

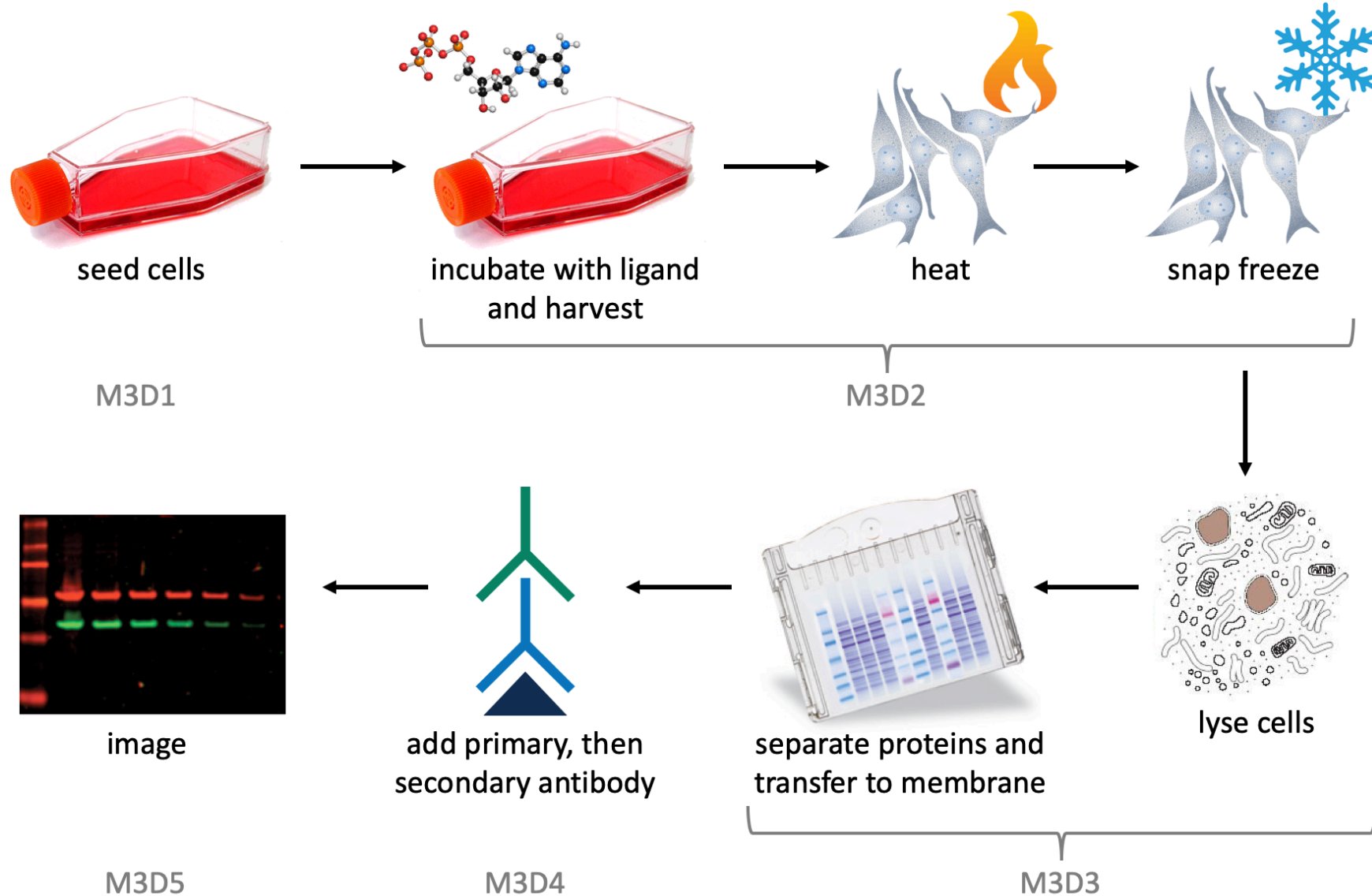
M3D5: Data analysis and assignment preparation

