Measurements

miMeasure

- Tecan - Plate reader
- Olympus - plate scanning microscope
- Confocal Microscope

TECAN

- GFP was detected „well“
- BFP is hardly higher than background
  (not as bright as tested before)

Transfection efficiency?
Cell seeding?
Same construct as tested before?

TECAN

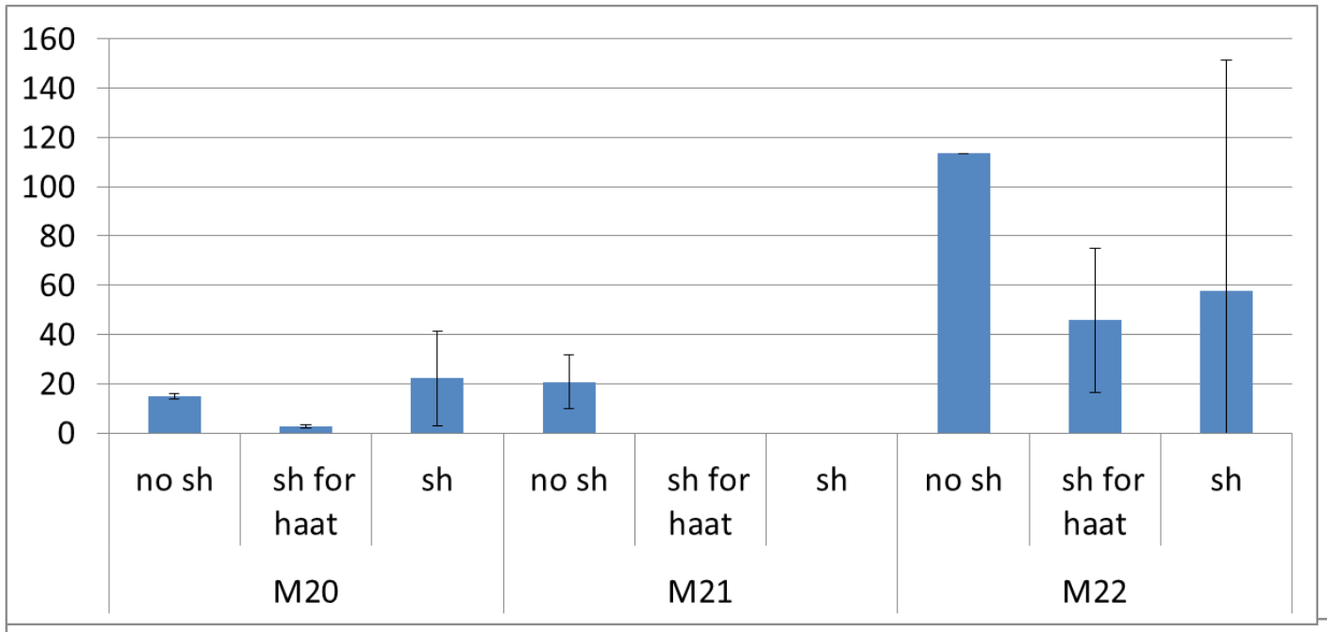
- 11 samples over 3 plates in 8 replicates
- On each plate also: control (non-transfected cells) in 8 replicates

- Selecting wells with higher expression by eye (on the plate)
- Selecting wells having BFP higher than the averaged control (in excel)

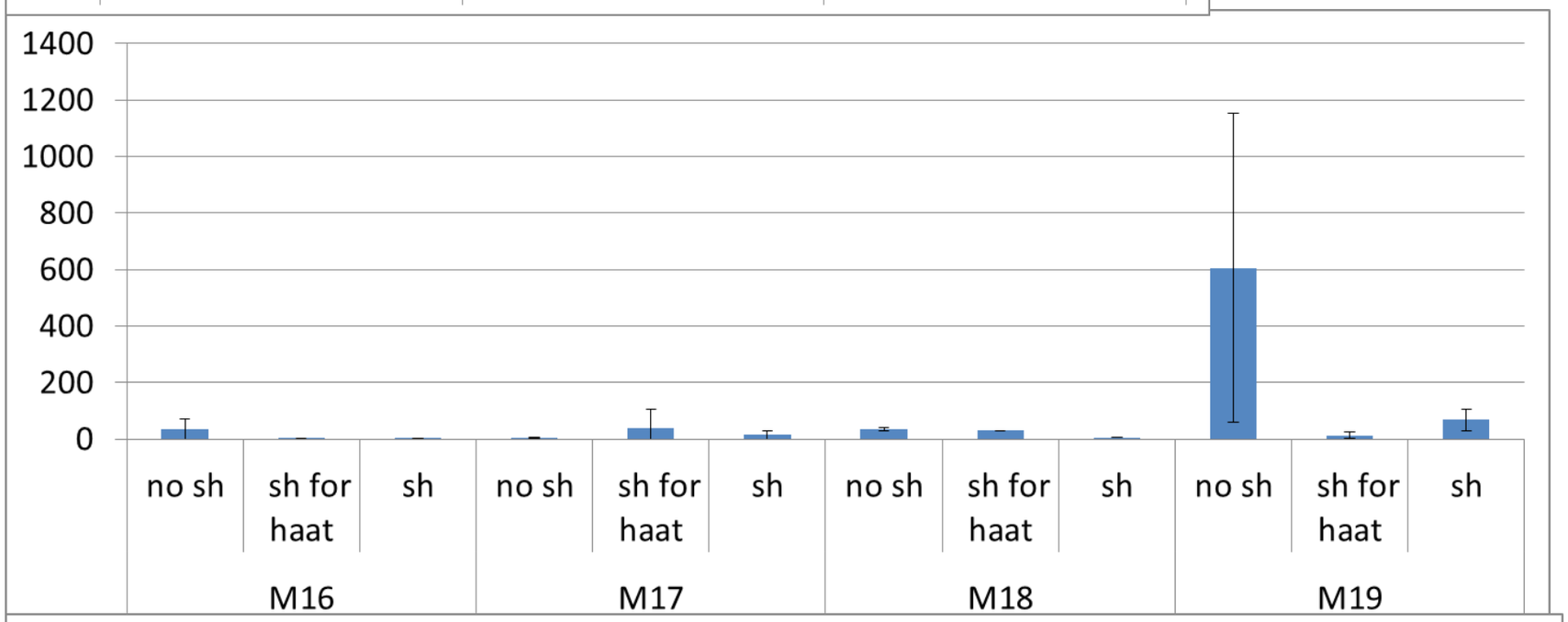
Measurement for each well of a sample was done as follows:

