Imaging to the rescue: understanding courtship in plants

Ravi Palanivelu

School of Plant Sciences
University of Arizona

(http://www.ag.arizona.edu/research/ravilab)

Outline of today's lecture

Biological question

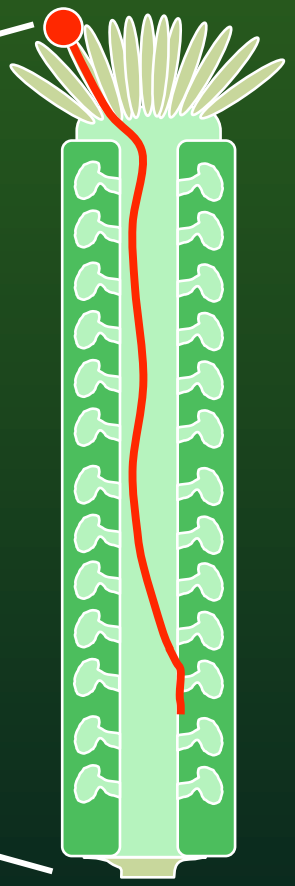
Experimental system

Completed experiments using imaging

Modeling pollen tube growth and behavior using images

Intricate cell-cell interactions are essential for plant reproduction

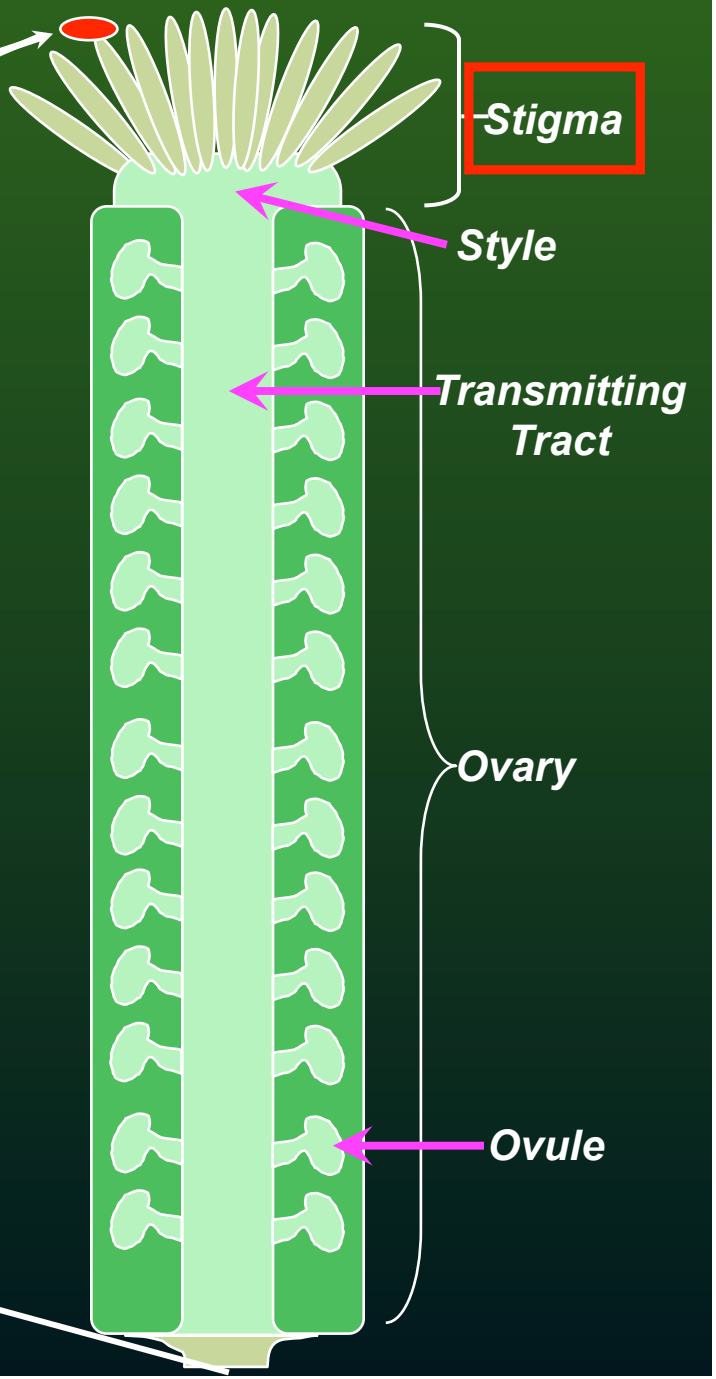
Arabidopsis thaliana



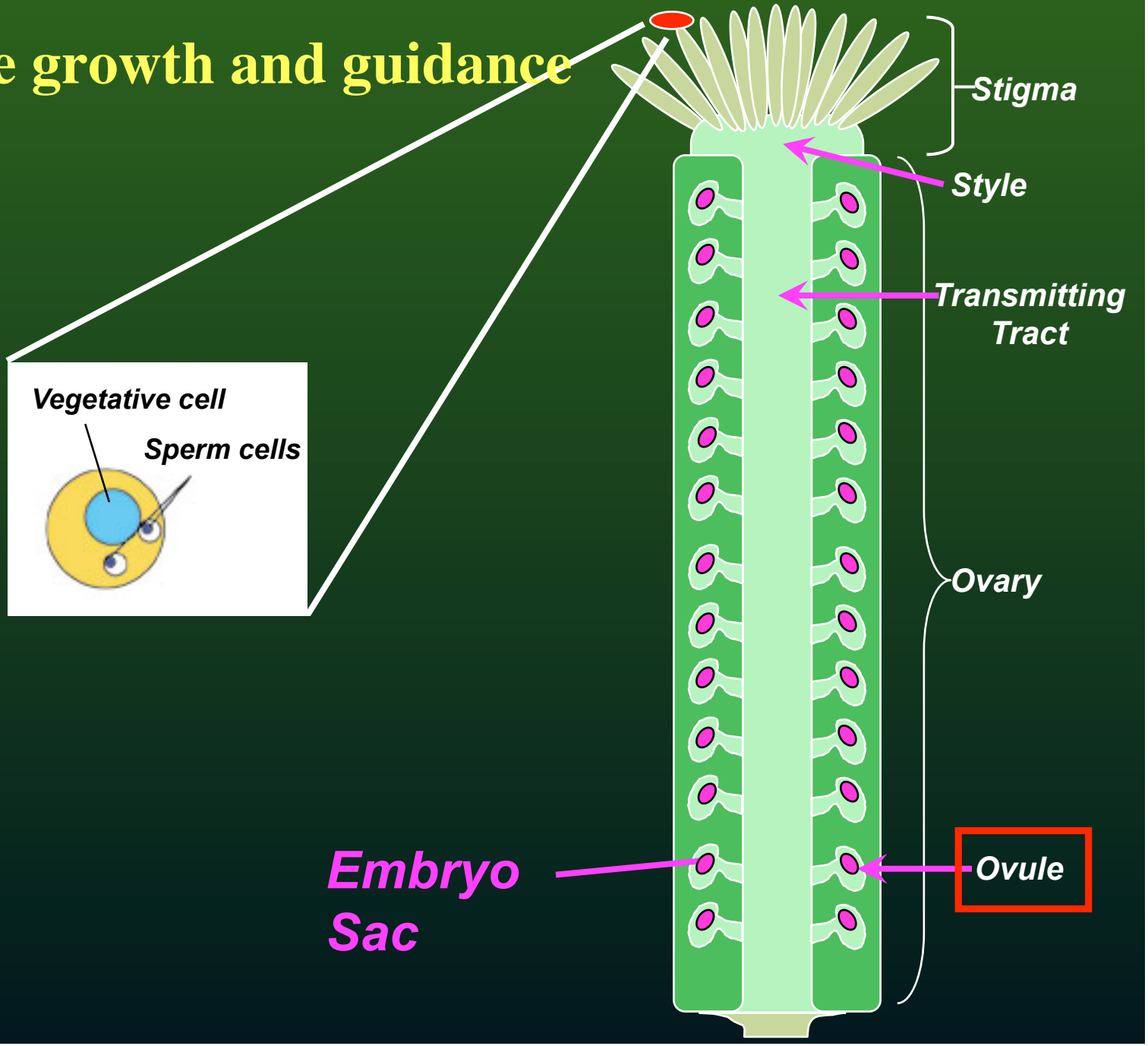
Pistil

Pollen tube growth and guidance

Pollen tube growth and guidance



Pollen tube growth and guidance



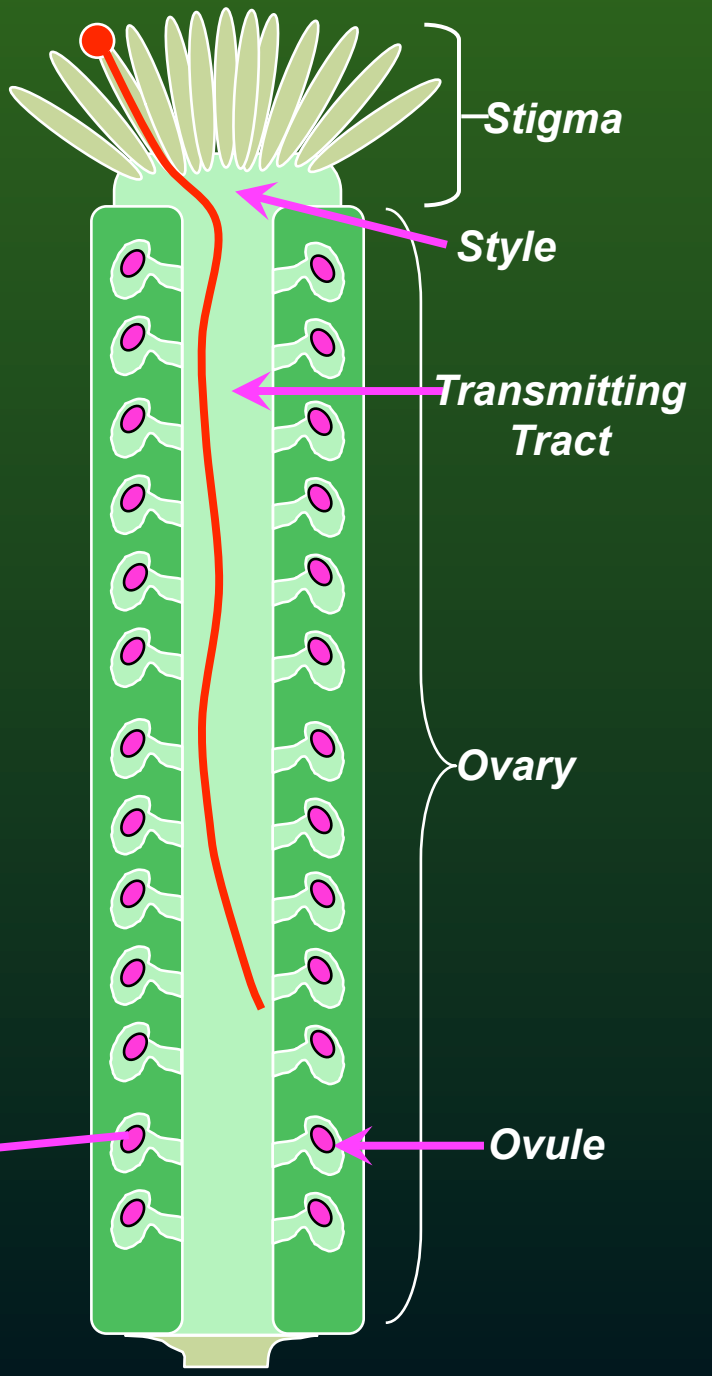
Pollen tube growth and guidance

Pollen grain lands on the stigma

- **Receives water from the stigma**

The tube then…

- **Swells, forming a pollen tube**
- **Descends down the transmitting tract**



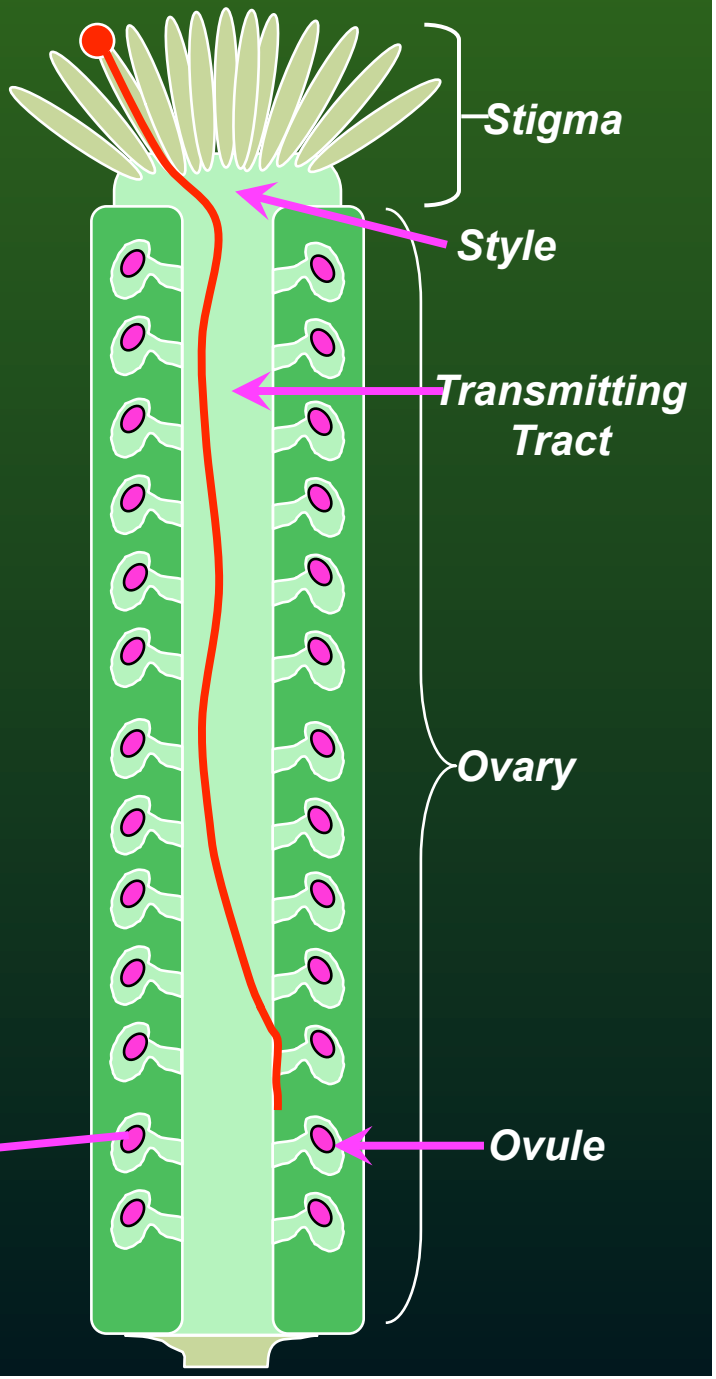
Pollen tube growth and guidance

Pollen grain lands on the stigma

- **Receives water from the stigma**

The tube then…

- **Swells, forming a pollen tube**
- **Descends down the transmitting tract**
- **Emerges into an ovary chamber**

