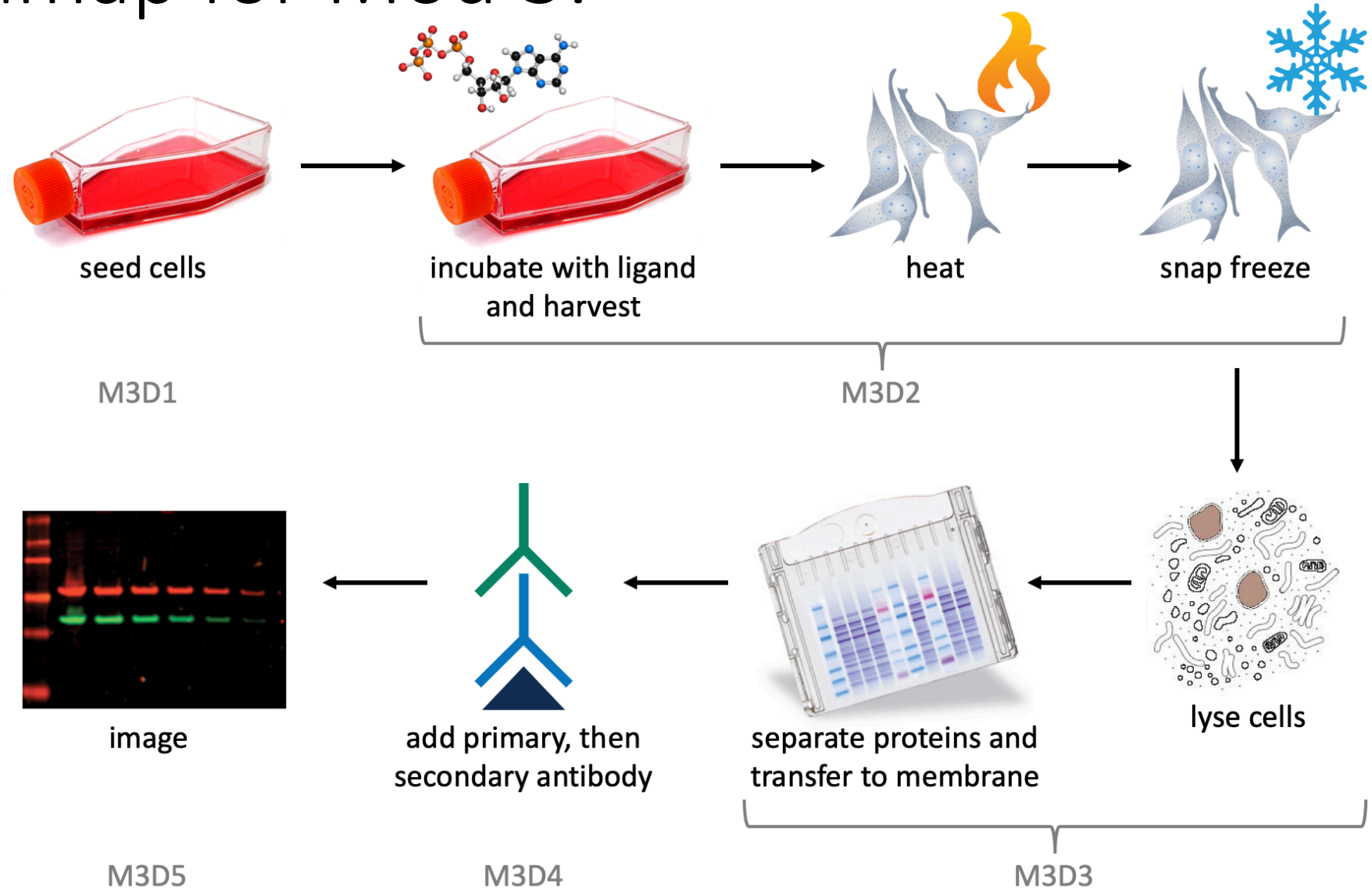
M3D3:
Data analysis and assignment preparation

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

Notebook entry due: M3D4

Roadmap for Mod 3:



How will we analyze the data?