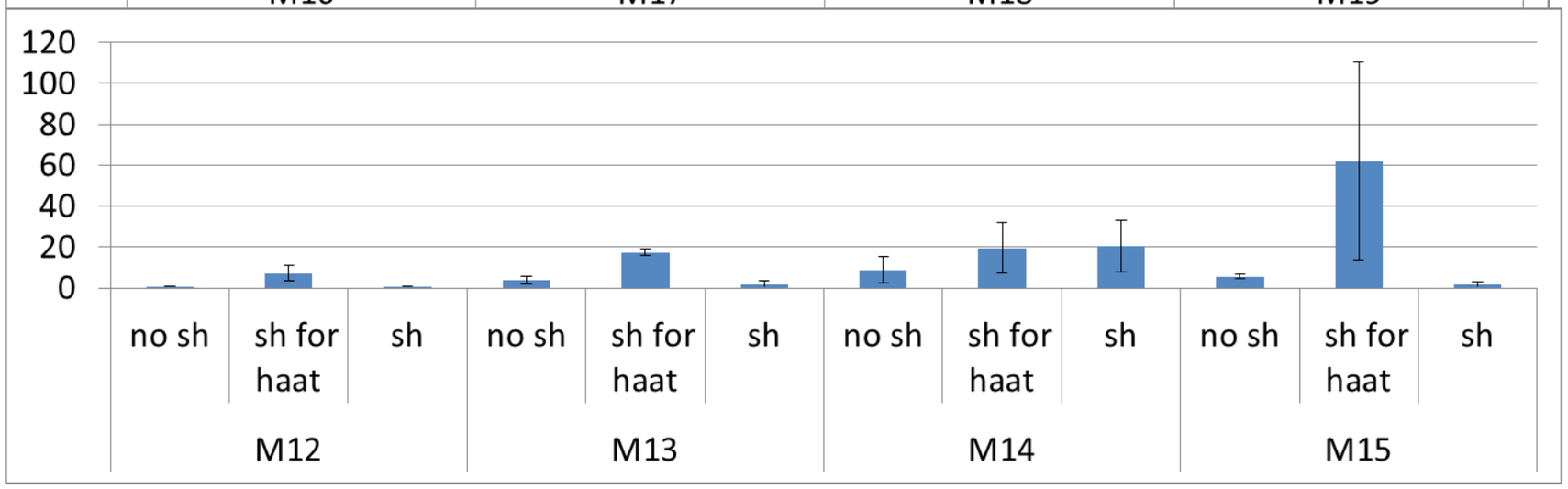
$$\frac{\frac{I(sample_{GFP})}{I(sample_{Draq5})} - \left\langle \frac{I(control_{GFP})}{I(control_{Draq5})} \right\rangle}{\frac{I(sample_{BFP})}{I(sample_{Draq5})} - \left\langle \frac{I(control_{BFP})}{I(control_{Draq5})} \right\rangle}$$

→ Gives no reasonable values, because BFP-Intensities are hardly higher than control. This can not normalize the GFP-Intensity per well as intended and seem to falsify values of one replicate (well).