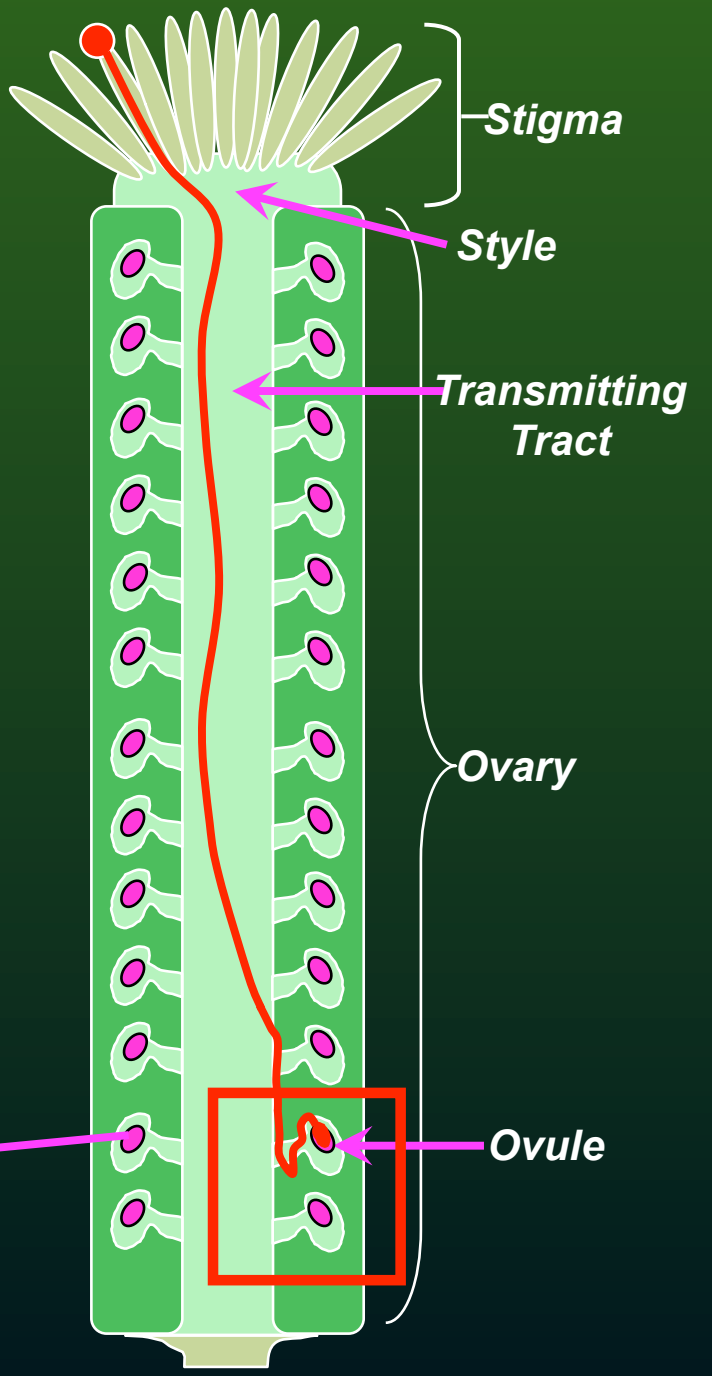


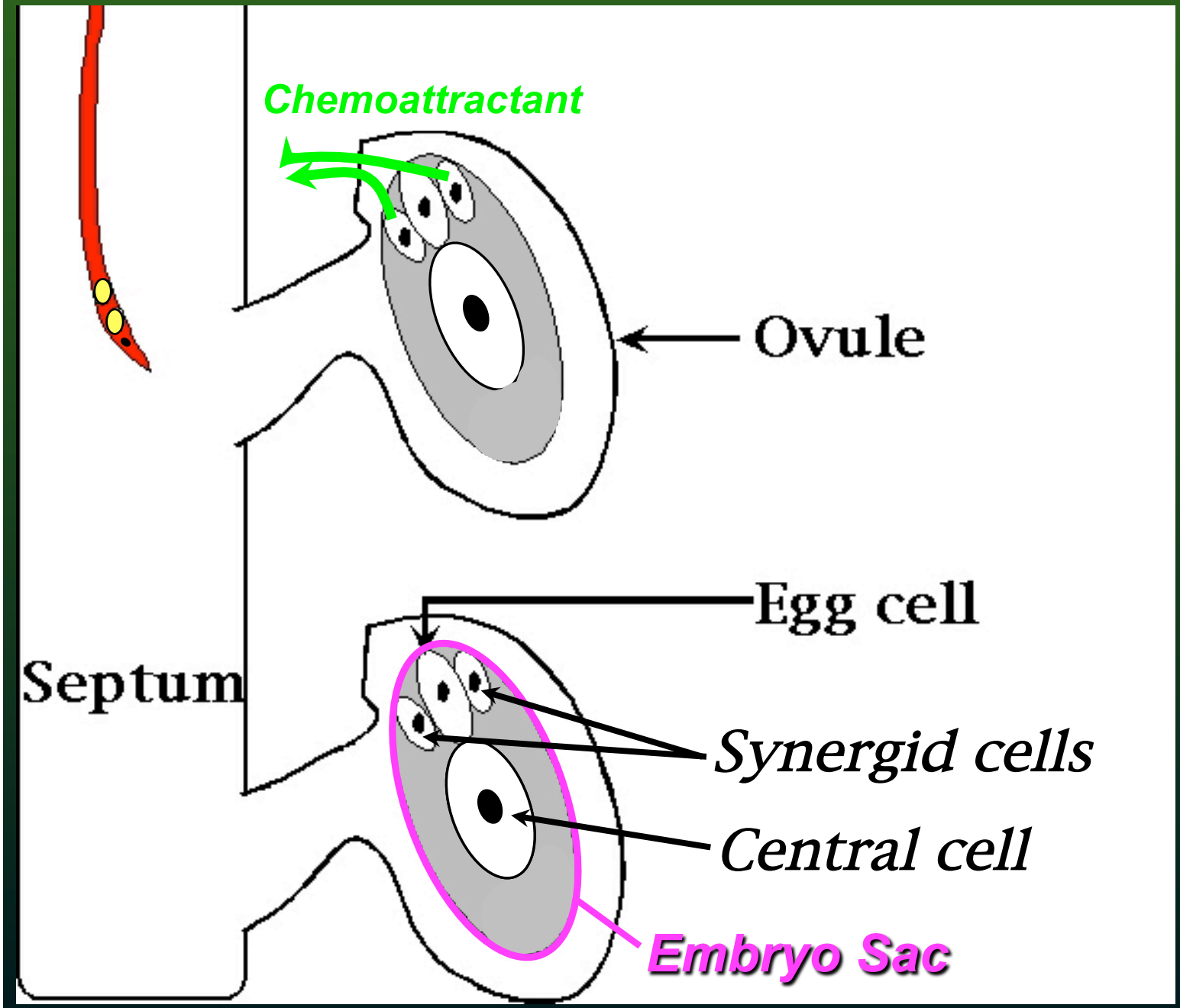
Pollen tube growth and guidance

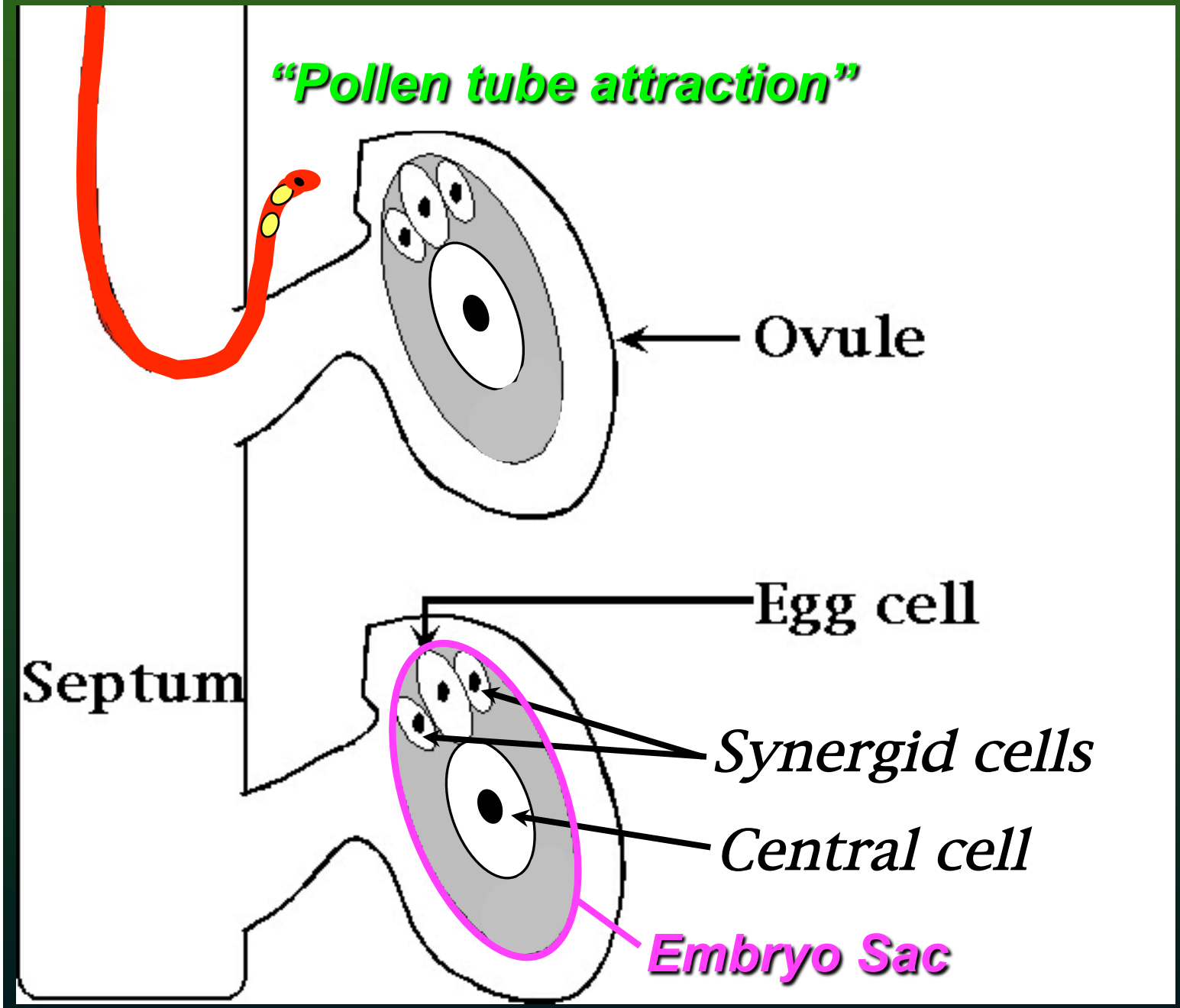
Pollen grain lands on the stigma

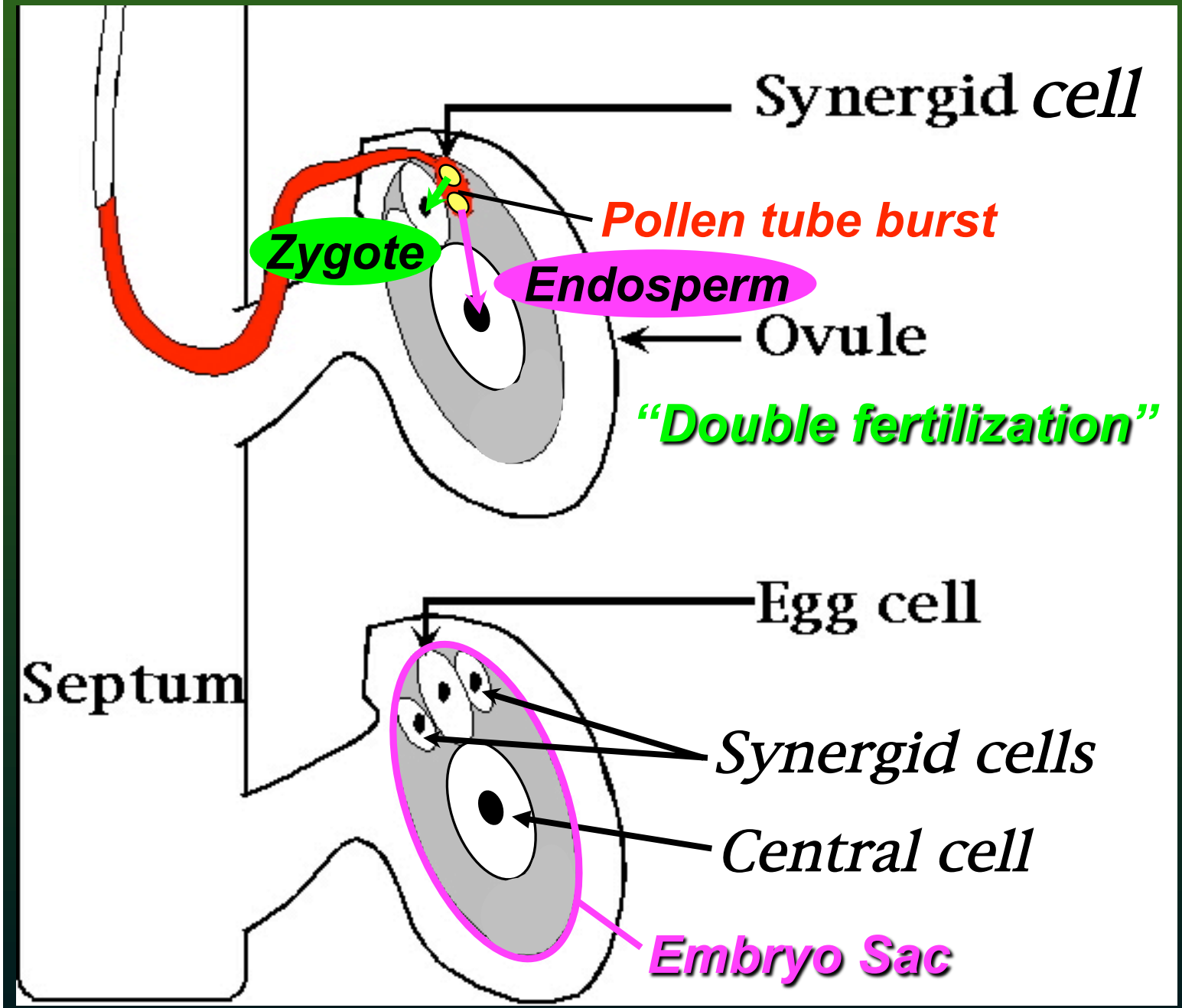
The pollen bb grain then…

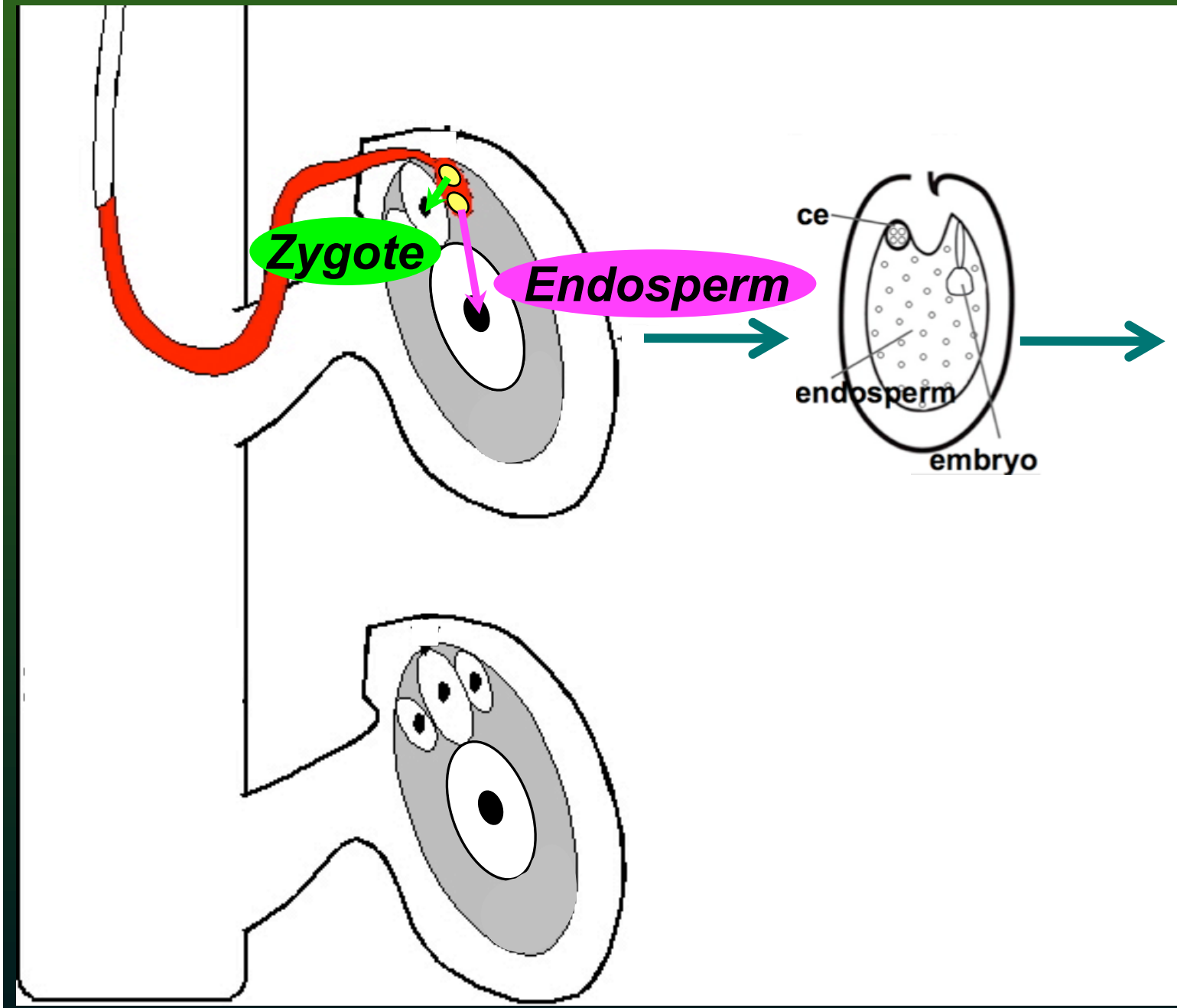
- Swells, forming a pollen tube
- Descends down the transmitting tract
- Emerges into an ovary chamber
- Locates and fertilizes an ovule