1. Last quiz!
2. Prelab discussion
3. Analyze CETSA results
4. Prepare Mini-report

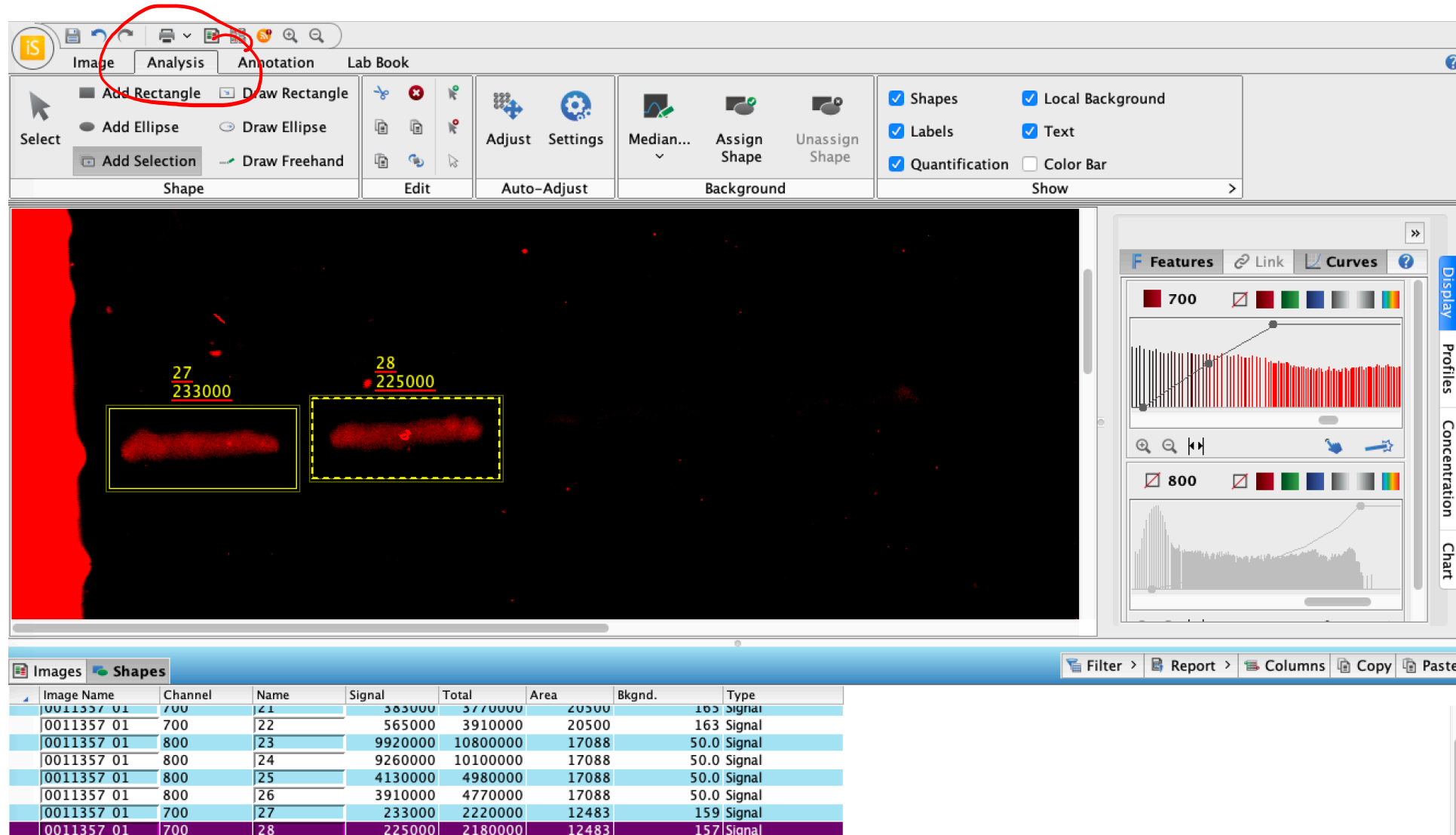
Thursday 04
10-5pm
56-302



Overview of Mod 3:



How will we analyze the data?