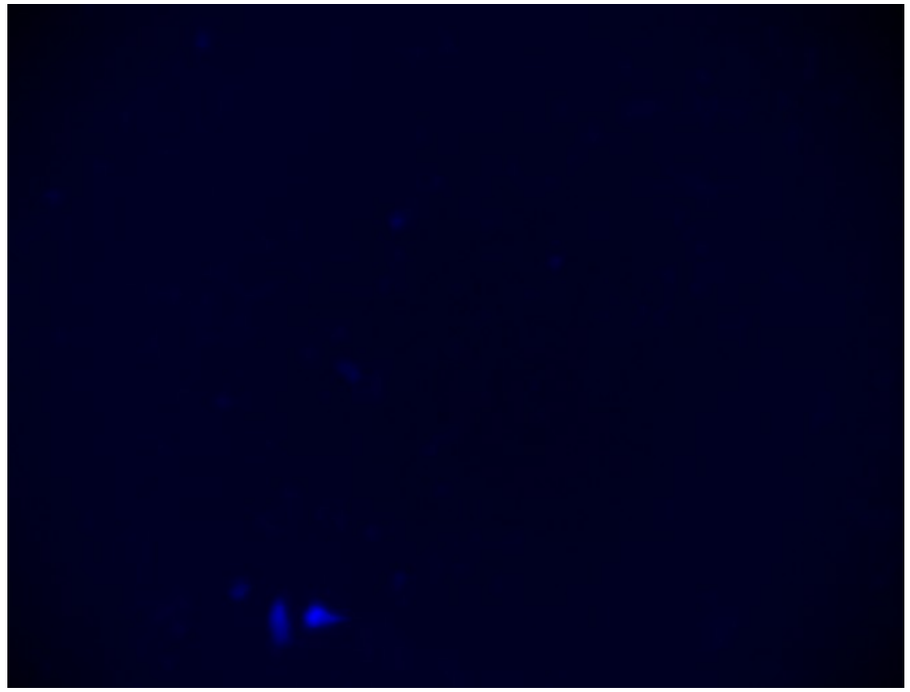
- Control also showing high intensities for BFP

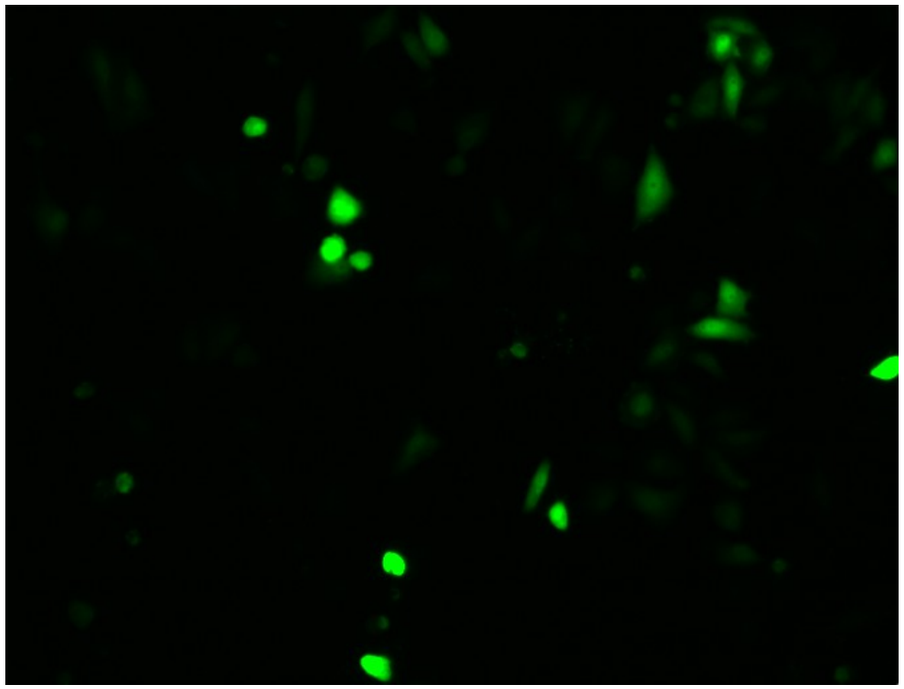




Measurement: Olympus ‘plate scanning microscope’