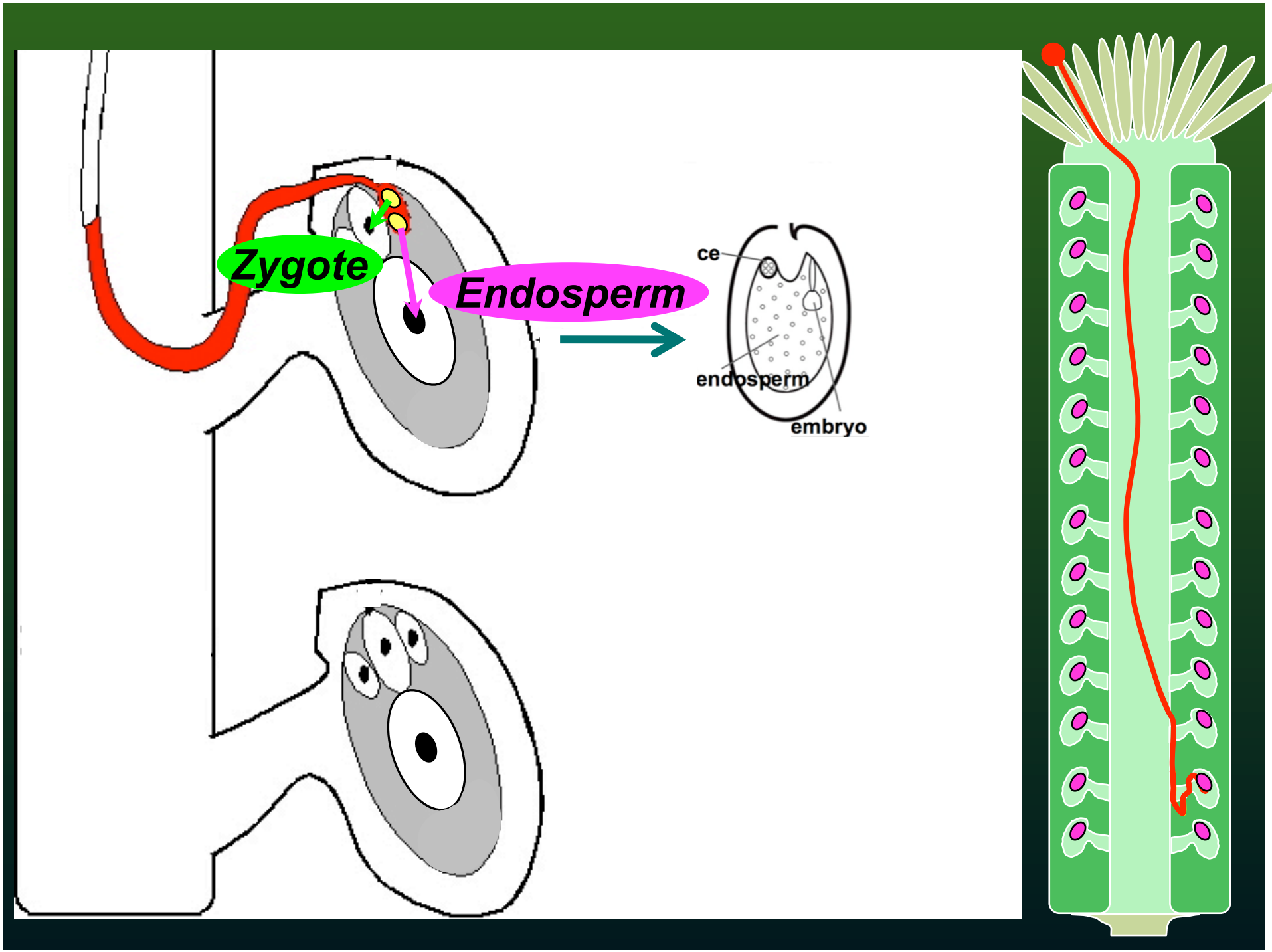

Chemoattractant
Ovule
Egg cell
Septum
Synergid cells
Central cell
Embryo Sac

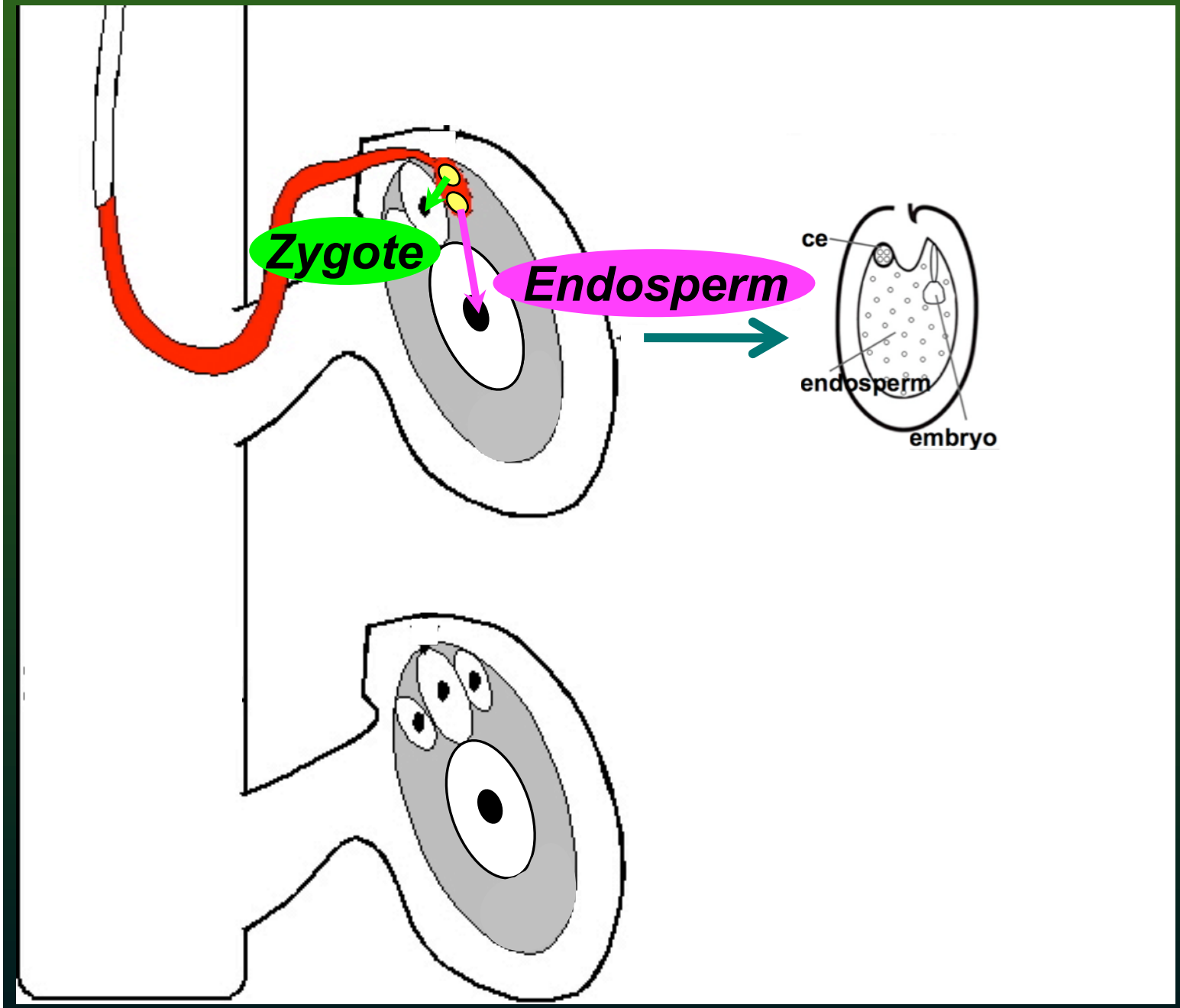

"Pollen tube attraction"
Ovule
Egg cell
Septum
Synergid cells
Central cell
Embryo Sac


Synergid cell
Pollen tube burst
Zygote
Endosperm
Ovule
"Double fertilization"
Egg cell
Septum
Synergid cells
Central cell
Embryo Sac


Zygote
Endosperm

ce
endosperm
embryo


Zygote
Endosperm
ce
endosperm
embryo


Zygote
Endosperm
ce
endosperm
embryo

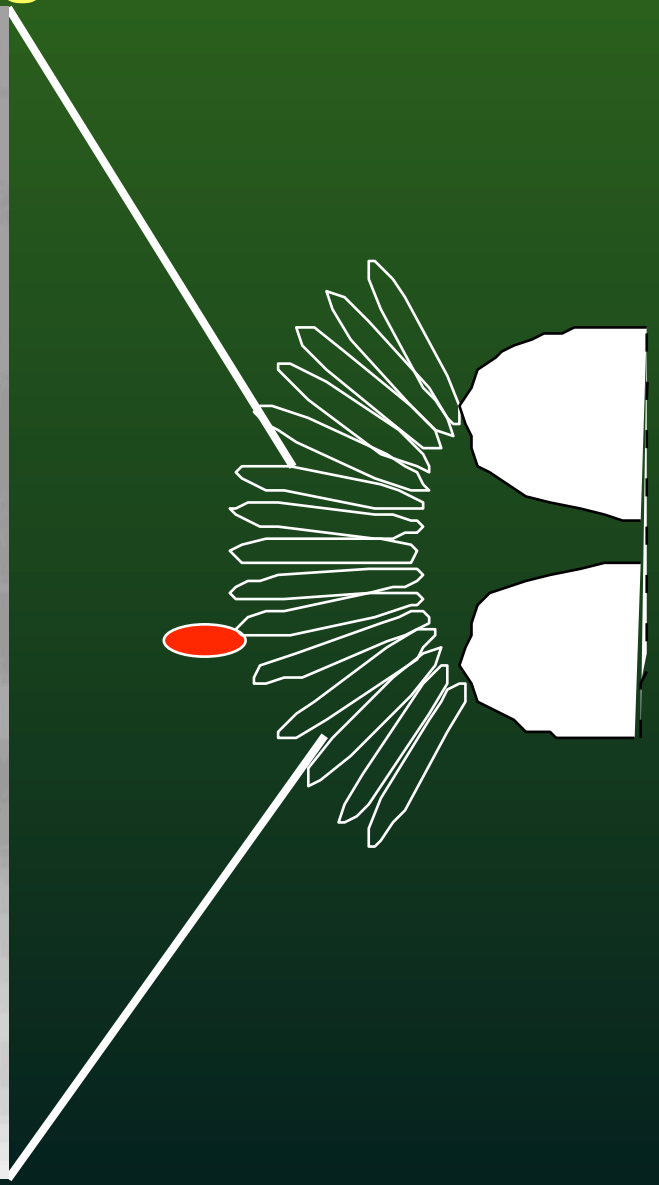
Arabidopsis reproduction

How are pollen tubes precisely guided to their target?

Yuan Qin

Jessica Franco

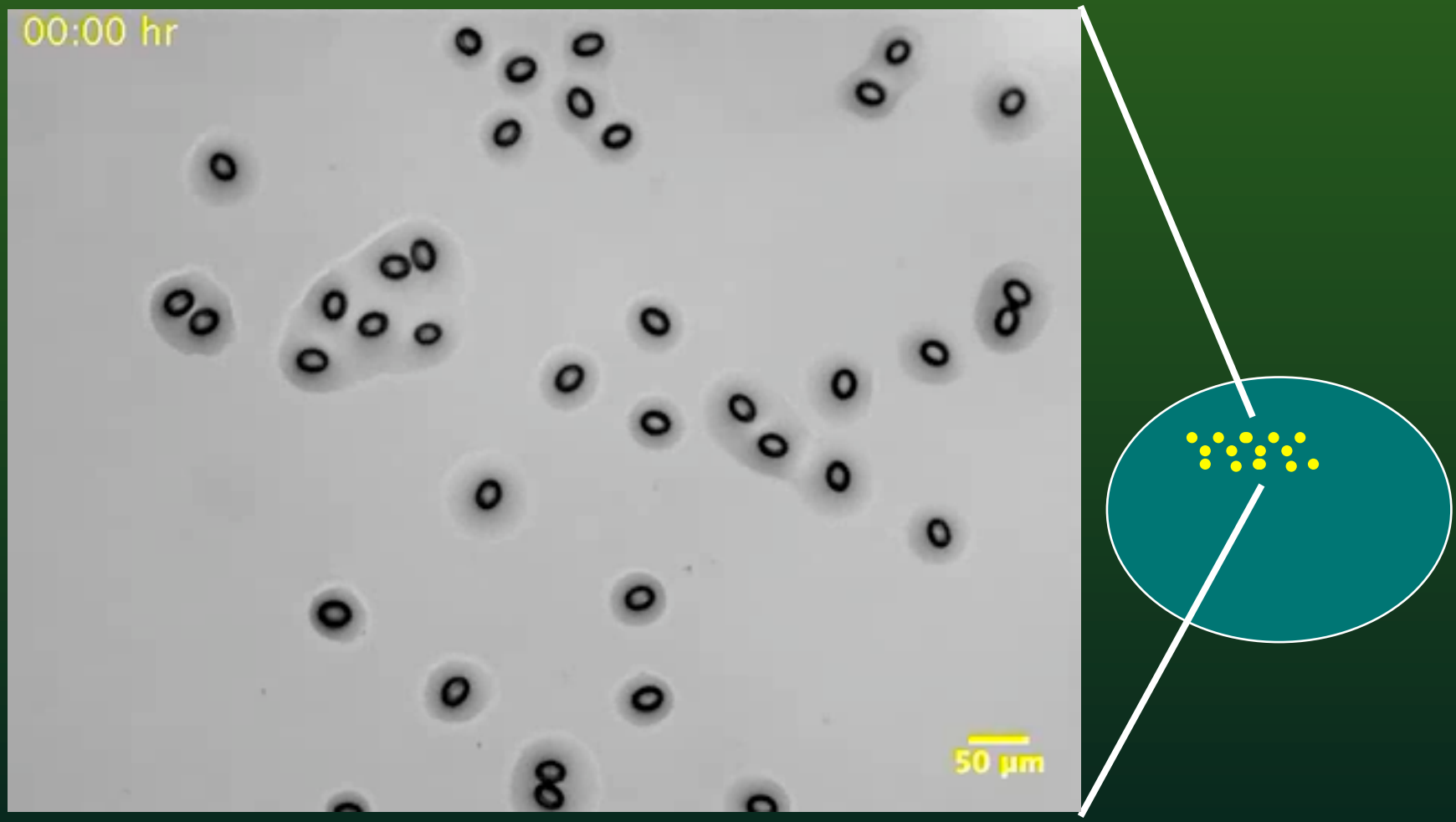
Pollen germination on a stigma cell



Total time elapsed: 30’

Tube emerges within 15’

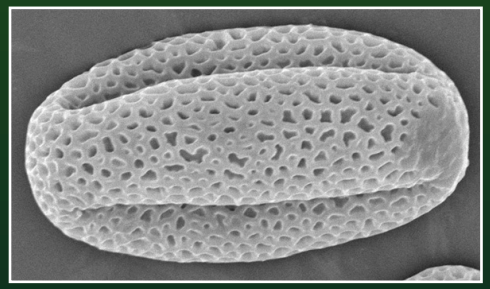
In vitro Arabidopsis pollen germination



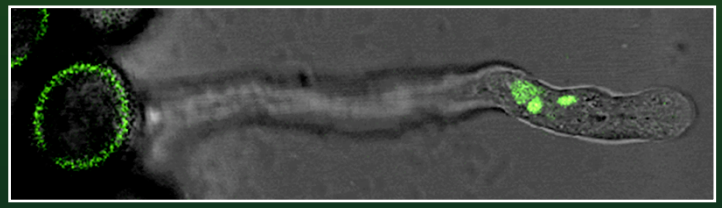
1st tube emerges only after *3 hrs and 45'*

