Using fusion proteins to study cellular and subcellular processes

Par3-GFP within the tissue, approx 6hr timelapse

Par3-GFP within individual cells, approx 10 mins timelapse
Live Imaging of Zebrafish Neurulation - Methods

- Sparse distribution of mRNA coding for fusion protein(s)
  e.g. Pard3-GFP, ZO1-GFP, Rab11-GFP

- Live Confocal Imaging

![Diagram of 32 cell stage with imaging views](image)
Does your fusion protein match endogenous protein distribution?

Par3-GFP

ZO1-GFP

ZO1 antibody

Par3-GFP

ZO1-GFP

ZO1 antibody
Segregation of molecules at cell division reveals native protein localization

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Check your fusion protein doesn’t affect other endogenous proteins!
The demo
ANDY
WT embryos at approx 16 to 32 cell stage injected with a cocktail of 3 RNAs:

RAB11A-EGFP
H2B –RFP
Cherry-CAAX

- screen through embryos to find nice mosaic distribution in tissue of interest
- dechorionate and mount in low melting point agarose in embryo chambers
- image on confocal microscope (probably Zeiss Spinning Disk)

- should see many cells dividing in neural rod and intracellular traffic of RAB11-EGFP
Observing RAB11A-GFP endosomes through neural rod divisions and at the apical pole of neuroepithelial cells.

Rab11-GFP endosomes shown in other systems to be required for trafficking Crumbs - a transmembrane regulator of apical epithelial junctions, also required for new membrane delivery at abscission plane.