+ mean intensity analysis

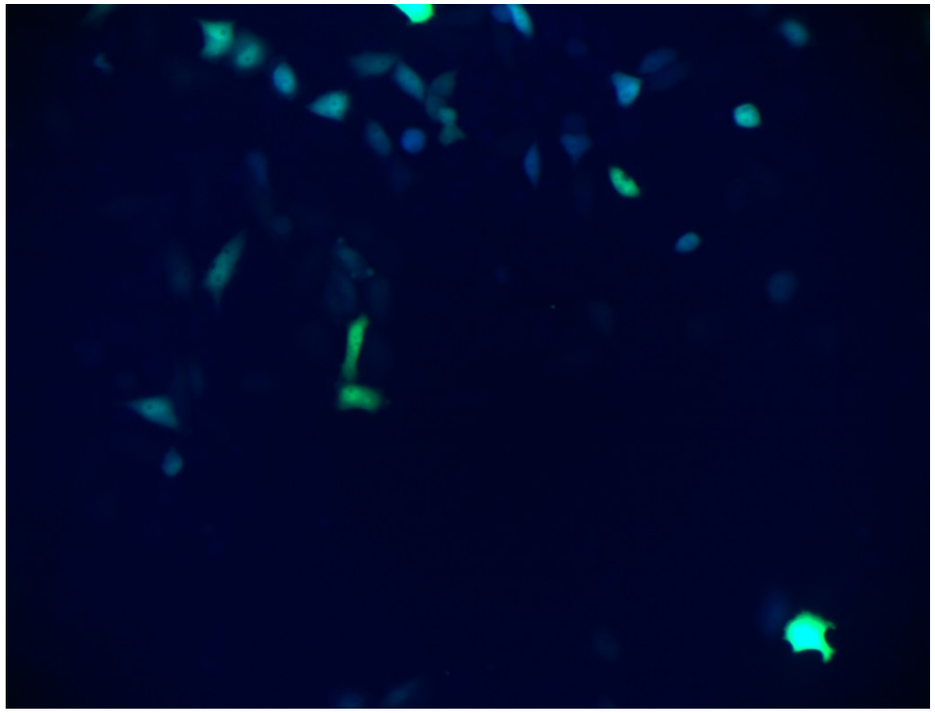




- Signal/noise problems for BFP channel
- Better sensitivity than TECAN if single cell analysis
- relatively fast

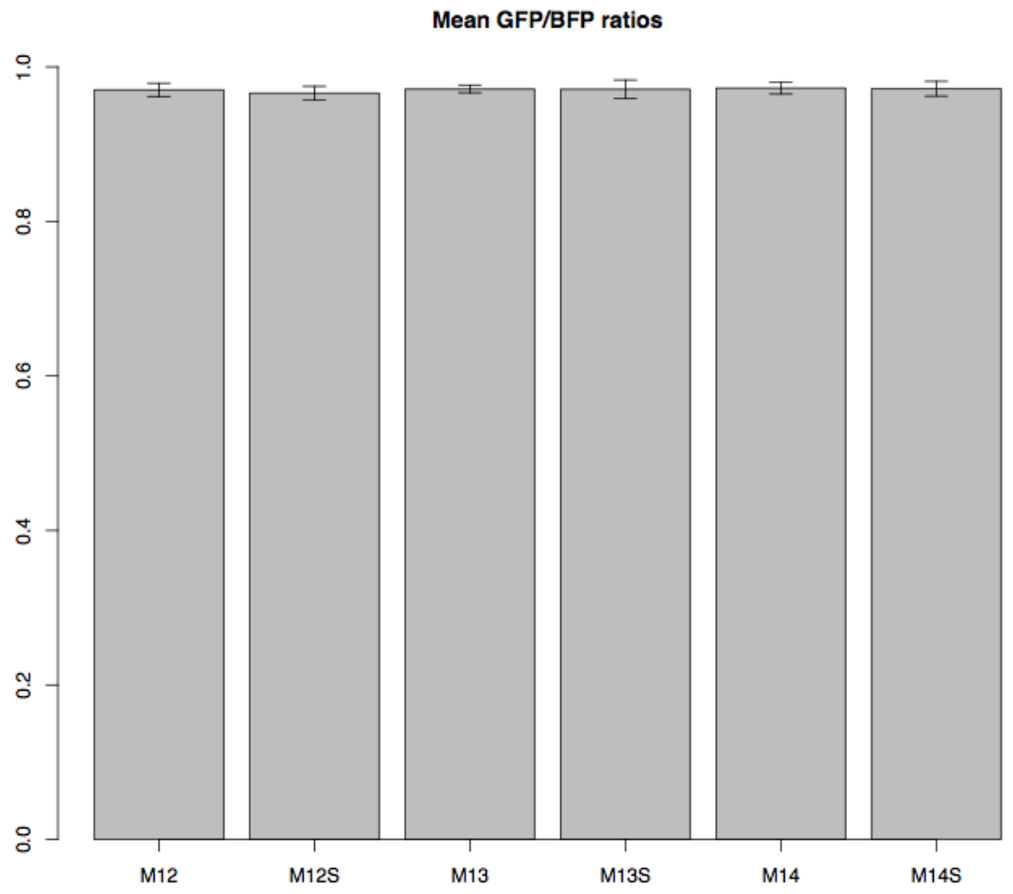
Measurement: Olympus ‘plate scanning microscope’