Hypothesis:

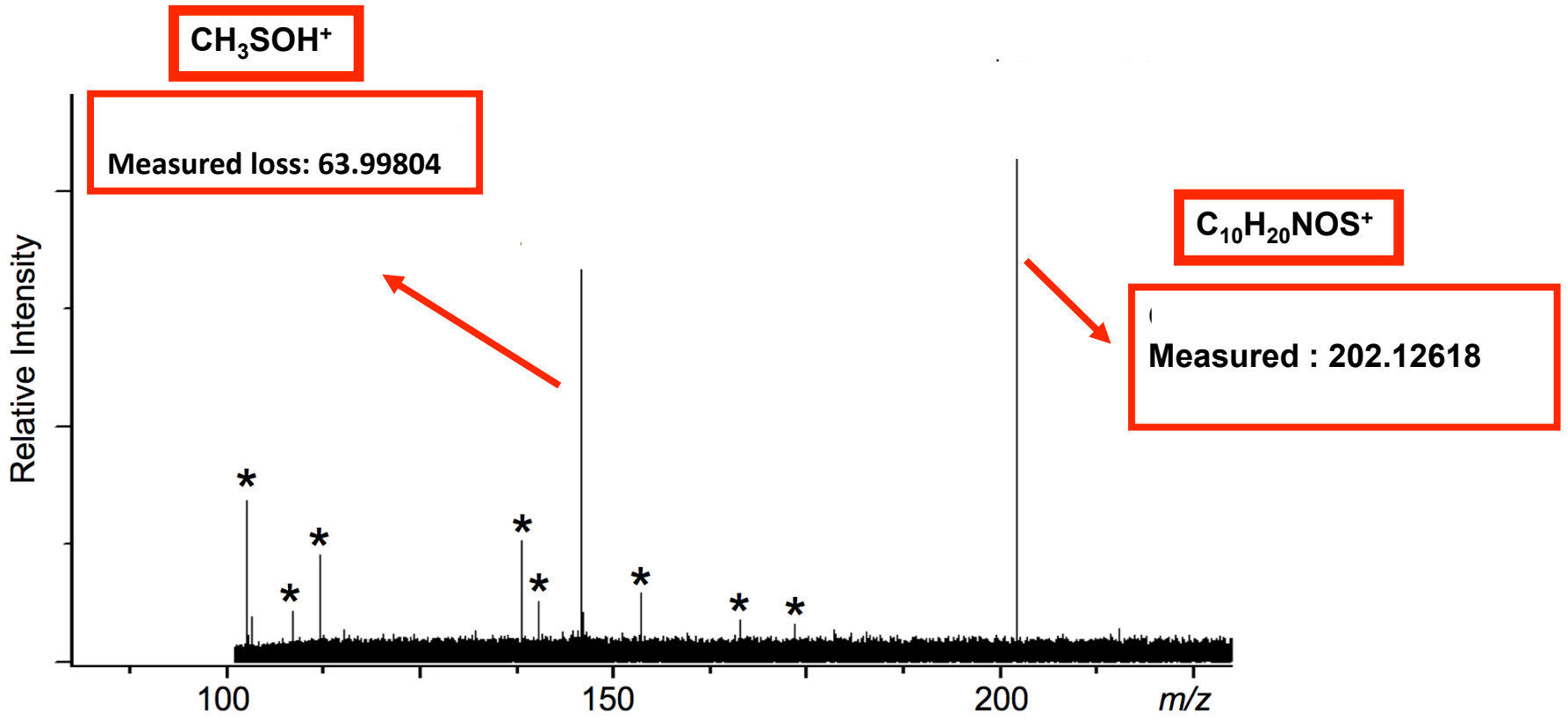
Pistil produces factors that promote pollen germination



Pistil factors

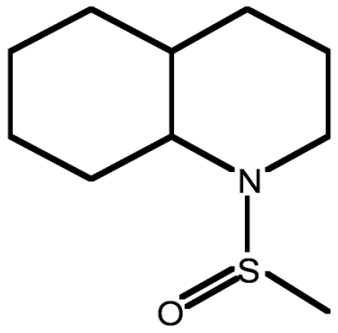


MS/MS fragmentation spectrum of *m/z* 202 in pistil extracts

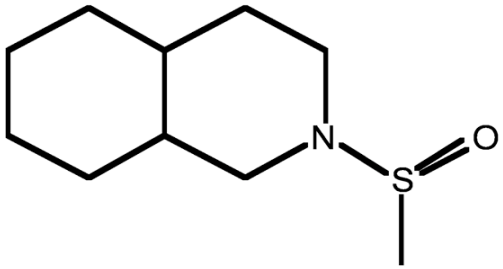


Arpad Somogyi

Synthesis of *m/z* 202 *sulfinylated azadecalins*



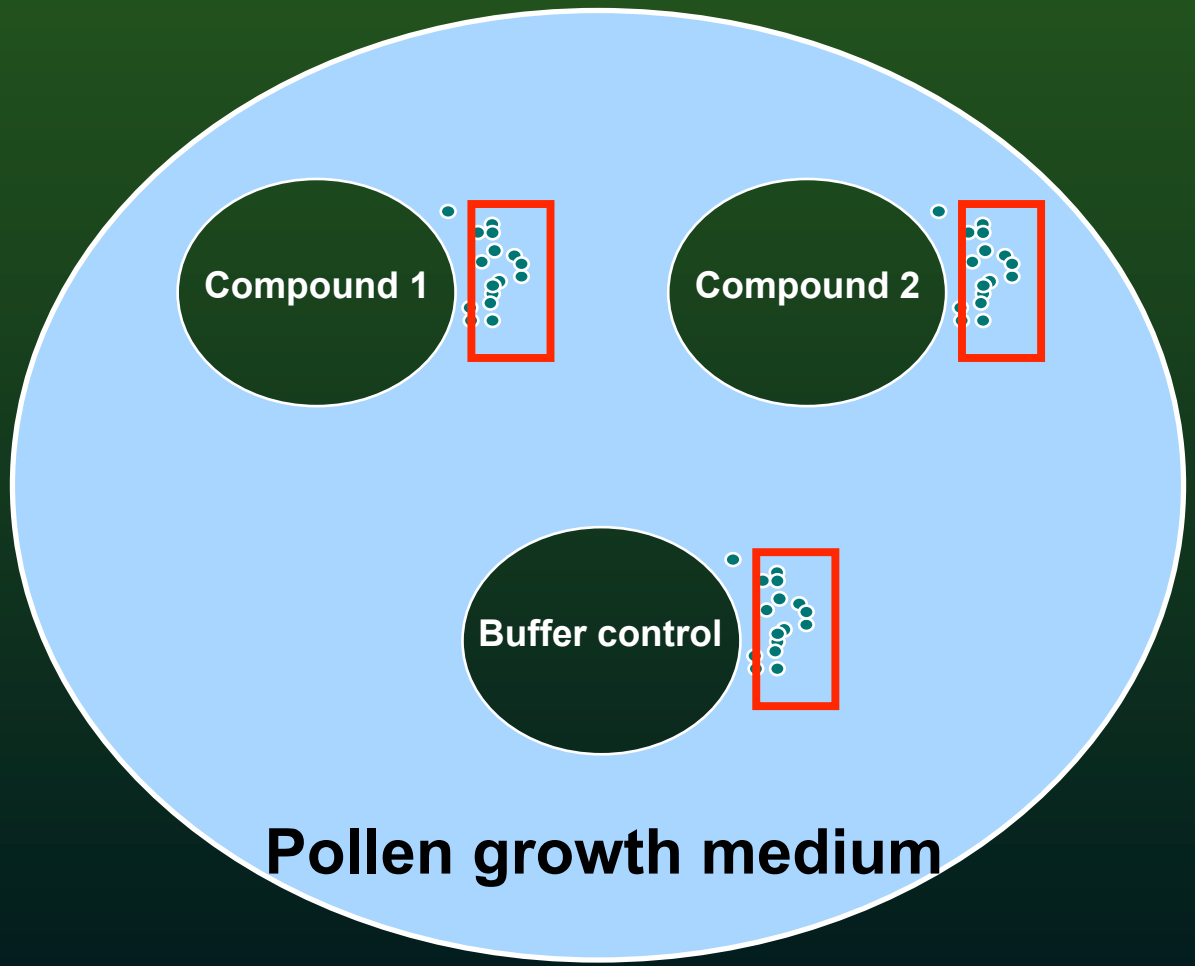
N-methylsulfinyl 1- azadecalin



N-methylsulfinyl 2- azadecalin

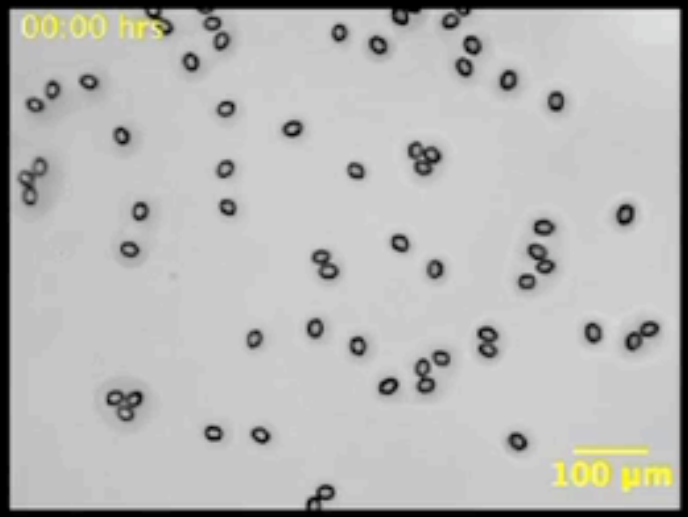
Ron Wysocki

Synthesized *sulfinylated azadecalins* stimulate pollen germination

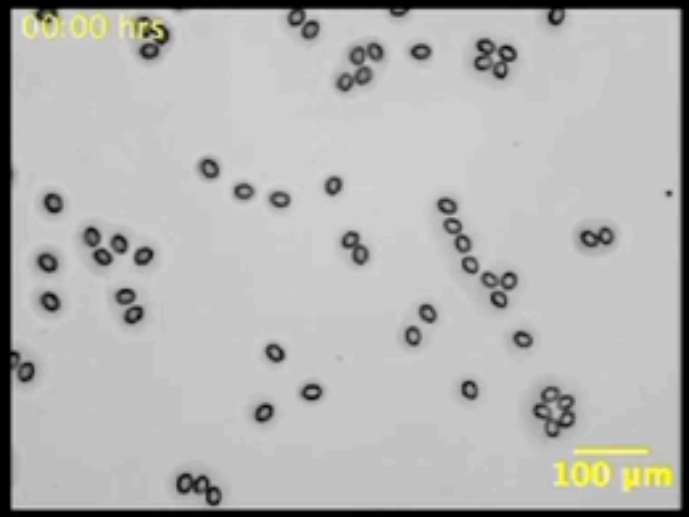


Qin et al (2011), Plant J 68 (5): 800-815

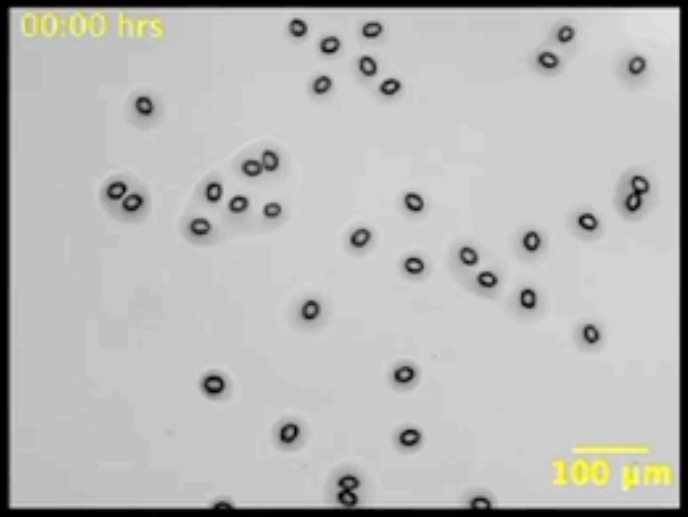
Sulfinylated azadecalins stimulate pollen germination



