

Imaging:

- Sample groups: Positive control * 3; negative control * 3; ATG GFP * 3; ACG GFP * 3
- Parameters:
 - Magnification: 5X objective
 - Exposure: 20.98 (green fluorescence channel); 1733 (red fluorescence channel)
- 2 different fields with relatively even distribution of cells were chosen for each slide
- 1 photo was taken under each channel for every field chosen
Total: 4 groups * 3 samples each * 2 fields per sample * 2 channels per field = 48 photos

ImageJ Analysis:

- “Analyze” → “Set Measurement” → select “Area”, “Integrated density”, “Mean gray value” → “OK”
- For each image, “Analyze” → “Measure” (Results should include “Area”, “Mean”, “IntDen”, and “RawIntDen”)
- Save “Measure” results (as .xls, etc.)

Excel Analysis:

- For each entry, calculate the green/red ratio:

$$\frac{Mean_{green}}{Mean_{red}}$$

- For each of the 4 groups of samples: Average the 6 entries of green/red ratio
- Eliminate background: subtract Negative control from each entry

$$Average_{WT/Mutant/Positive} - Average_{Negative}$$

- Normalization:

$$\frac{Average_{WT/mutant(negative\ subtracted)}}{Average_{Positive(negative\ subtracted)}}$$

(Normal of positive control = 1)