+ ‘single cell’ analysis

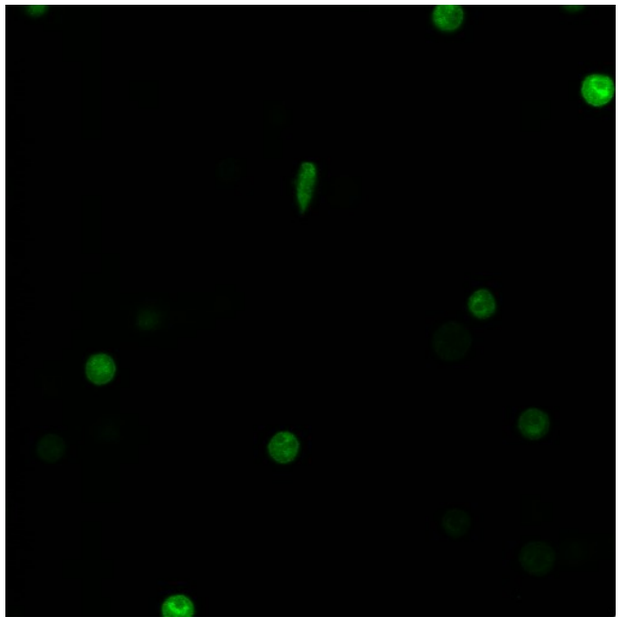


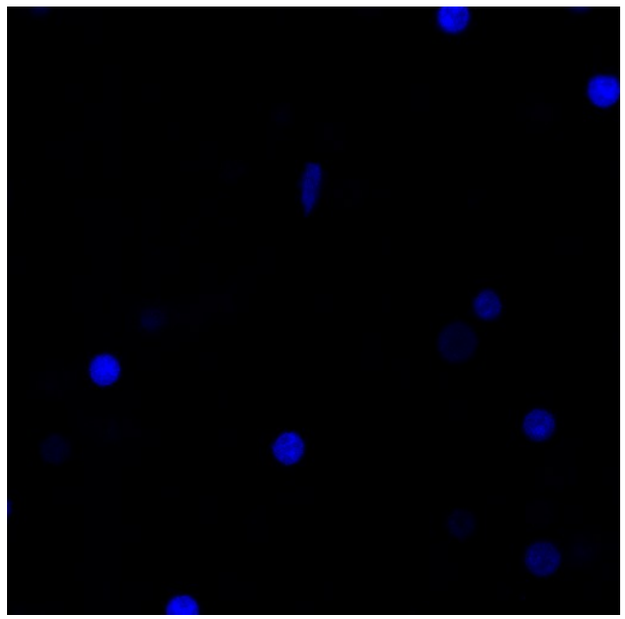
- Signal/noise problems for BFP channel
- Better sensitivity than TCAN if single cell analysis
- relatively fast

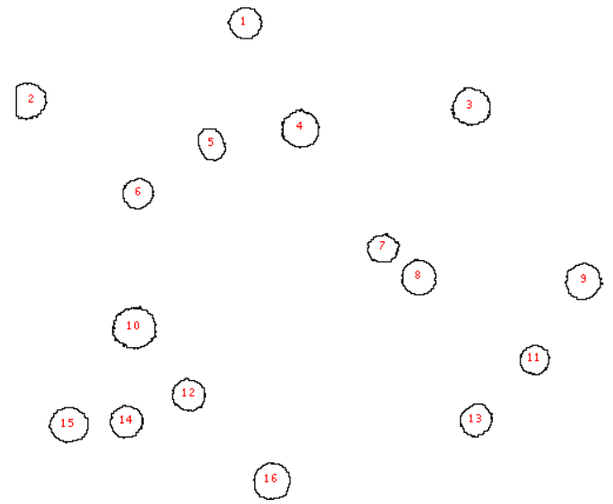
But...