Stimulated with
N-methanesulfinyl 1-azadecalin

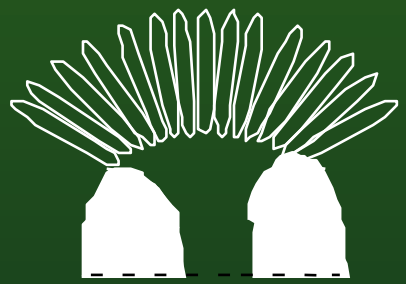


Stimulated with
N-methanesulfinyl 2-azadecalin

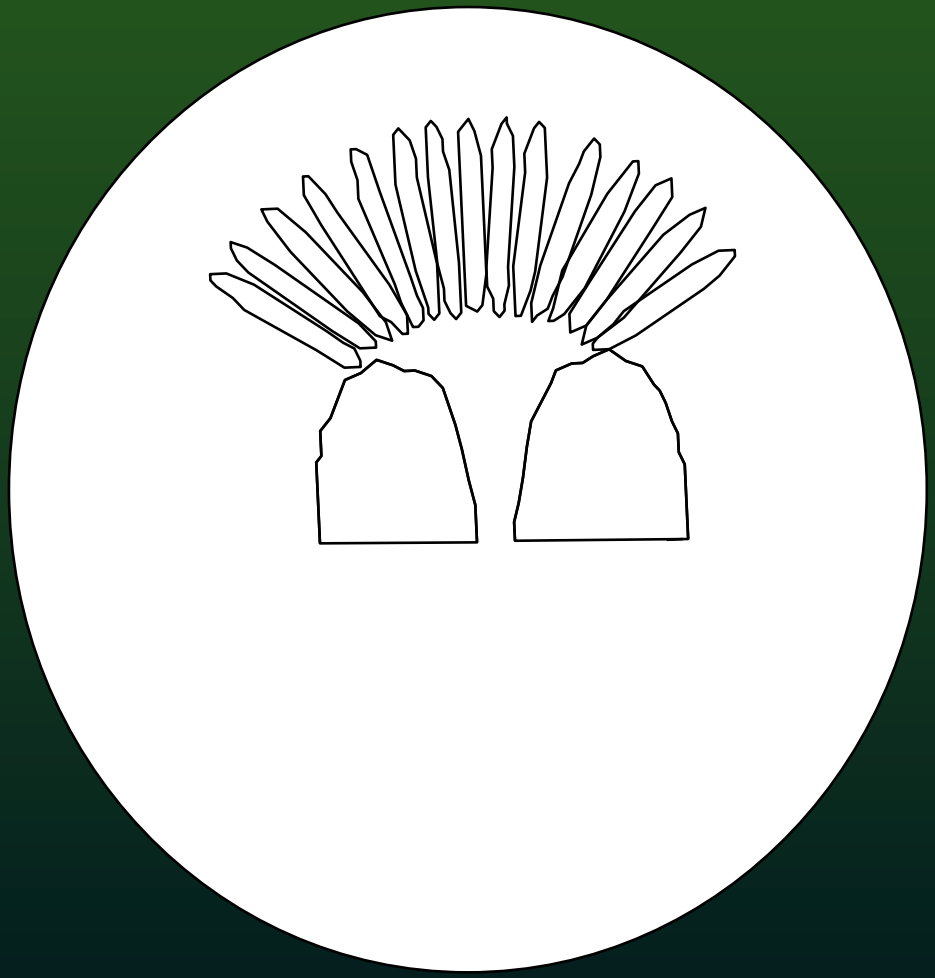


No stimulant control

in vitro Arabidopsis
pollen tube guidance system



in vitro Arabidopsis
pollen tube guidance system



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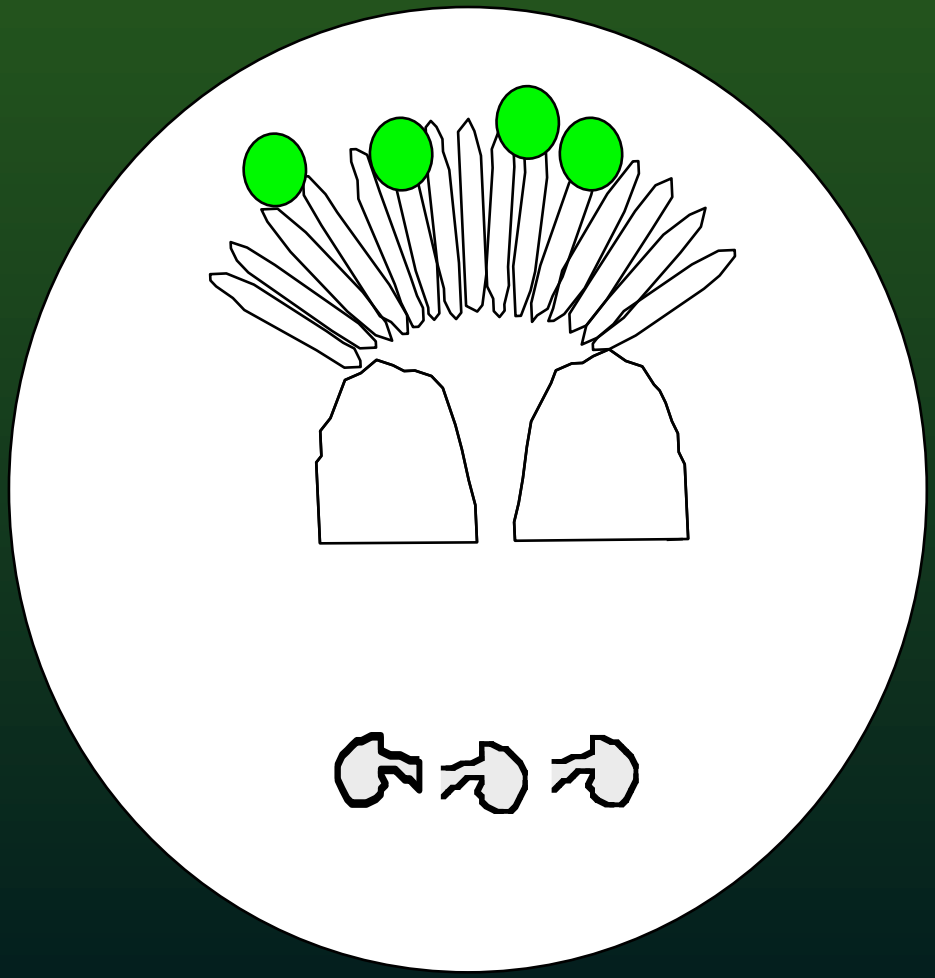

in vitro Arabidopsis
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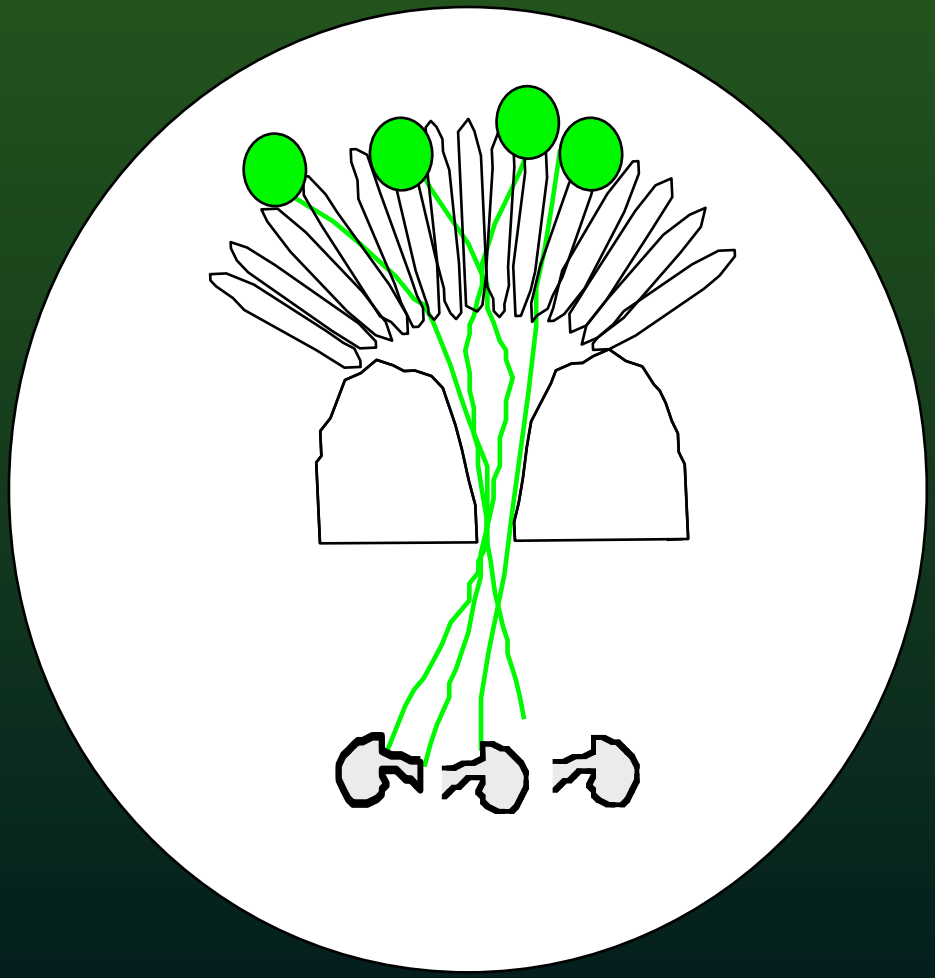


in vitro Arabidopsis
pollen tube guidance system

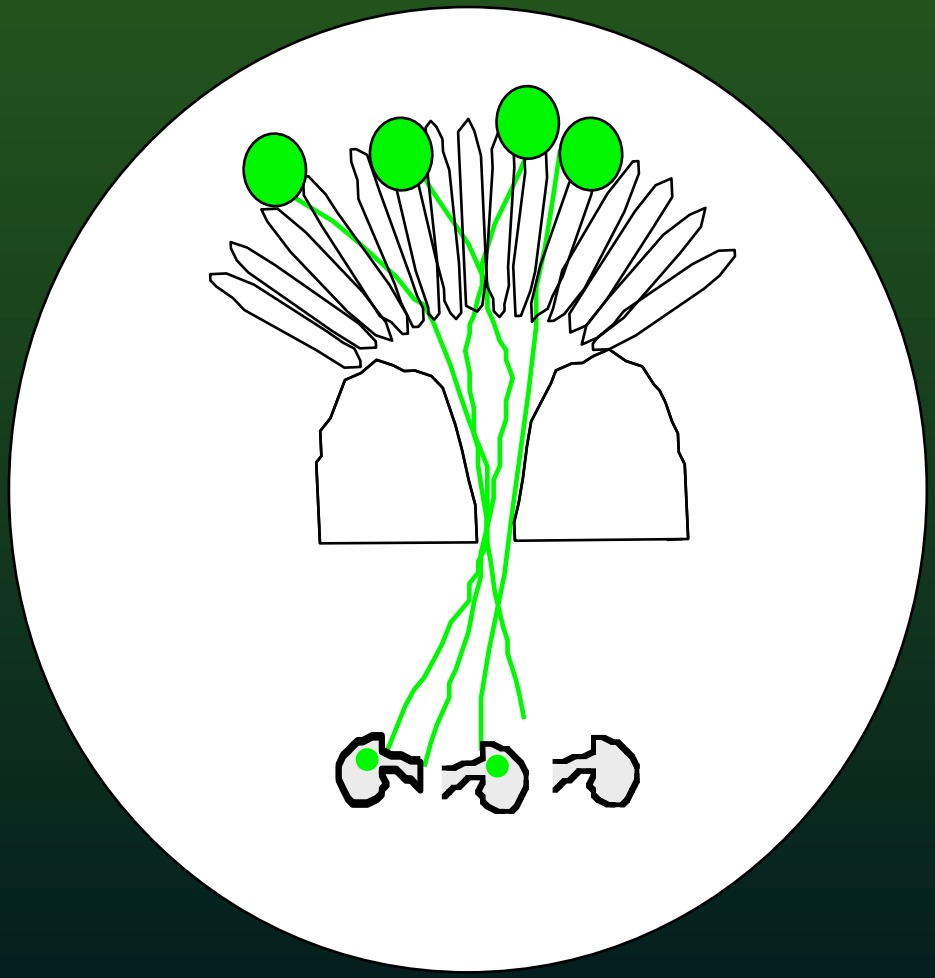


Image acquisition

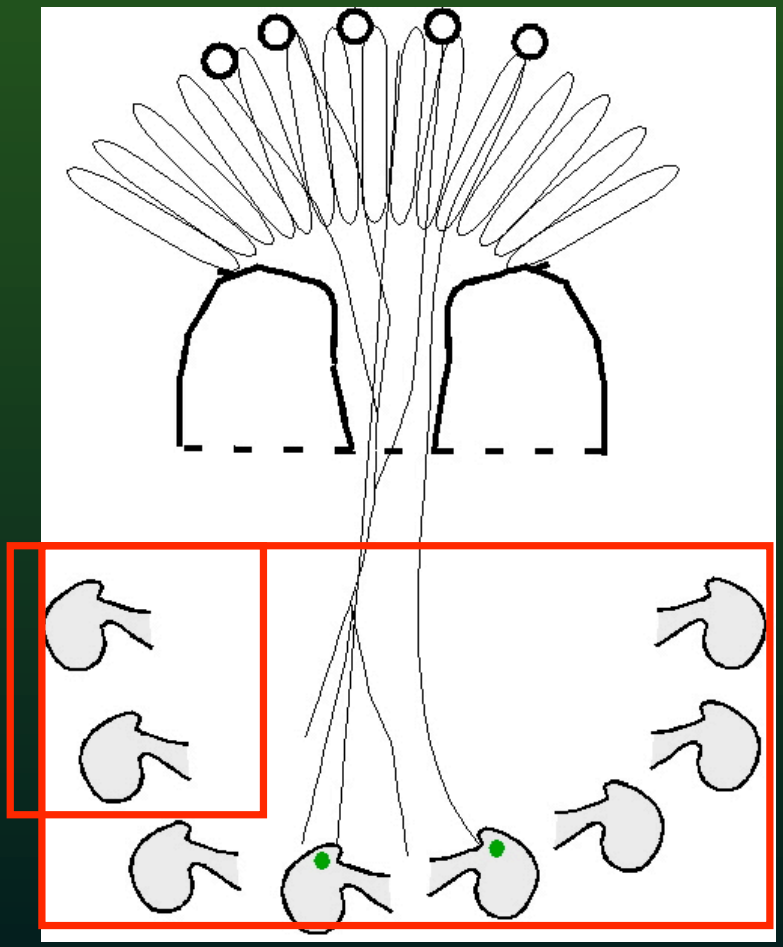
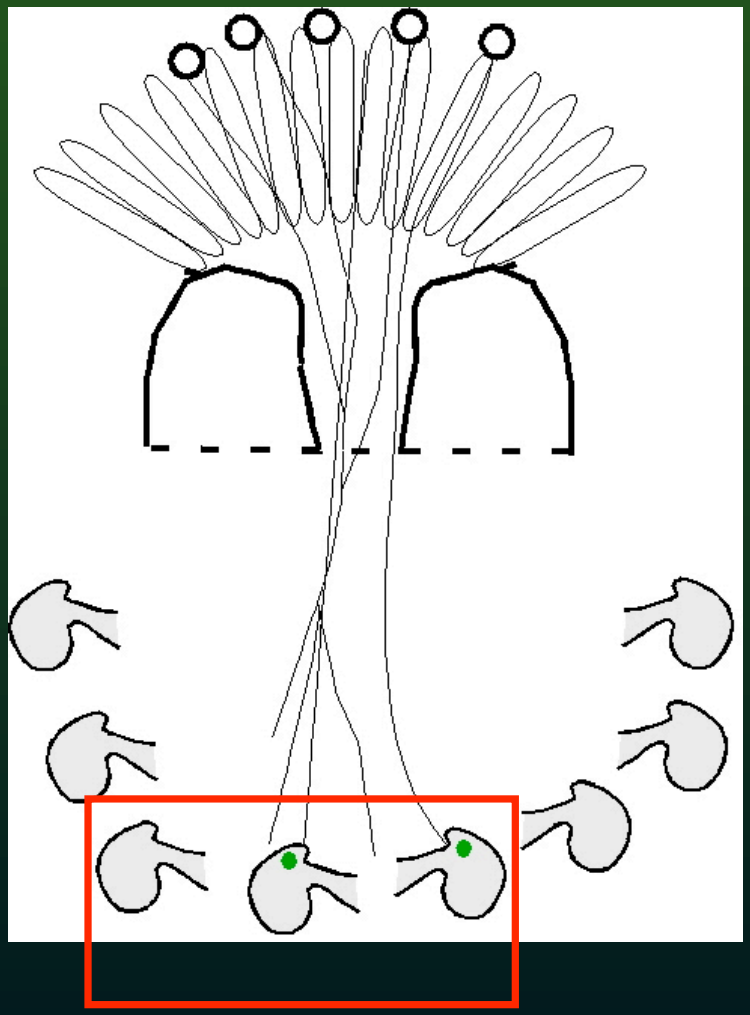


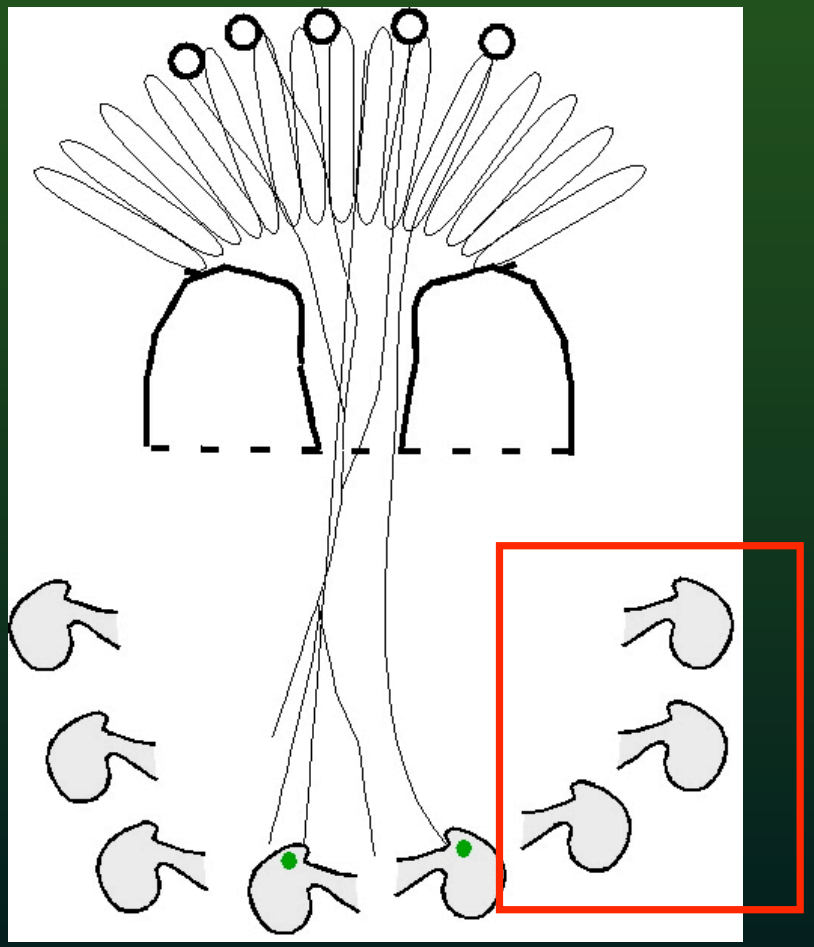
Image is taken once in every 10 minutes, once in brightfield and once under UV light

