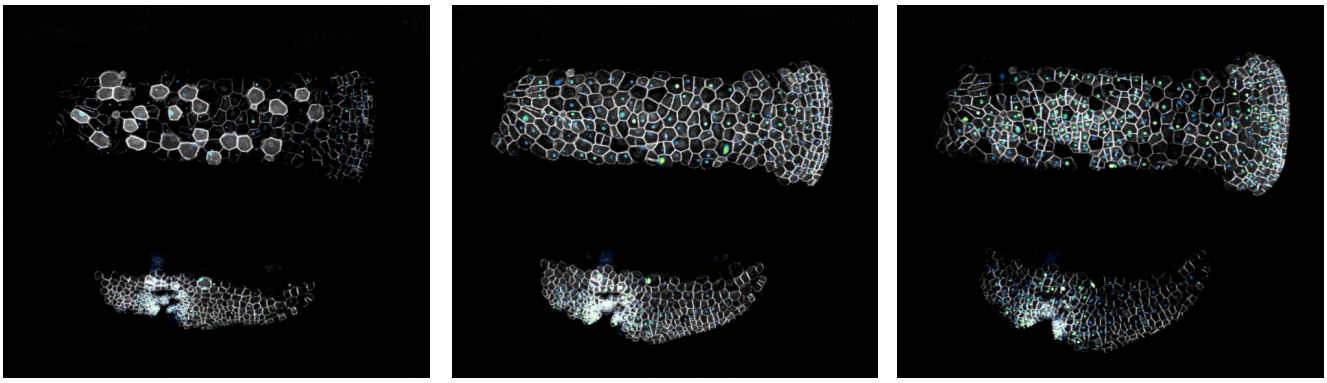


An Auxin-signalling reporter was constructed by expressing two nuclear-localised different-coloured fluorescent proteins. One of these fluorescent proteins was tagged with the DII degron sequence from MpIAA, the Marchantia AUX/IAA protein. Auxin response triggers degradation of this DII-tagged protein. The ratiometric levels of the two fluorescent proteins were measured, assigned a colour-coded value and plotted on the images of two excised gemmae central domains. Increased degradation of the DII marker produces a lower signal. These were imaged one day apart. The dashed lines (green) denote the borders of emerging meristematic regions. (data from Mihails Delmans).

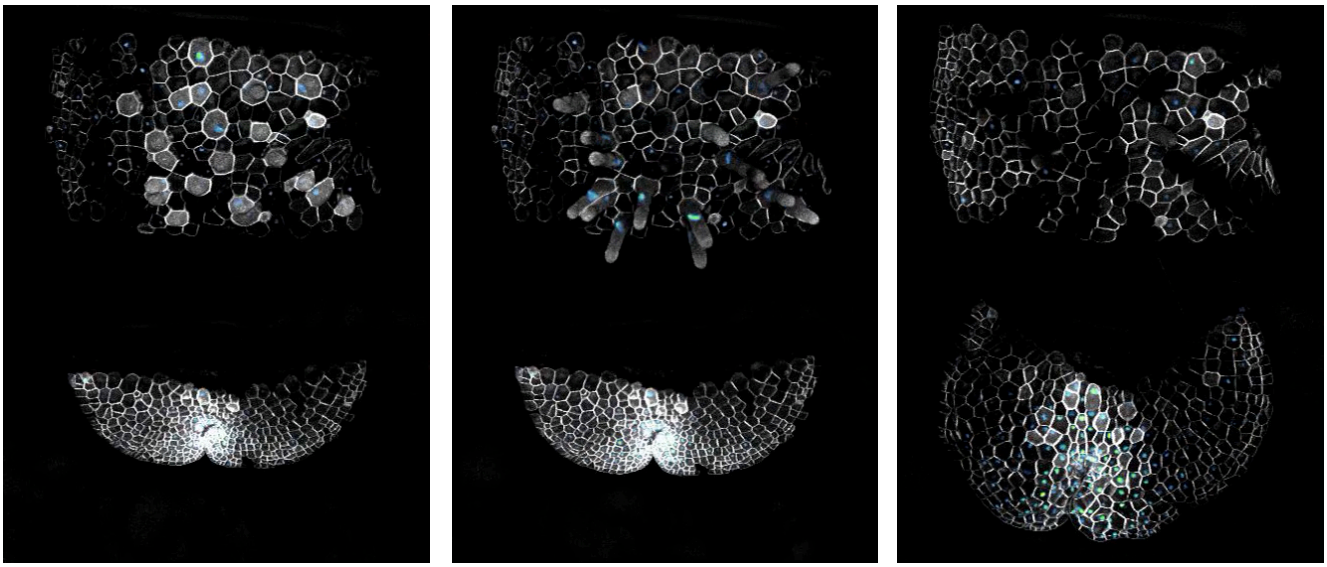
Experiment 5:

Time course of regeneration with a Yucca2 : cyan fluorescent protein marker gene

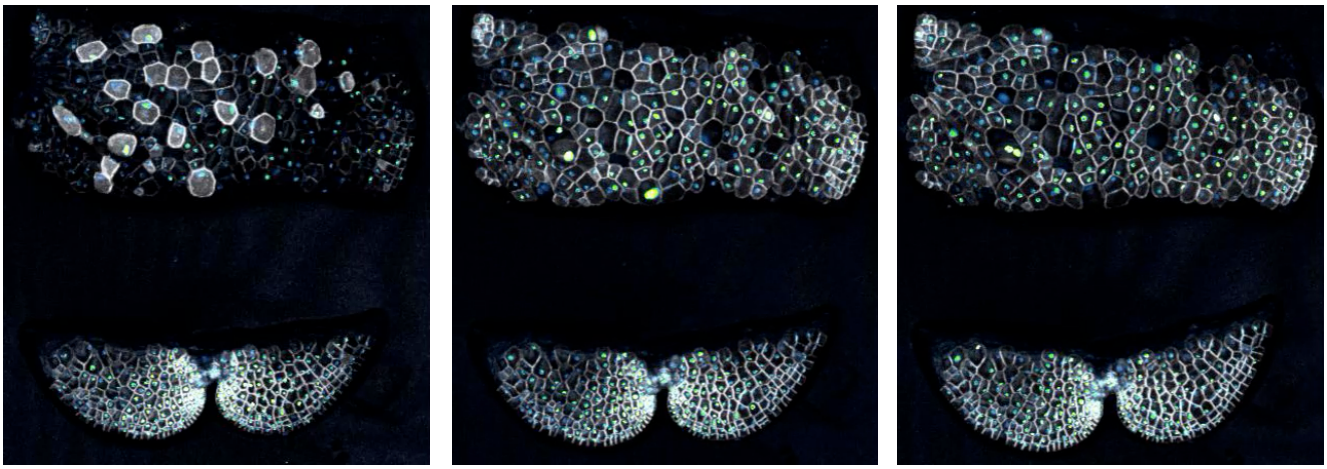
0h 12h 24h



A. Marchantia gemma after surgery (untreated).



B. Marchantia gemma treated with 10µM NAA auxin



C. Marchantia gemma treated with 100 µM L-kynurenine (inhibitor of auxin synthesis) (data from Mihails Delmans)