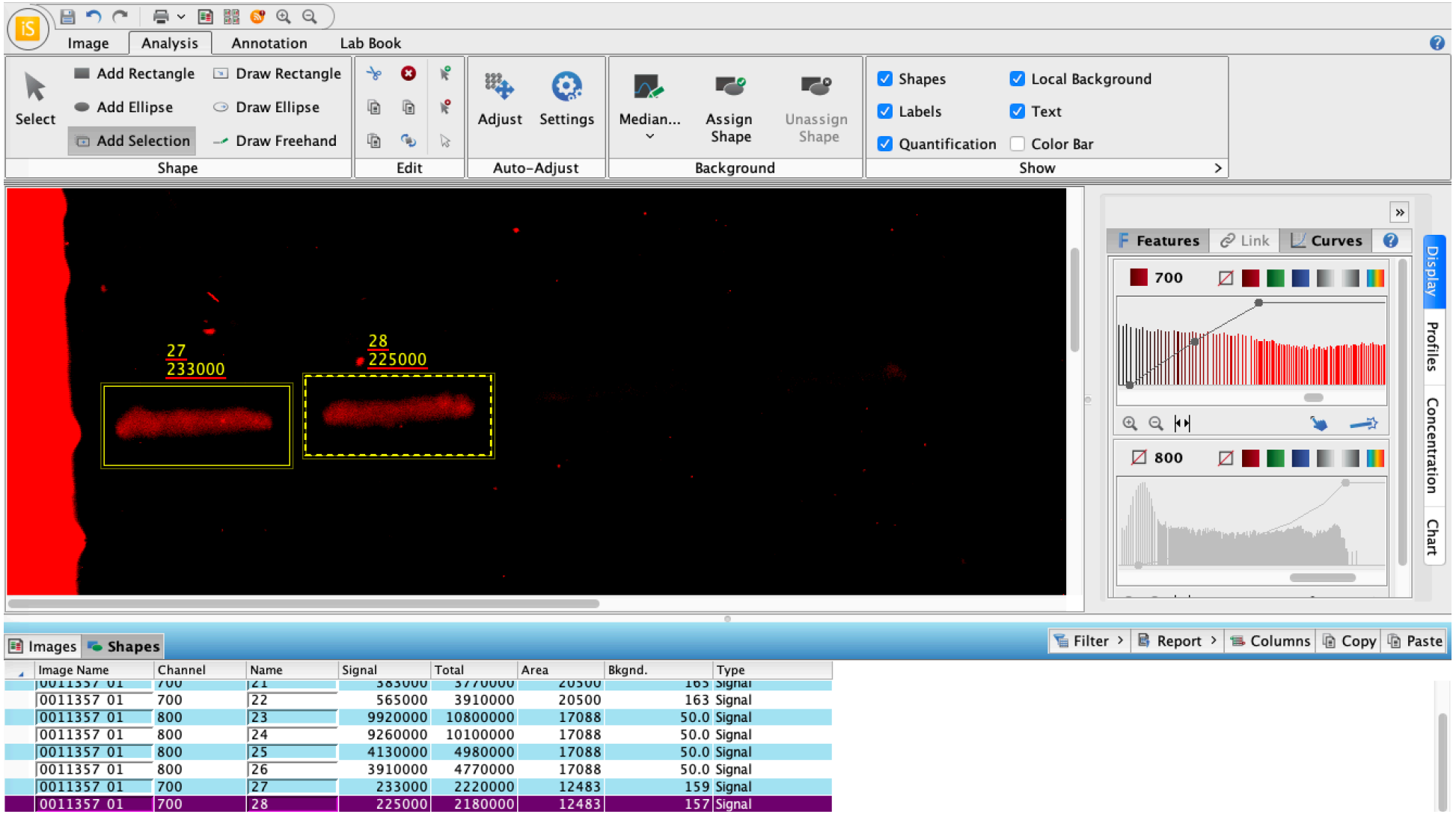


Image Name	Channel	Name	Signal	Total	Area	Bkgnd.	Type
0011357 01	700	21	383000	3770000	20500	163	Signal
0011357 01	700	22	565000	3910000	20500	163	Signal
0011357 01	800	23	9920000	10800000	17088	50.0	Signal
0011357 01	800	24	9260000	10100000	17088	50.0	Signal
0011357 01	800	25	4130000	4980000	17088	50.0	Signal
0011357 01	800	26	3910000	4770000	17088	50.0	Signal
0011357 01	700	27	233000	2220000	12483	159	Signal
0011357 01	700	28	225000	2180000	12483	157	Signal

