LECTURE THEATRE
TEACHING LAB
LAB 19
IMAGING SUITE

DAY	MORNING	AFTERNOON	NOTES
Sunday 15th March, 2015		**ARRIVAL**	
Monday 16th March 2015	Welcome; Lecture on plant genetic transformation (Ute); Lecture on calcium signalling and calcium measurement / imaging (Marc).	Preparation of buffers / solutions, reconstitution with coelenterazine (**1**) Setting up Agrobacterium cultures for infiltration (**2**) Setting up protoplasts from transgenic lines expressing fluorescent proteins Imaging fluorescent proteins in protoplasts (**3**).	**1.** These will be Arabidopsis pMAQ2 (cytosolic) and pMAQ6 (stromal) aequorin lines, and any other aequorin-expressing materials sent by the ESRs/ERs; **2.** These will be fluorescent protein constructs from Ute, pMAQ2 and pMAQ6 aequorin constructs from Gioia, and any other fluorescent protein/aequorin-expressing binary vectors sent by the ESRs/ERs; **3.** These will be fluorescent protein expressing Arabidopsis from Ute, and any other fluorescent protein-expressing transgenic plants sent by the ESRs/ERs
Tuesday 17th March 2015	Harvesting Agrobacterium cells, resuspension, infiltration of tobacco; Calcium measurements: luminometer and camera of Arabidopsis seedlings expressing aequorin (**4,5**); Imaging fluorescent proteins in transgenic Arabidopsis seedlings (**4,5**).	Calcium measurements: luminometer and camera of Arabidopsis seedlings expressing aequorin (**4,5**); Imaging fluorescent proteins in transgenic Arabidopsis seedlings (4,5); Reconstituting more seedlings and ESR/ERs own material; preparation of thin slices of Plantago major leaf stems, fixation, blocking and incubation with the primary antibody overnight.	**4**. ESR/ERs can image their own material, transgenic Arabidopsis seedlings will be provided for those without their own material. **5**. Roughly half the ER/ESRs will do either fluorescence or luminescence in the morning and swap in the afternoon.

Wednesday 18th March 2015	Calcium measurements: luminometer and camera; infiltrating coelenterazine into tobacco leaves; washing, incubation with 2nd antibody (Cy2 conjugated) for 1 hour. Analysis by fluorescence microscopy. (**6**).	Afternoon off/sight seeing etc.	**6**. ESR/ERs can chose which type of imaging, and which material they wish to practice with, allowing them a chance to experiment.
Thursday 1 9th March 2015	Imaging of fluorescent proteins in tobacco leaves on confocal / imaging of aequorin in tobacco leaves on photon camera (**5**)	Imaging of fluorescent proteins in tobacco leaves on confocal / imaging of aequorin in tobacco leaves on photon camera (**5**)	**5**. Roughly half the ER/ESRs will do either fluorescence or luminescence in the morning and swap in the afternoon.
Friday 20th March 2015	Lecture on other imaging approaches/techniques(Tim); Feedback, questions and discussion; Exam :-(	**DEPARTURE**	
Saturday 21st March 2015			