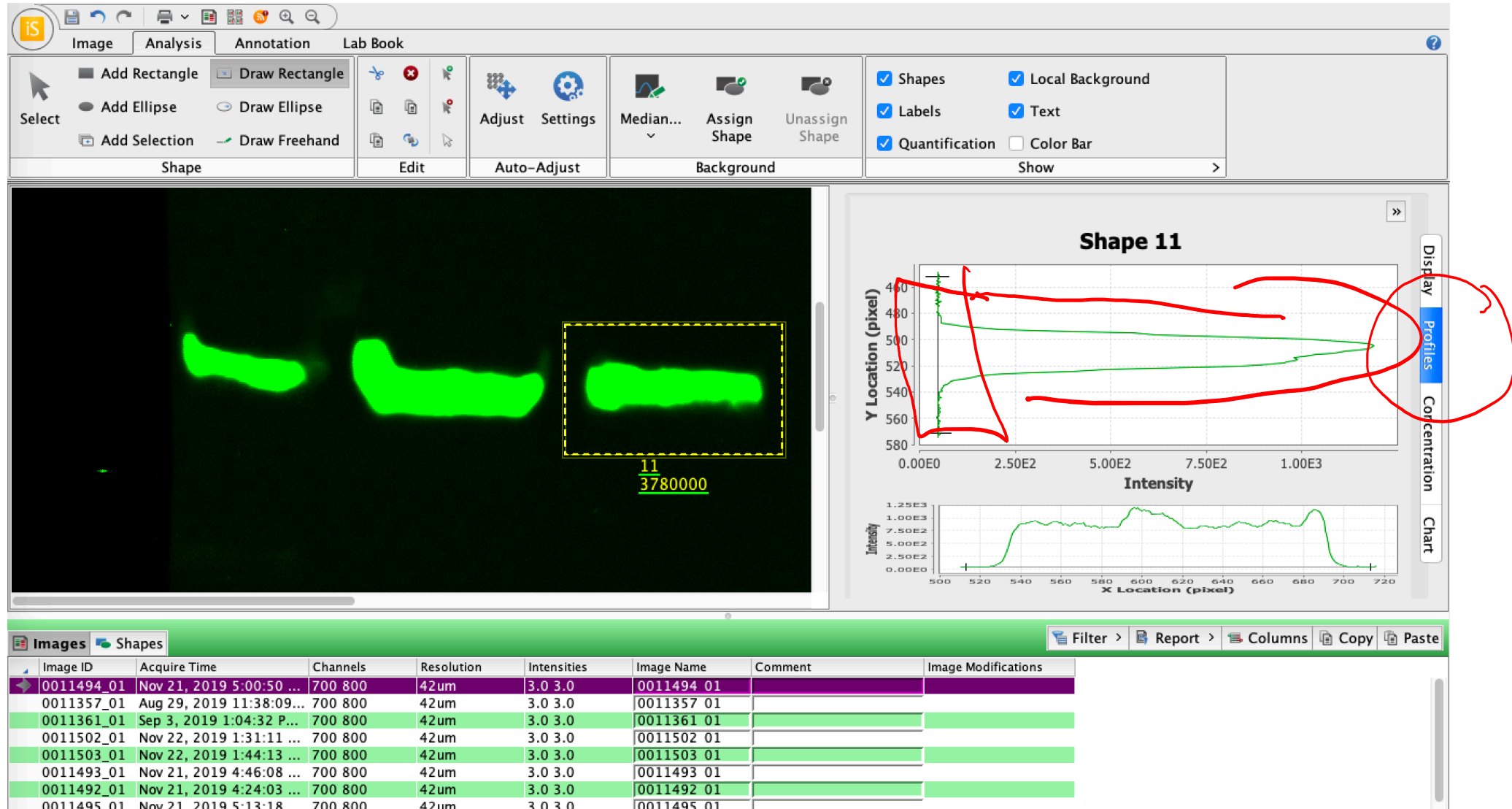
Analyzing CETSA WB with Image Studio

Image Name	Channel	Name	Signal	Total	Area	Bkgnd.	Type
0011361_01	700	29	719000	2820000	11394	184	Signal
0011361_01	700	30	939000	2920000	11394	174	Signal
0011361_01	700	31	348000	2180000	11394	161	Signal
0011361_01	700	33	868000	2700000	11394	161	Signal