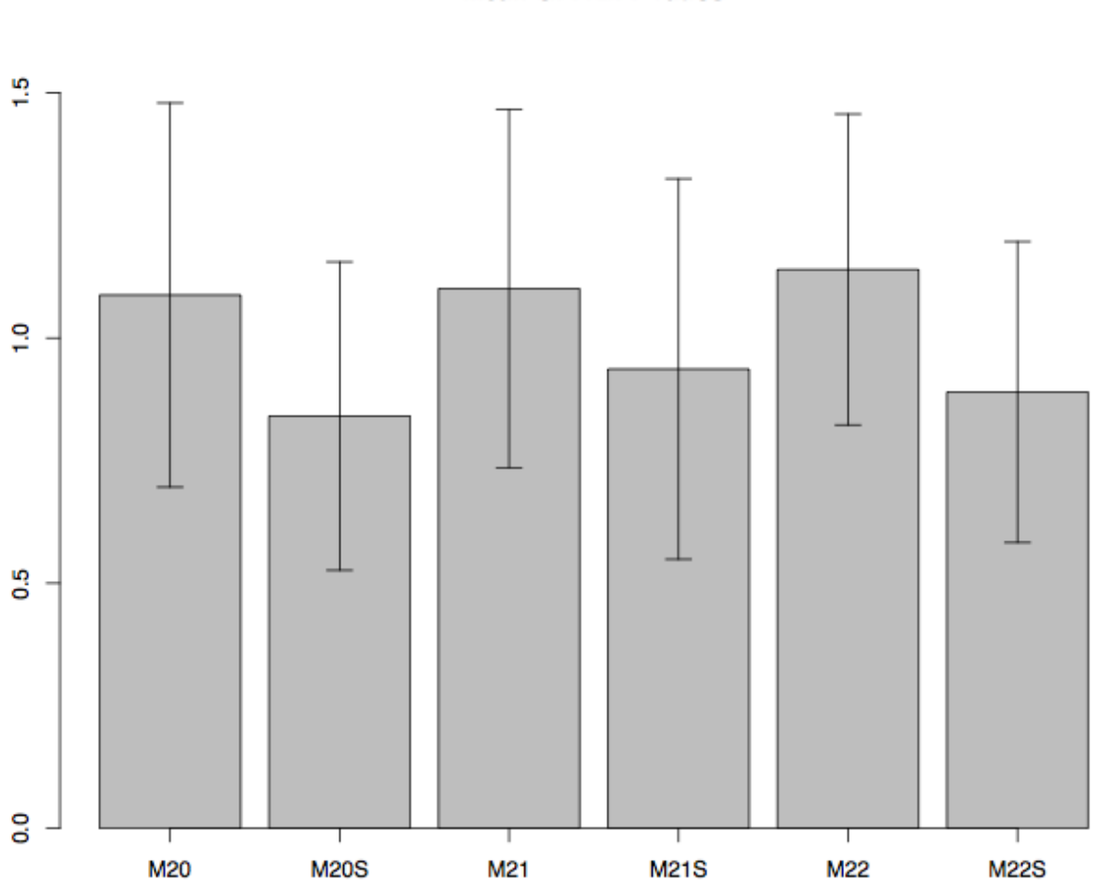


Measurement: Confocal microscope





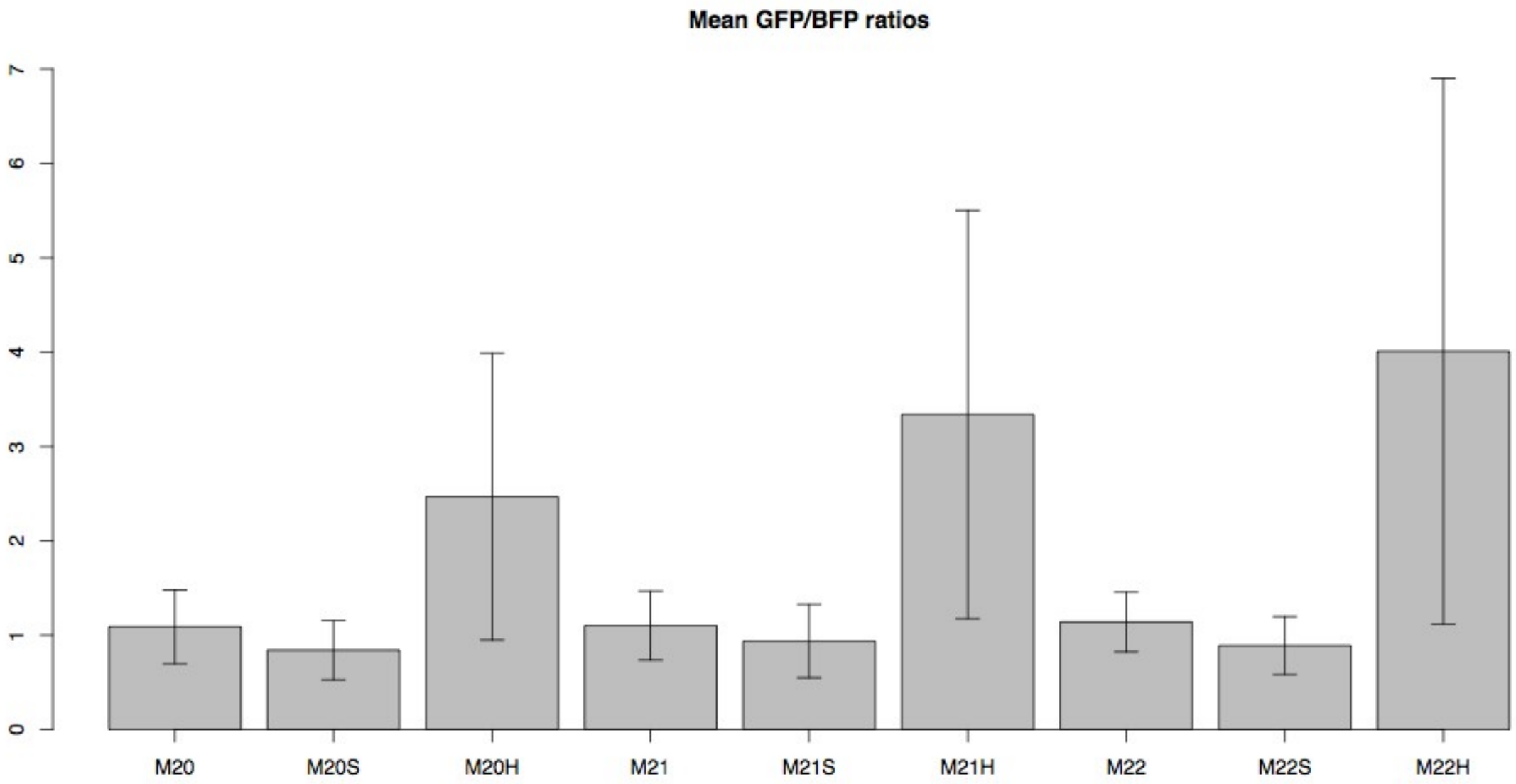




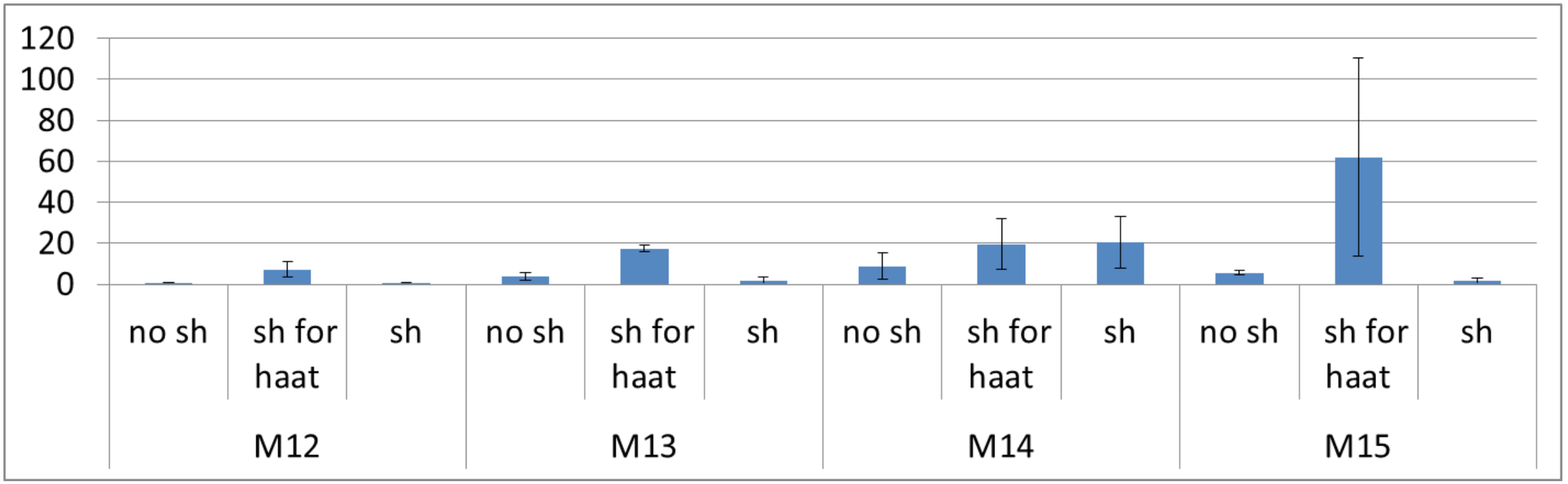