Image acquisition



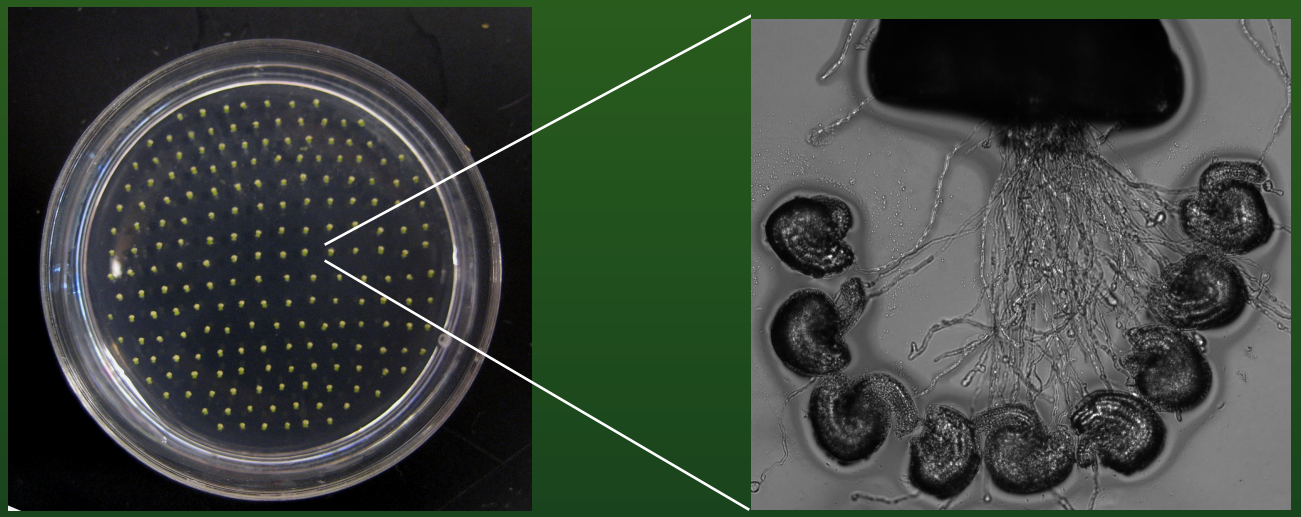
This process is repeated after automatically moving over to the next position

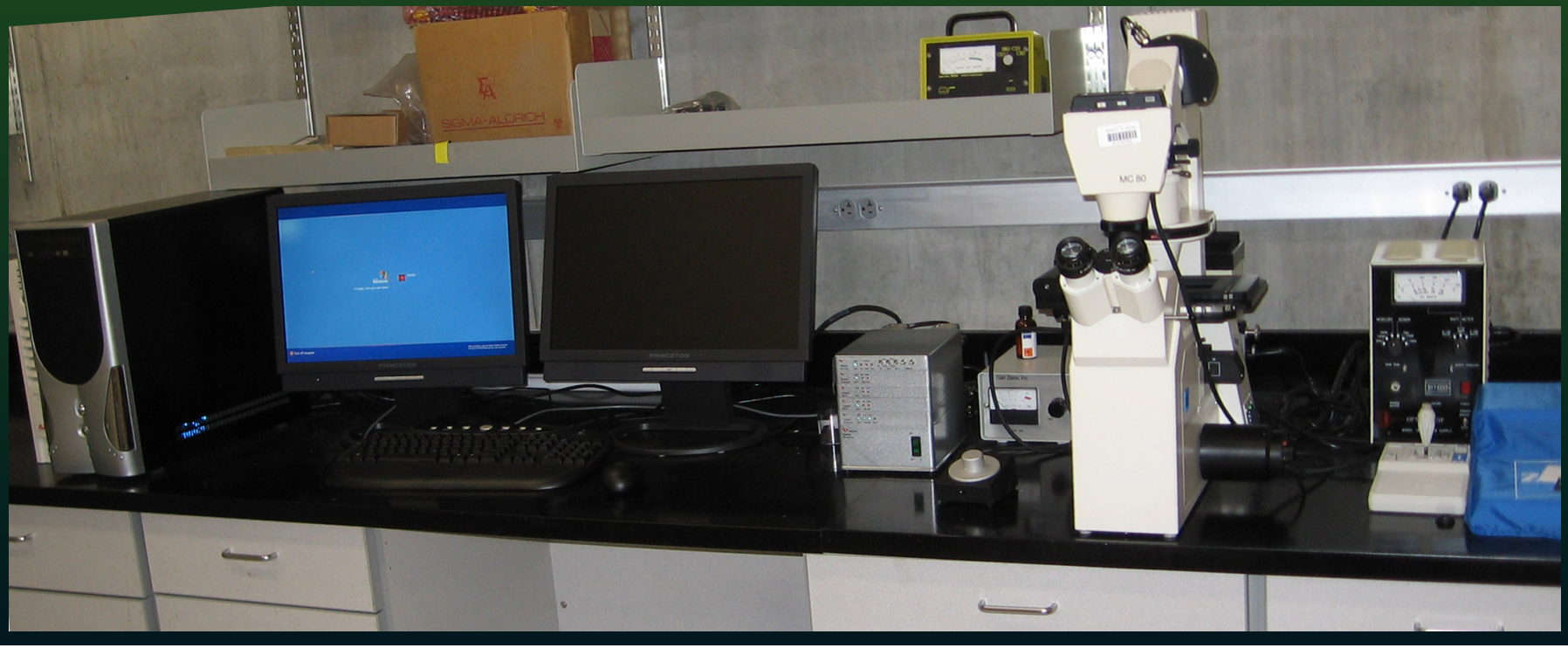
Image acquisition



This process is repeated after automatically moving over to the next position

High throughput time-lapse microscopy

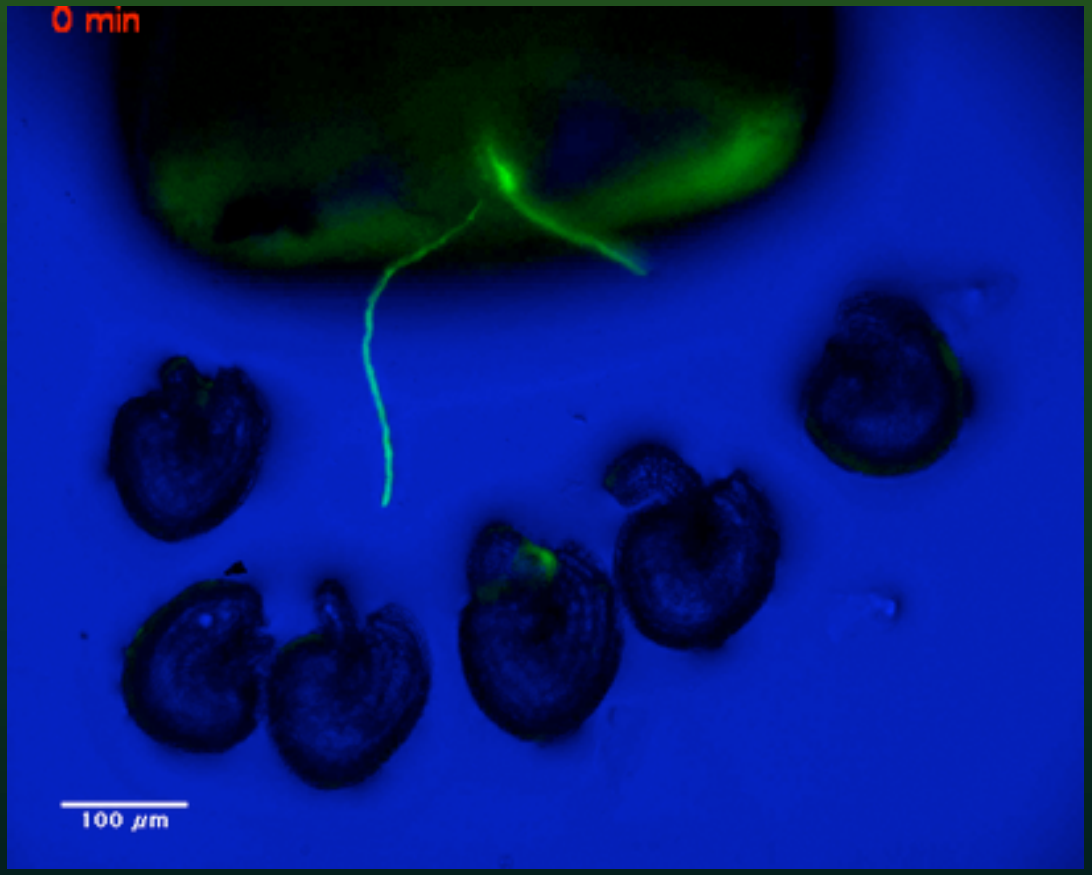


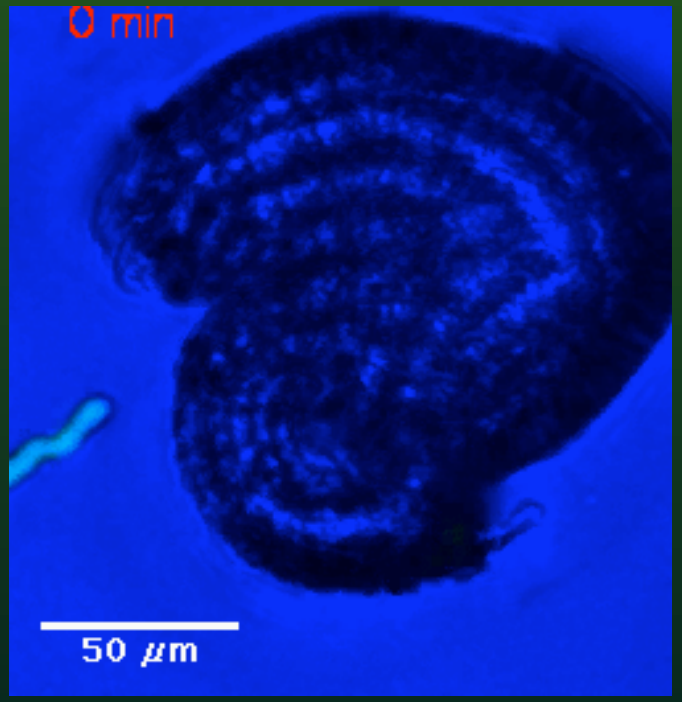


High throughput time-lapse microscopy

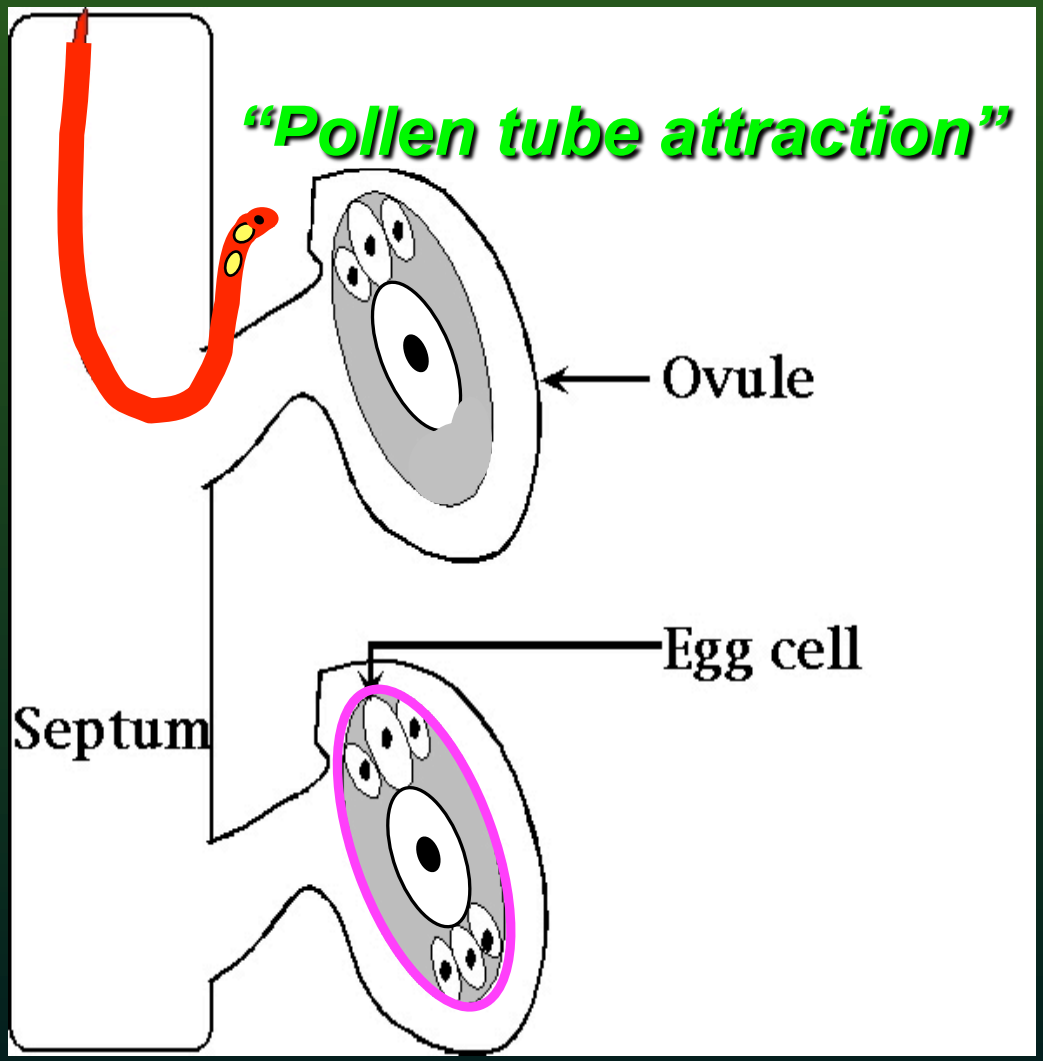
Ultimately, time-lapse series of each type of exposures (UV & bright field) are merged for each position in metamorph software and assembled as a movie using ImageJ software