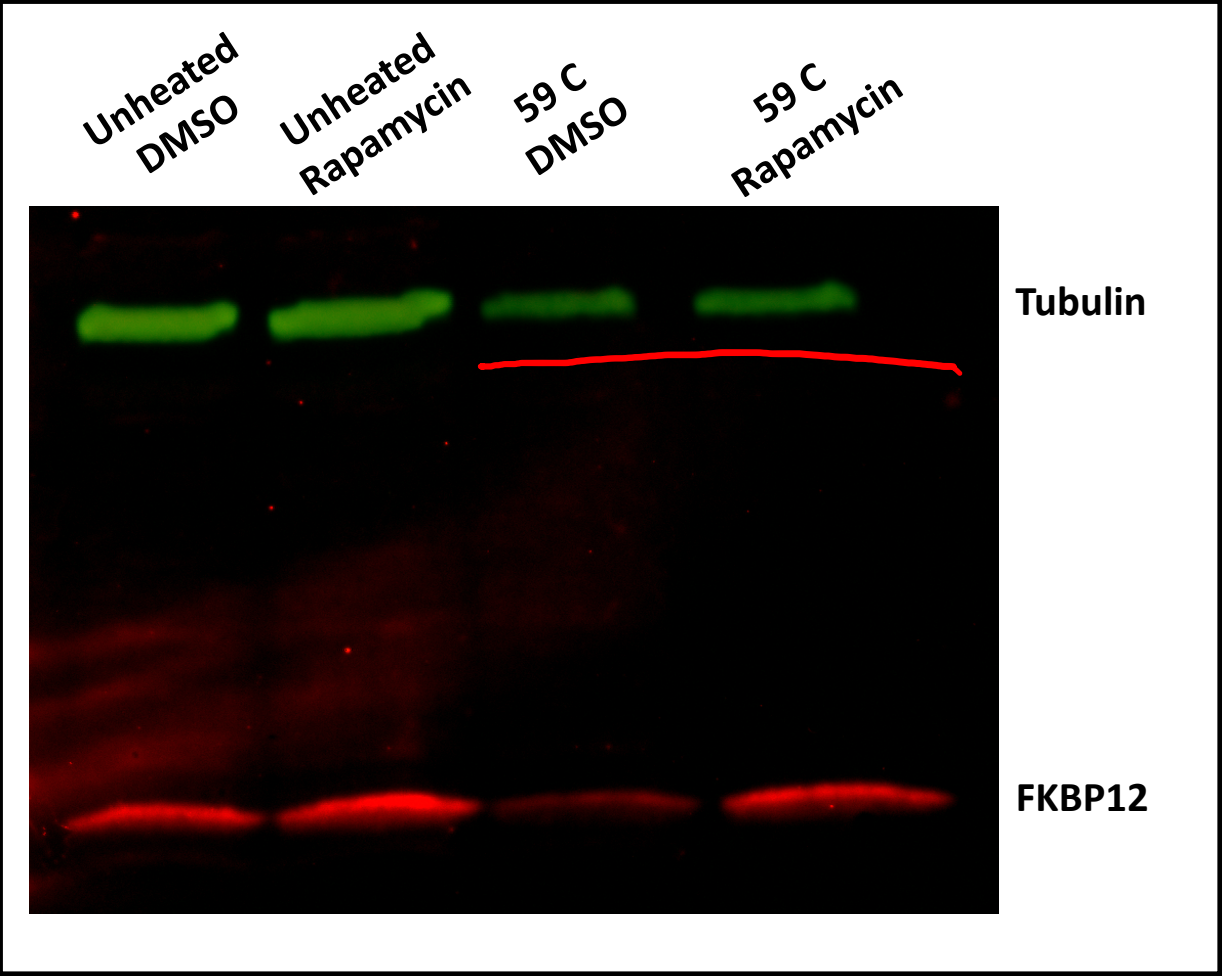
- Image Name: Name of entire image in Image Studio
- Channel: Wavelength of signal detection (700 or 800)
- Name: Number assigned to rectangle drawn around a band. Each rectangle for each channel will have a unique number
- Total: Sum of individual pixel intensities in the rectangle
- Area: Total number of pixels enclosed by the rectangle
- Bkgnd: Value assigned for background subtraction (default= mean pixel intensity of background)
- Type: What being measured (i.e. signal or background). More relevant for manually determining background.
- Signal: Sum of the pixel intensity values in the rectangle minus the product of the background and area

$$\text{Signal} = \text{Total} - (\text{Background} \times \text{Area})$$

Assessing signal quality of the protein bands



Example analysis from pilot data



Data:

	FKBP12 Signal Ratio to Unheated DMSO
Unheated DMSO	1
Unheated Rapamycin	1.305980529
59 C DMSO	0.484005563
59 C Rapamycin	1.207232267

Analysis:

- The heated DMSO treated group shows a 50% loss of FKBP12 signal compared to the unheated DMSO group.
- Rapamycin treatment stabilized the FKBP12 protein so that it maintained unheated levels of expression.
- Tubulin decreased with heat, but there was no apparent effect of Rapamycin on Tubulin stabilization.

Mini-Report details

- Introduce your investigation (1-2 paragraphs)
- Represent your data in figures / tables / text
 1. Ligand structure (figure)
 2. Western blot (figure)
 3. Analysis of Western blots bands (figure or text)
 4. Comparison of ligand fold-change values (table)
- Evaluate the data
 - Does it match the DSF?
 - What technical changes would you make to improve on the preliminary experiment?
 - What are the next steps for this project?

Important Mod 3 dates

- Research proposal presentation due **Friday, Dec 6 by 1 pm**
 - Completed in teams!
 - 12 minute presentation, submitted to Stellar
- Lab Notebook due **Thursday, Dec 5 by 10 pm M3D4**
- Blog post due **Friday, Dec 6 by 10 pm**
- Mini-report due **Monday, Dec 9 by 10 pm**
 - Completed in teams!
 - 3 page word document, submitted to Stellar
- Feedback lunch on **Tuesday, Dec 10 at 11 am**