- highest sensitivity
- no background problems
- single cell analysis
- VERY time consuming

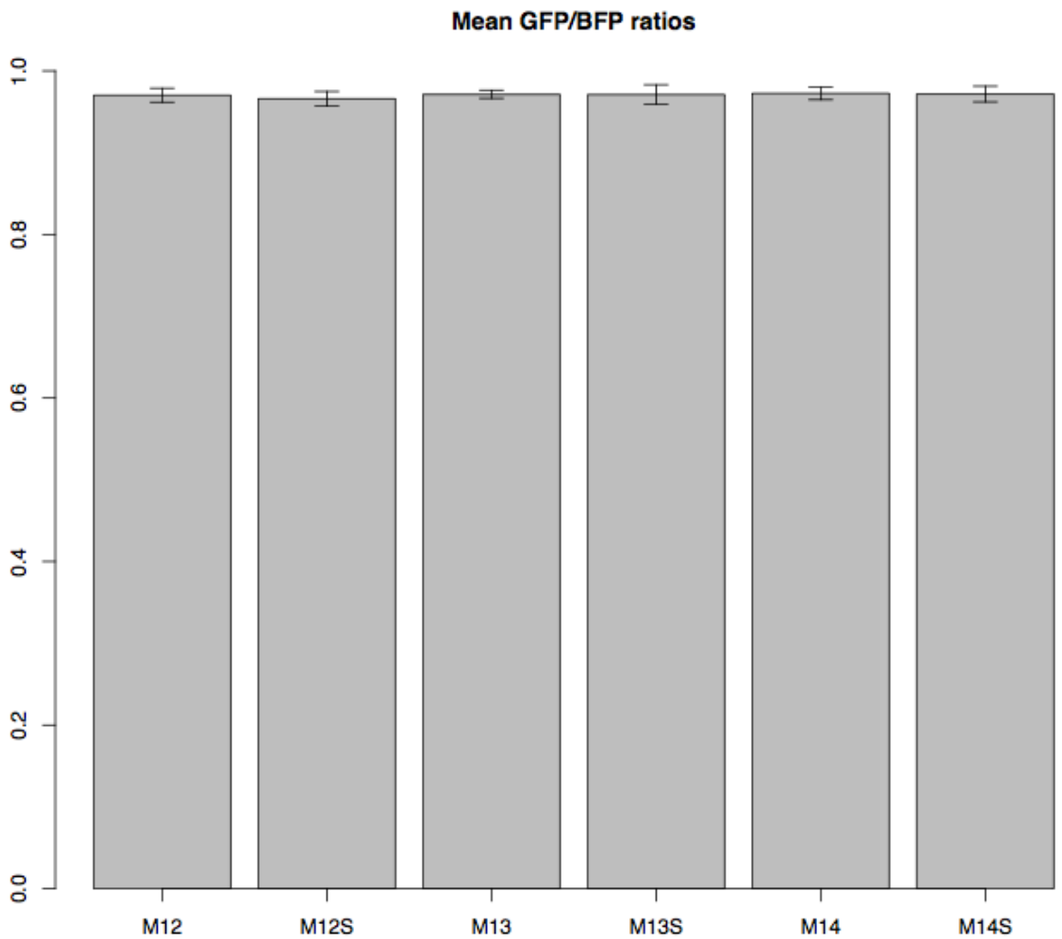
<- Down regulation tendency!

