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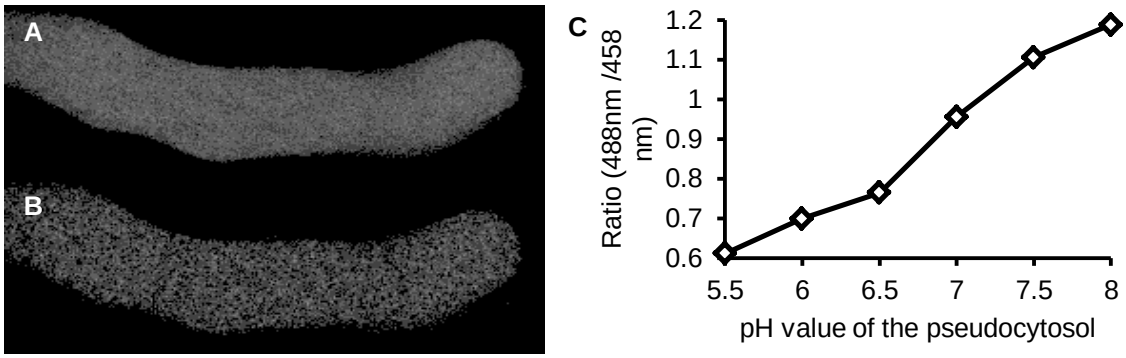
Supplemental Figure 1. Calibration of the cytosolic pH of *Papaver* pollen tube with the pH indicator BCECF AM



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A healthy, growing pollen tube was labelled with the pH indicator BCECF-AM prior to ratiometric imaging using confocal microscopy. A ratio value was made of the two images using Image J; this ratio was used to determine the pH of the pollen tube cytosol using a calibration curve (**C**), collected during the same experiment. This pollen tube has a cytosolic pH of 6.8.

(**A**) BCECF-AM signal when excited at 488 nm, emission at 510-550 nm. (**B**) BCECF-AM signal when excited at 458 nm, emission at 510-550 nm. (**C**) Pseudocytosol solutions of between pH 5-8, at approximately 0.5 intervals, were labelled with BCECF free acid and sequential confocal images were collected (excitation 488nm, then 458nm, emission 510-550 nm). A ratio of the two images was calculated and plotted as ratio: pH values.