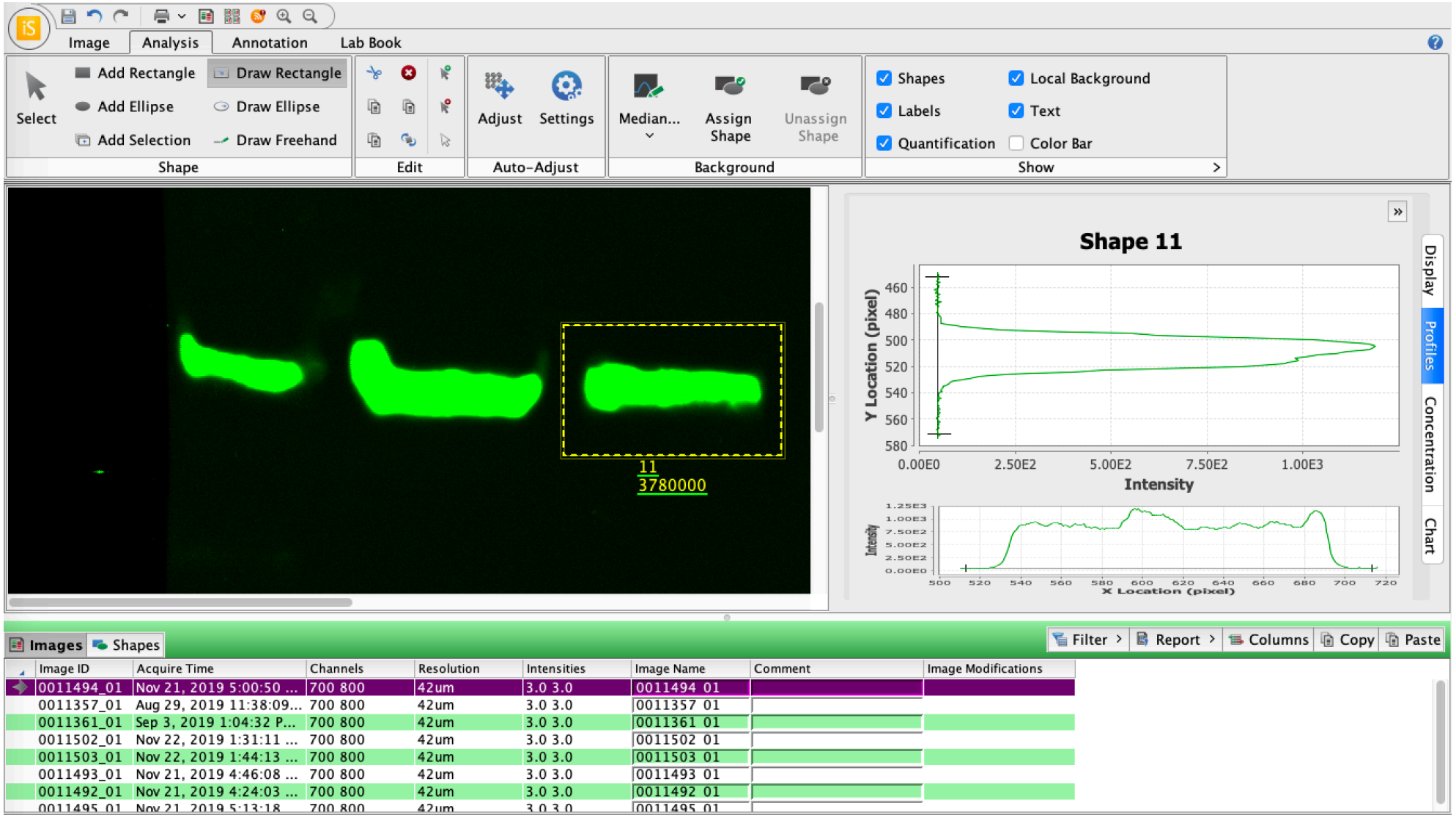
Analyzing CETSA (WB) with Image Studio

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

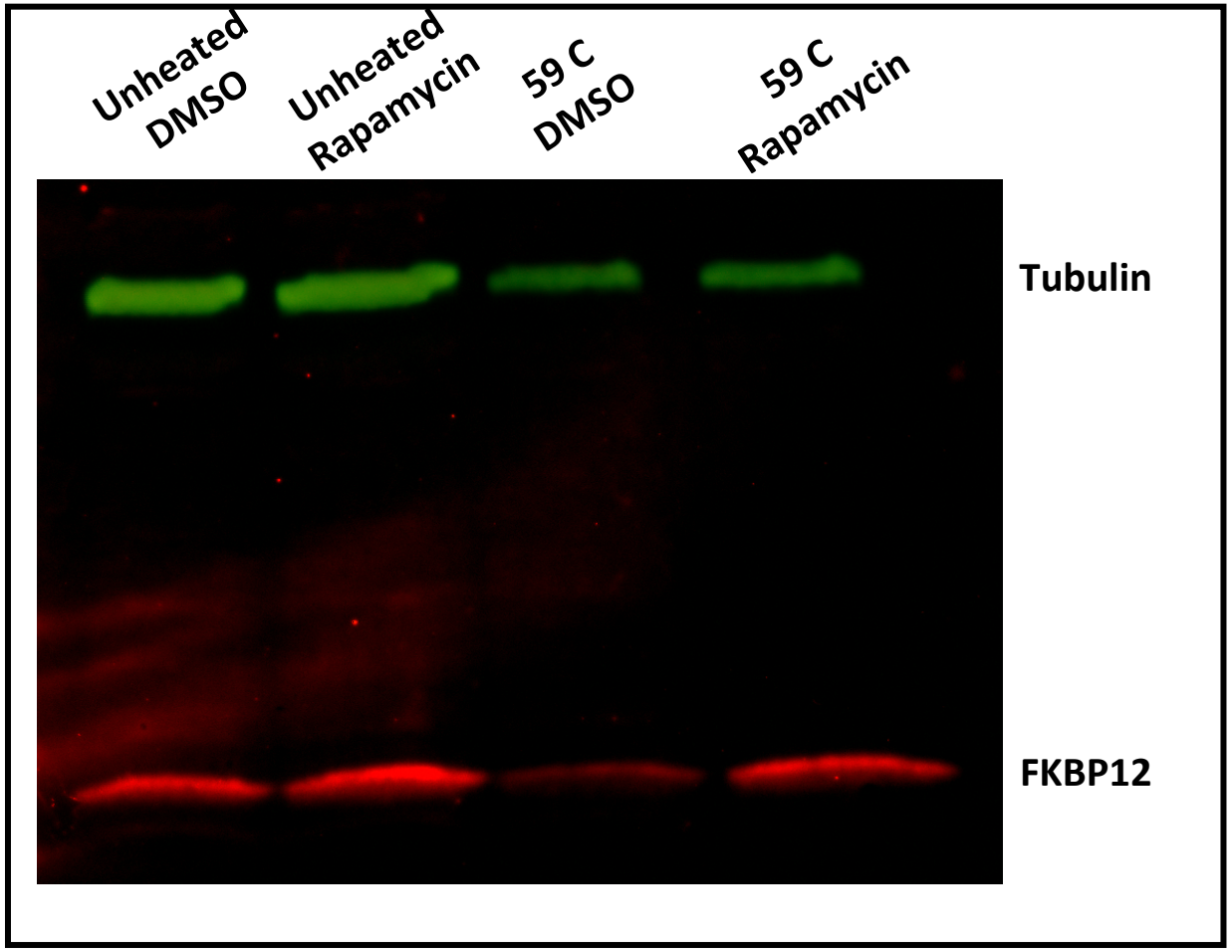
- Image Name: Name of entire image in Image Studio
- Channel: Wavelength of signal detection (700 or 800)
- Name: Number assigned to rectangle drawn around a band. Each rectangle for each channel will have a unique number
- Total: Sum of individual pixel intensities in the rectangle
- Area: Total number 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

Signal = Total – (Background x 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 T_m 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

Friday Dec 6

- Research proposal presentation due ~~Thursday, Dec~~ 5 by 1 pm
  - Completed in teams!
  - 12 minute presentation, submitted to Stellar
- 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!