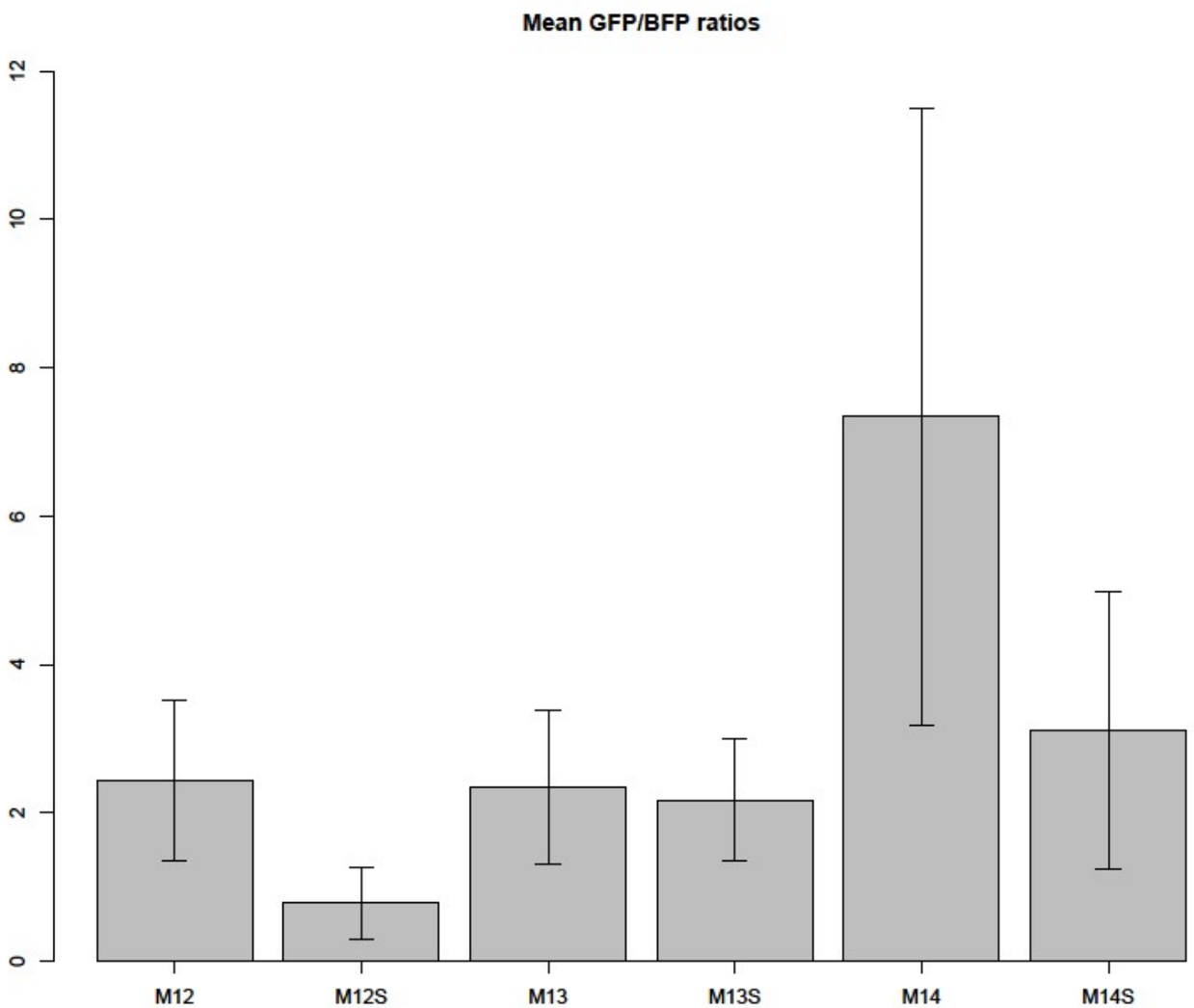
Measurement: Confocal microscope



What’s with these ‘H’ cotransfections?


120
100
80
60
40
20
0
no sh
sh for haat
sh
M12
M13
M14
M15


Mean GFP/BFP ratios
1.0
0.8
0.6
0.4
0.2
0.0
M12
M12S
M13
M13S
M14
M14S


Mean GFP/BFP ratios
12
10
8
6
4
2
0
M12
M12S
M13
M13S
M14
M14S