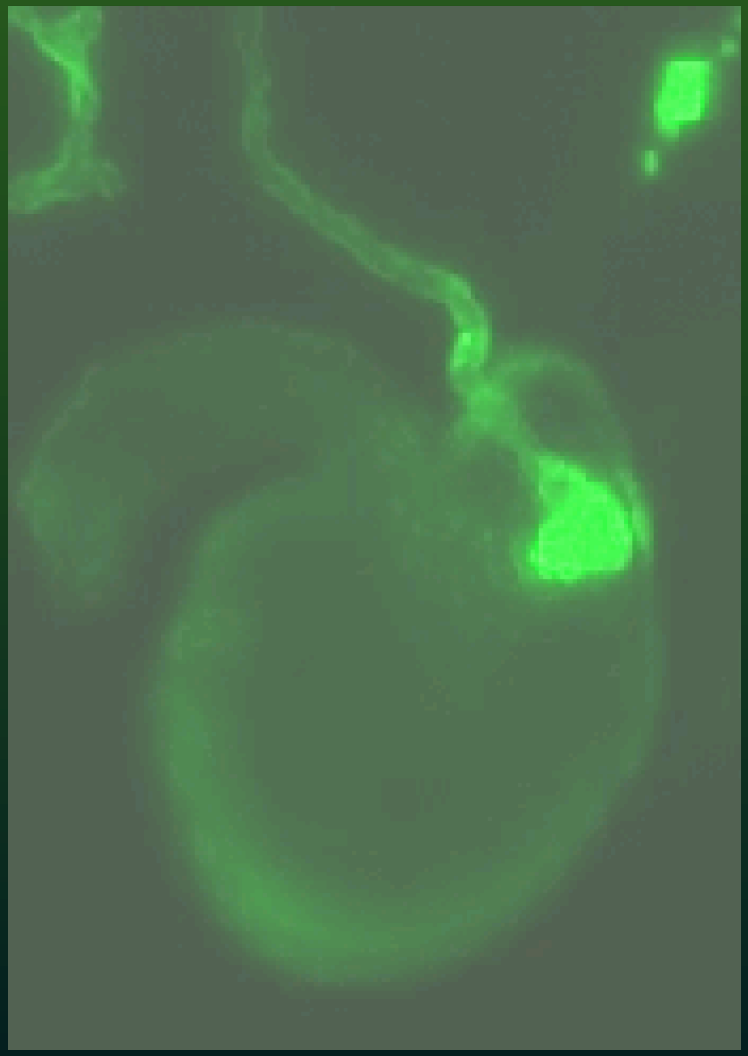
in vitro ovule targeting by pollen tube





Pollen tube attraction signals from ovules are unknown



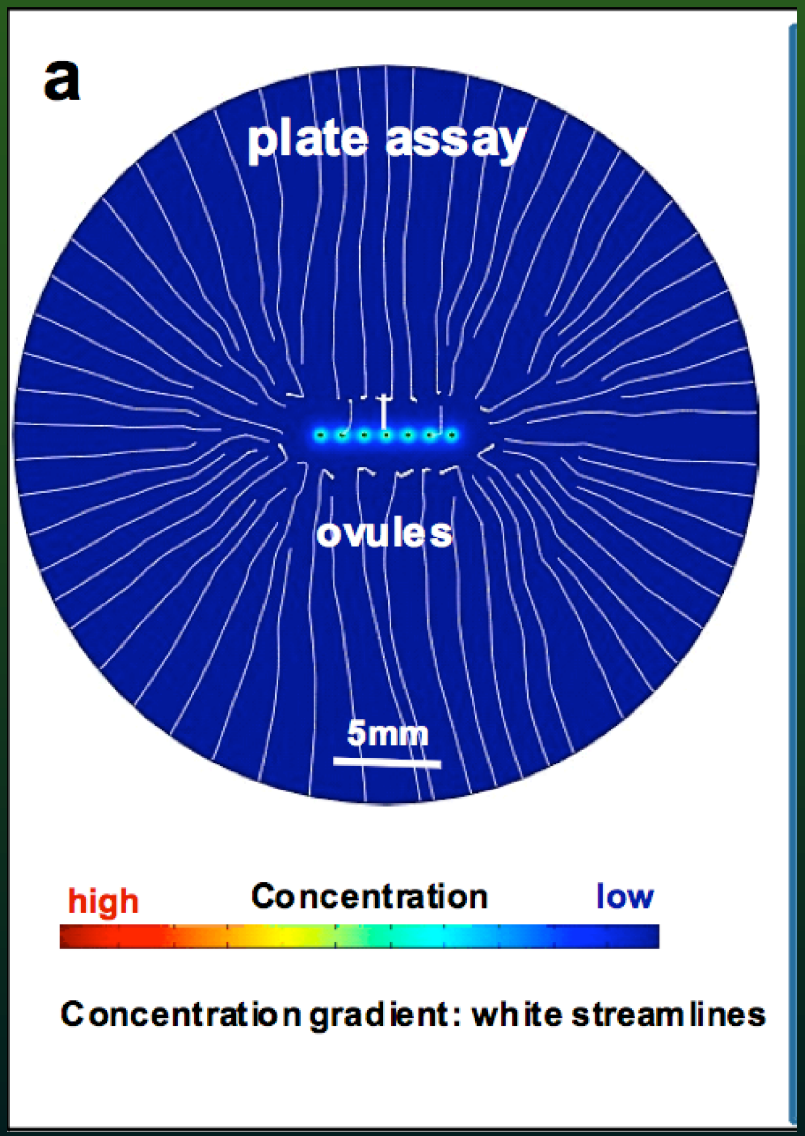


Pollen tube attraction signals from ovules are unknown

To biochemically isolate the attraction signals, it is necessary to develop a sensitive *in vitro* assay with ovule extracts rather than ovules themselves

Initial efforts to develop such an assay by spotting ovule extracts in pollen growth medium plates were not successful

Pollen tube attraction signals from ovules are unknown



The isotropic model of the plate assay is not suitable for quantitative investigation of pistil- or ovule-derived guidance signals' effect on Arabidopsis pollen tubes

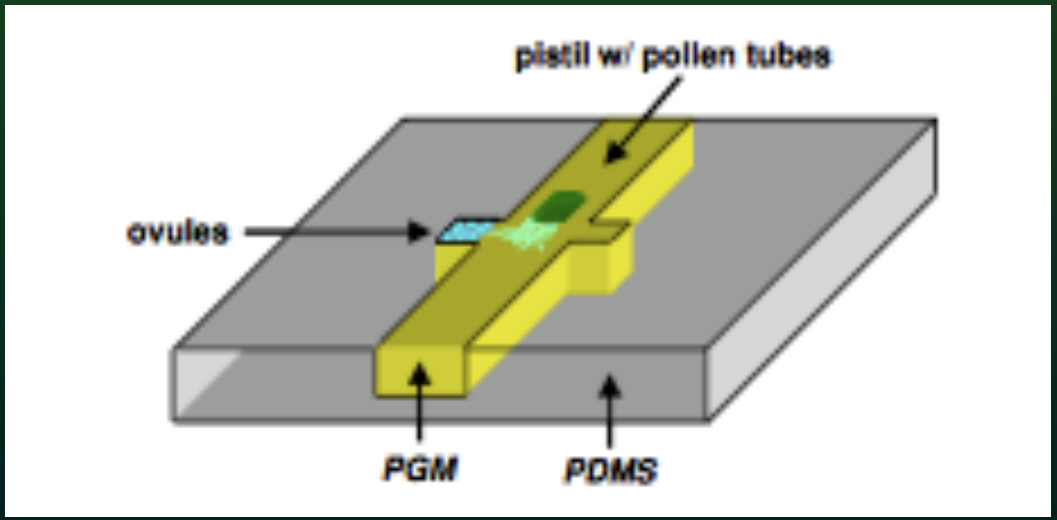
A microchannel-based assay to study pollen tube attraction

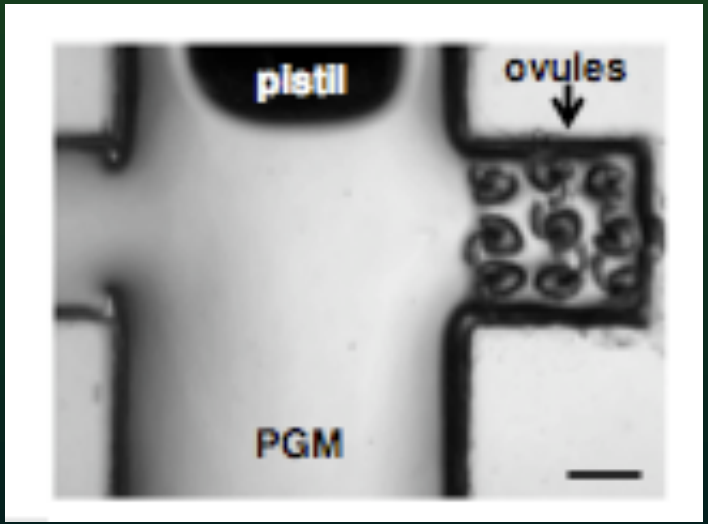
Ali Yetisen

Yitshak Zohar

A microchannel-based assay to study pollen tube attraction

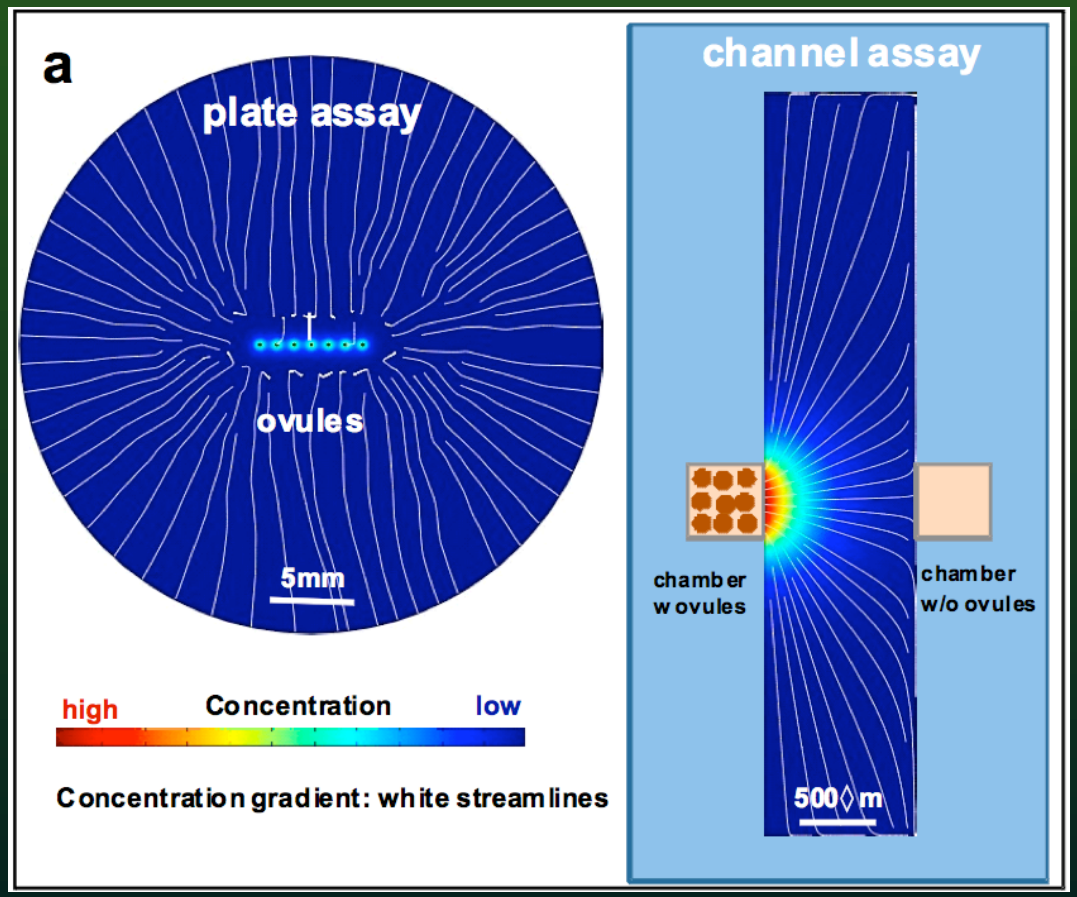
To model the micro-environment of ovule fertilization by pollen tubes, we designed a micro device consisting of a main groove and two ovule chambers on each side of the groove

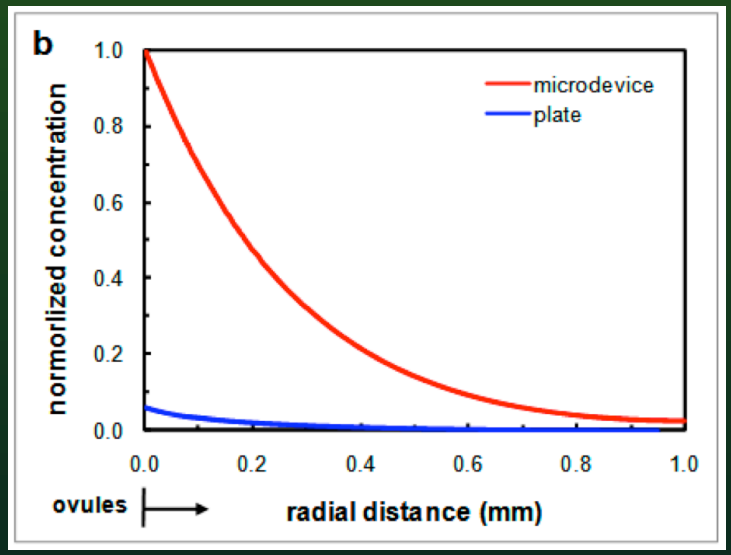


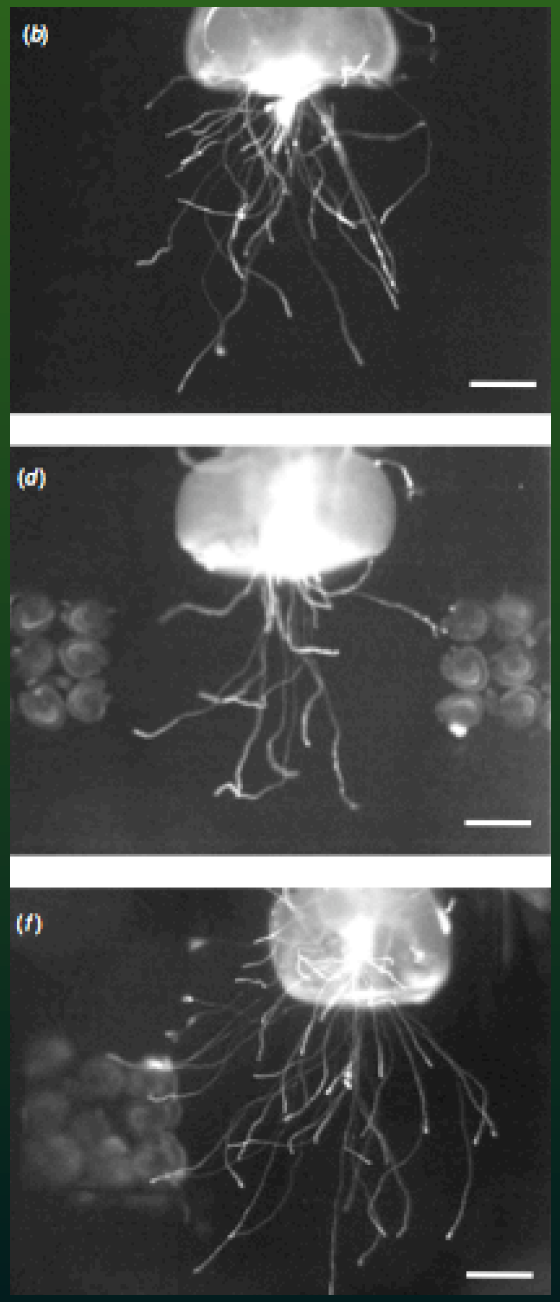


Yetisen et al (2011) JMM 21 (5): 054018

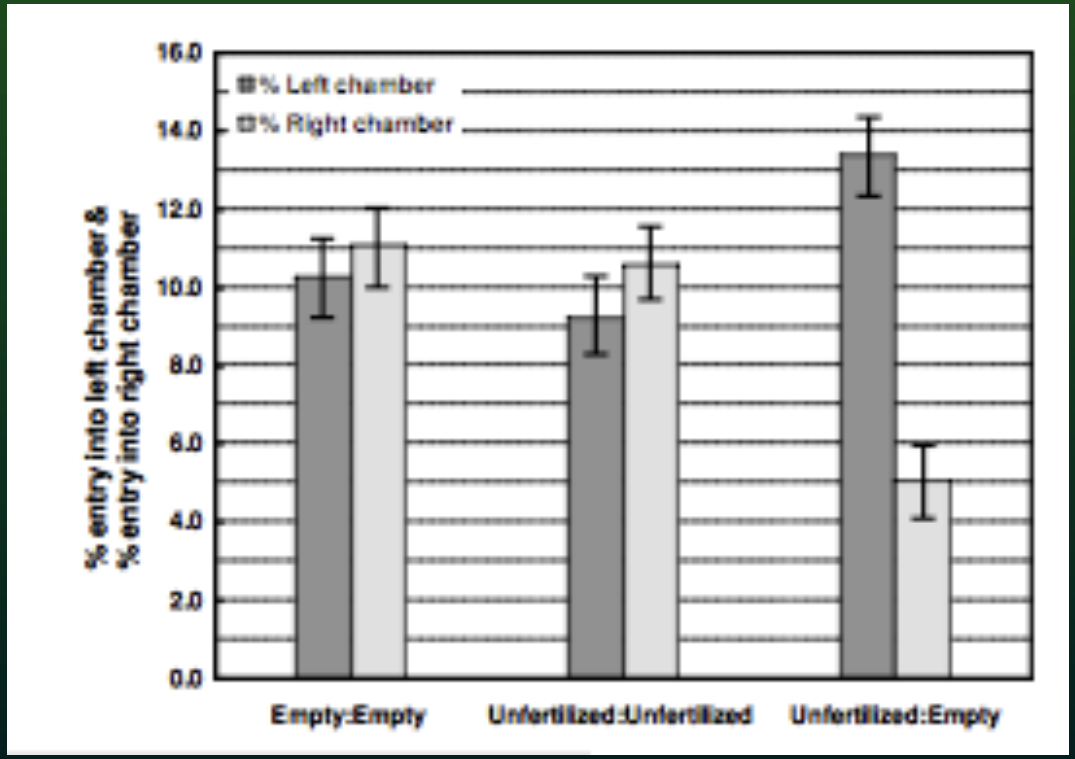
A microchannel-based assay to study pollen tube attraction



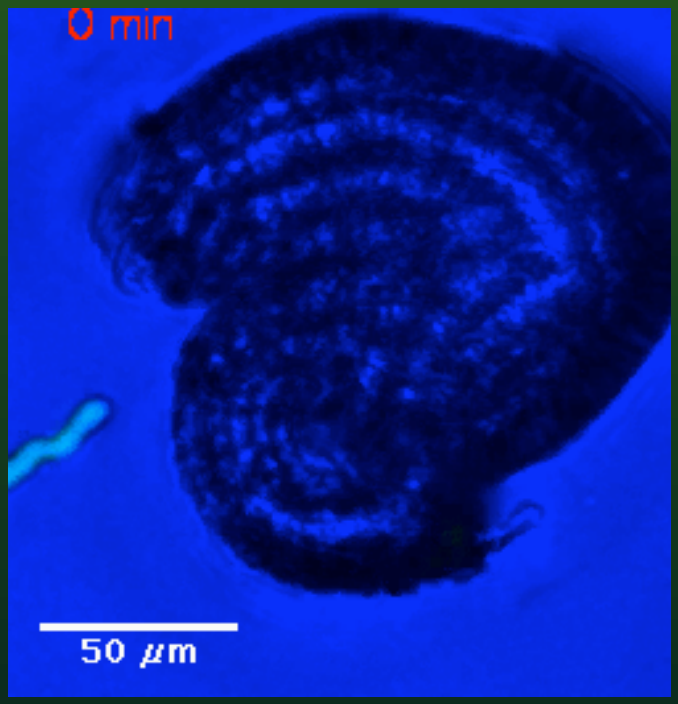




Preferential turning of pollen tubes into chambers with ovules



Pollen tube – ovule interactions



PT entry into ovule micropyle

↓

PT navigation to a synergid cell

↓

PT growth arrest at a synergid cell

↓

PT penetration of a svnergid cell

↓

PT lysis within a synergid cell

↓

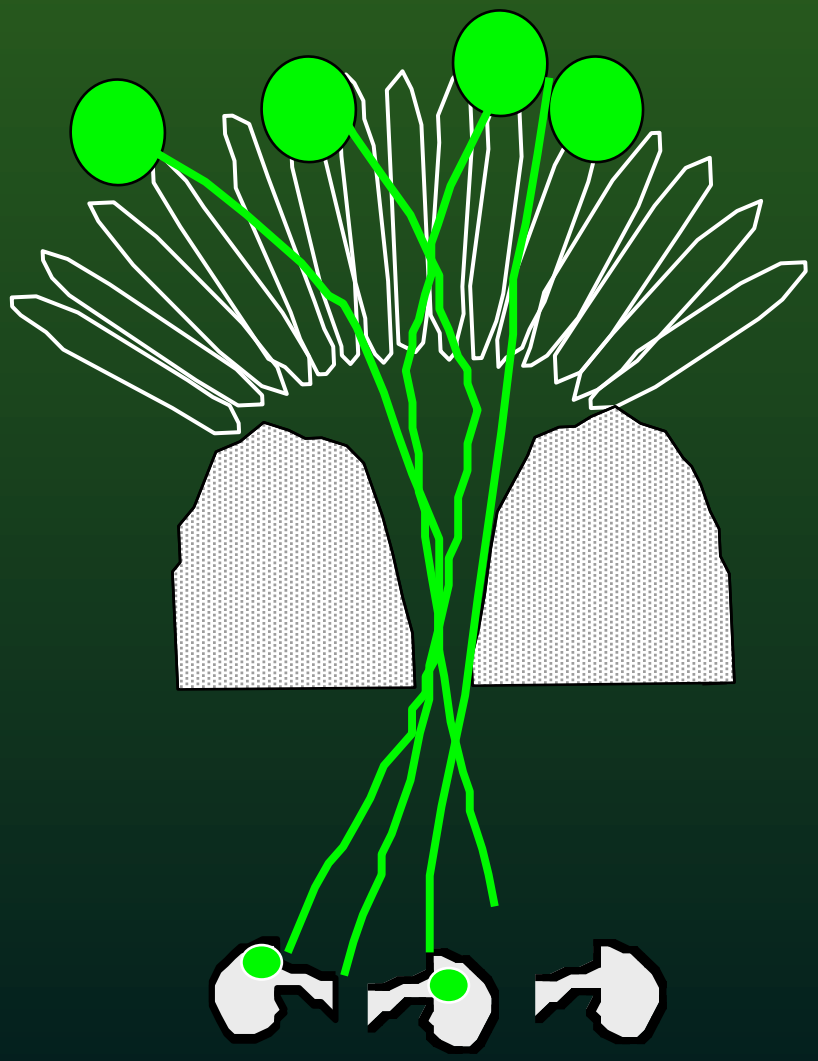
Synergid cell degeneration

↓

Double fertilization

Sandaklie-Nikolova et al (2007); Plant Physiol. 62 (4): 571-588

Pollen tube – ovule interactions



Mutants defective in pollen tube - ovule interactions are essential to understand the mechanism of these interactions

Screening would require high-throughput image acquisition

In turn this raises the need for… automated and high -throughput image analysis

Modeling pollen tube growth and behavior

Ernesto Brau

Kobus Barnard

Dami Dunatunga

Tatsuya Tsukamoto

Modeling pollen tube growth and behavior

- Generative model
- Characteristics of pollen tubes and ovules govern the states (*Z, W*) they go through, which stochastically 'generate' their observed physical features

Table 3. States for pollen tubes and ovules

State	Pollen tube type	State	Ovule type
0	Dead	0	Dead
1	Generic drift	1	Attracting tube to micropyle
2	Targeting ovule	2	Attracting tube to a synergid
3	Entered ovule	3	Receiving sperm
4	Targeting synergid		
5	Reached synergid		
6	Bursting		
7	Repelled from ovule		

Modeling pollen tube growth and behavior

- We can use the *in vitro* system to acquire images of pollen tube-ovules interactions
- Subsequently, we can understand the 'posterior distribution' of pollen tube and ovule states based on the tube tracks
- Prior to modeling its behavior, we need to be able to track multiple pollen tubes over a period of time

Computer Vision Tasks

- Track tubes
- Identify ovules and their orientation
- Classify behaviors

Pollen tube tracking

- Tip growth approximation makes this a classic tracking problem

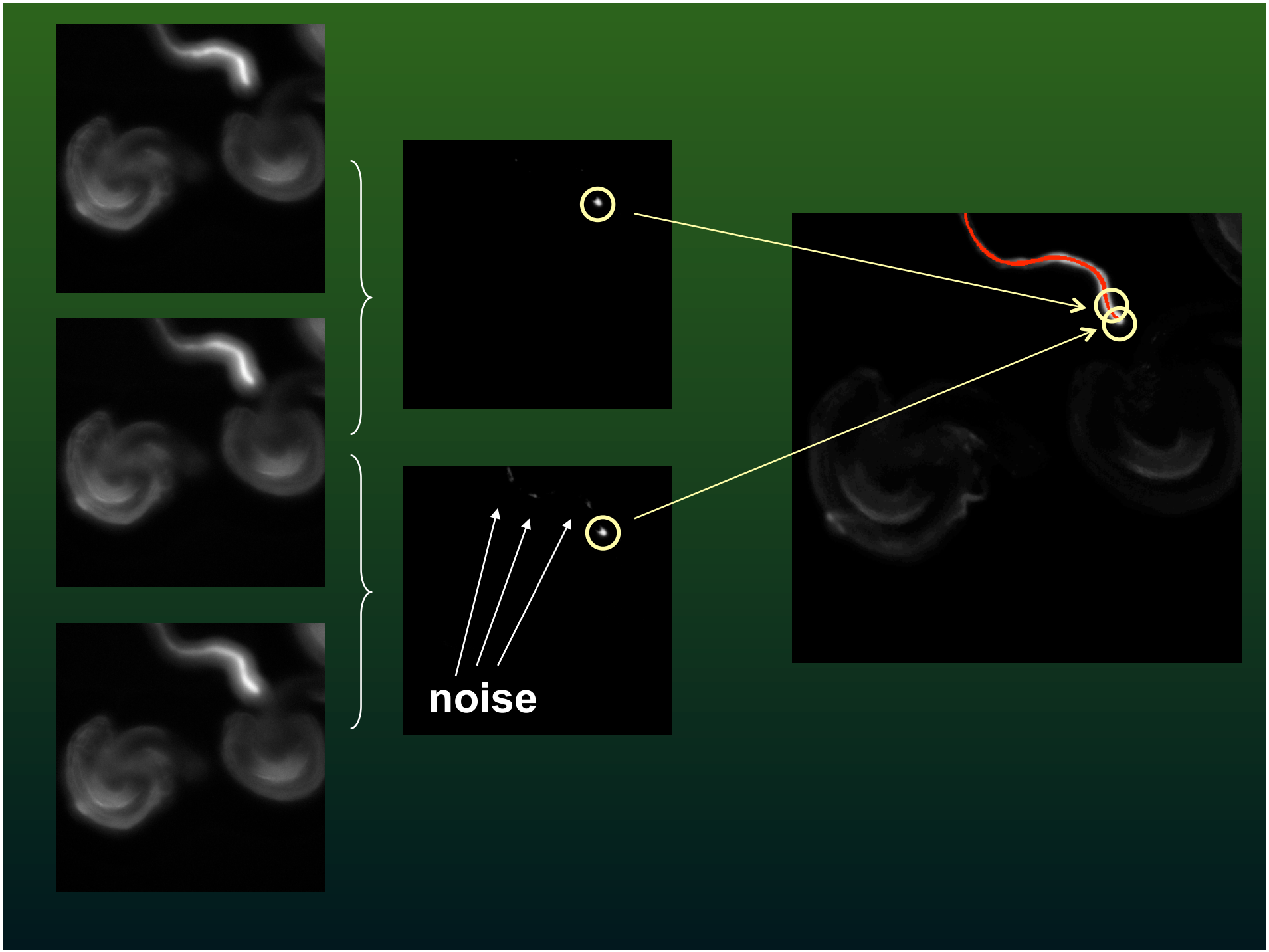
Pollen tube tracking

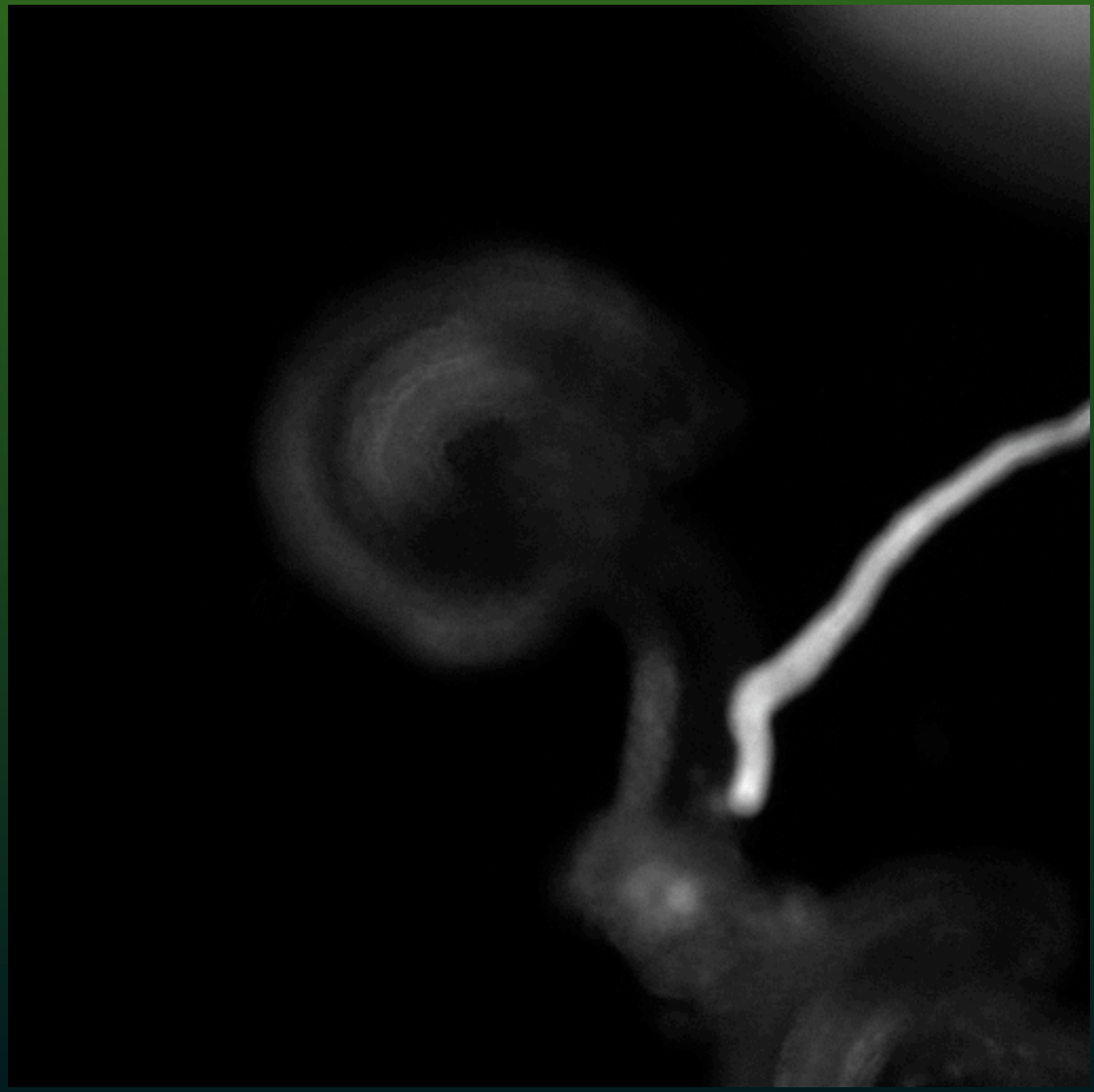
- Challenges
  - Multiple tubes
  - Overlapping trajectories
  - Unknown number of tubes
  - Missing data
  - Noisy data

Pollen tube tracking

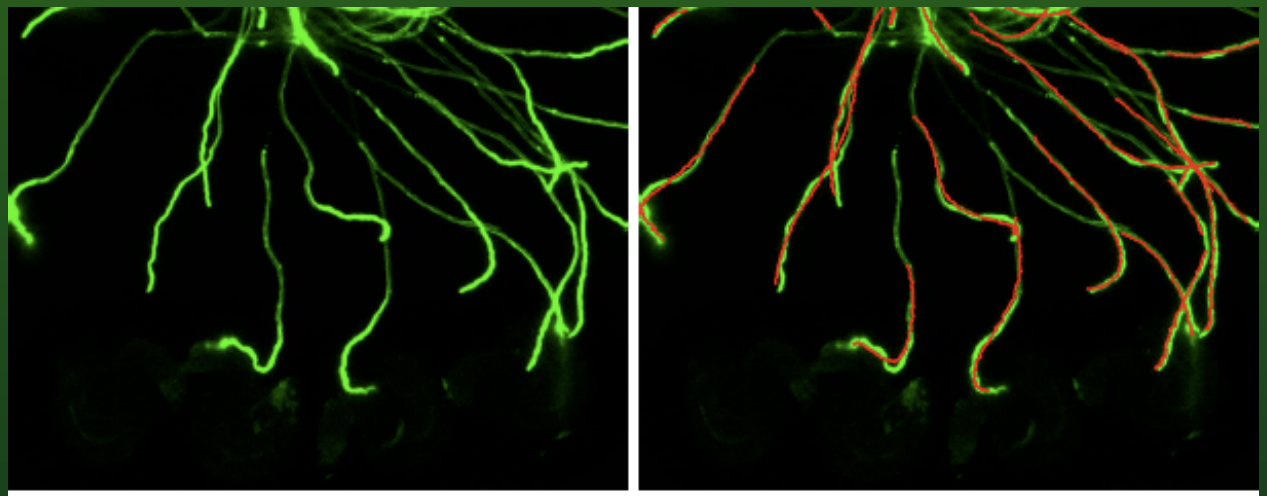
- **Solutions**
  - Assume that pollen tube trajectories are relatively smooth
  - Generate a generative statistical model
  - Model trajectories using a Gaussian process where the correlation of object position with preceding and subsequent position drops of exponentially with time

Brau et al (2011) CVPR 1137


noise



Pollen tube tracking



(a) Data image (b) Superimposed tracks

93% accuracy on images sequences with up to 10 trajectories (35 sequences)

88% accuracy when there are >10 trajectories (42 sequences)

This performance surpasses that of using an **LDS motion model** and far exceeds a **simple heuristic tracker**

Brau et al (2011) CVPR 1137

Next steps - Learning generative models for multiple pollen tube-ovule interactions from image sequences

- This is a very principled approach to understanding pollen tube and ovule behavior
- So far, most of it is already implemented
- Next, we it will be tested on image sequence data
- Eventually, we will use this for automatic classification of mutants

Brau and Barnard

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