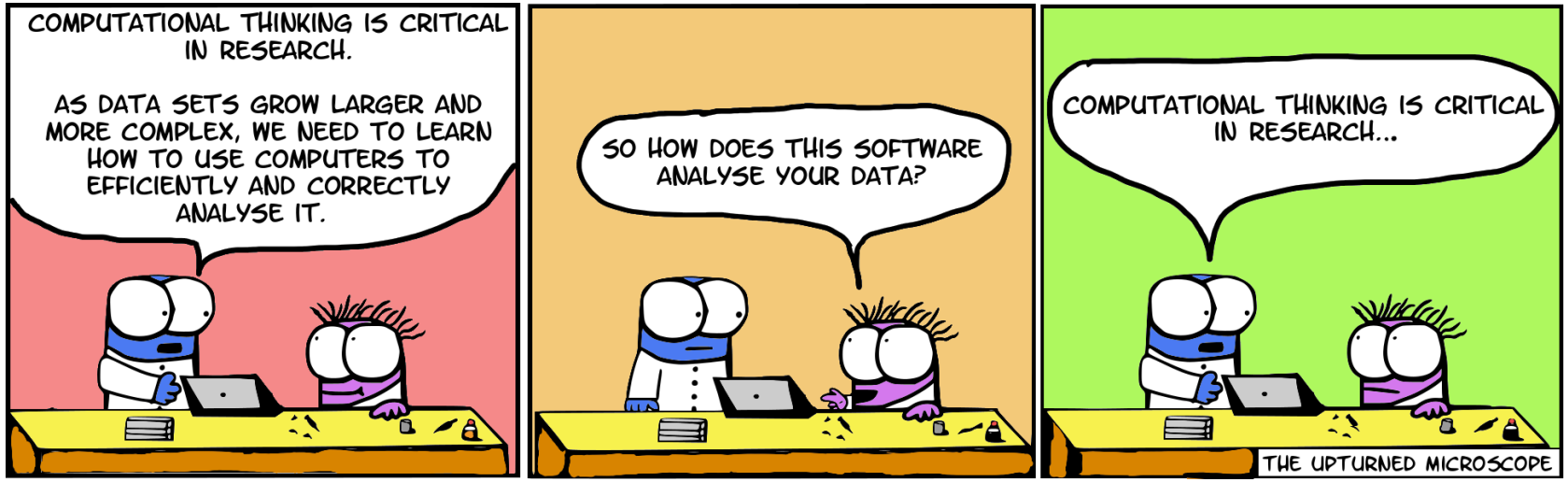
M1D7: Analyze SMM data to identify putative small molecule binders

1. Prelab
2. SMM analysis
3. Examine chemical structure of hits

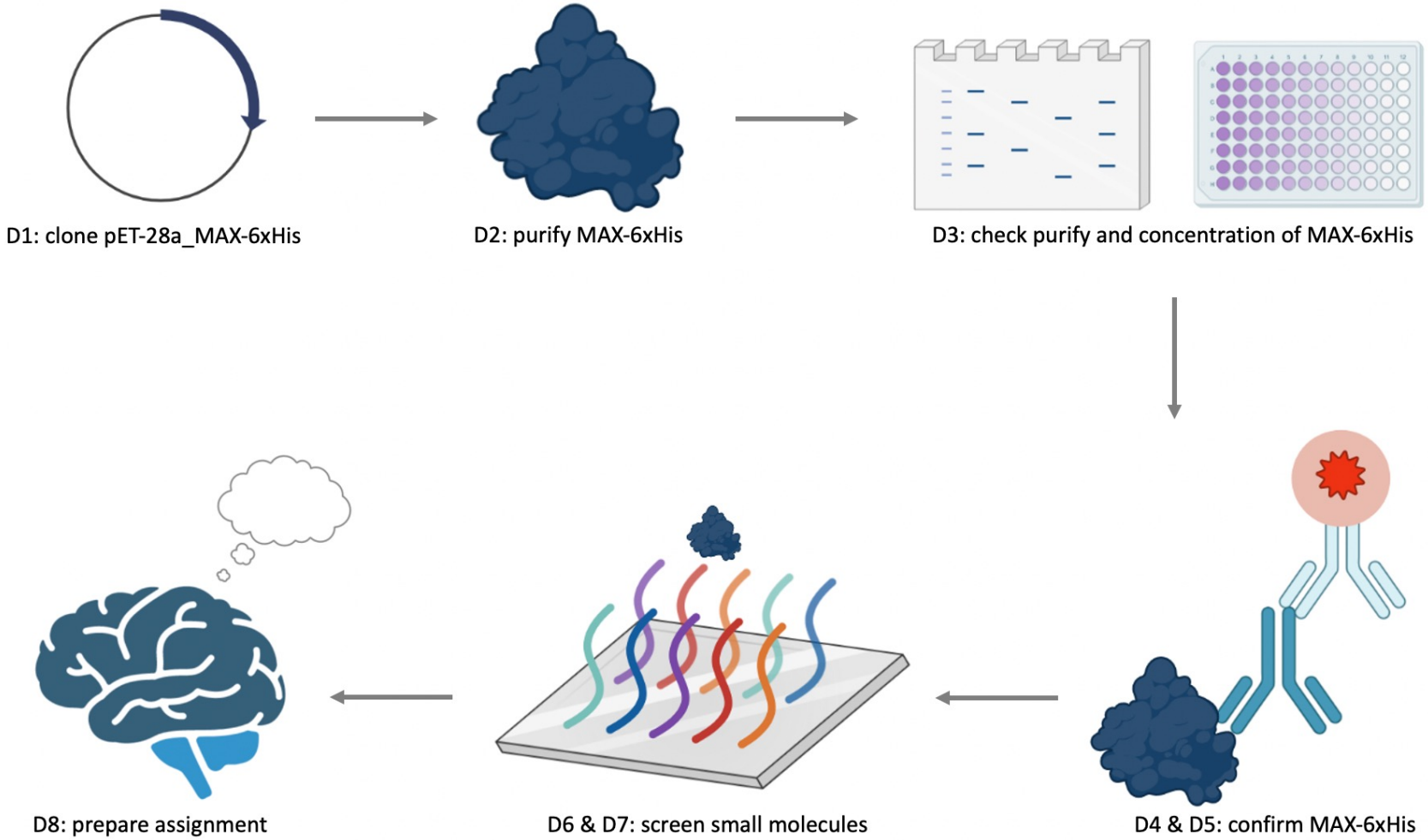


Mod 1 Overview

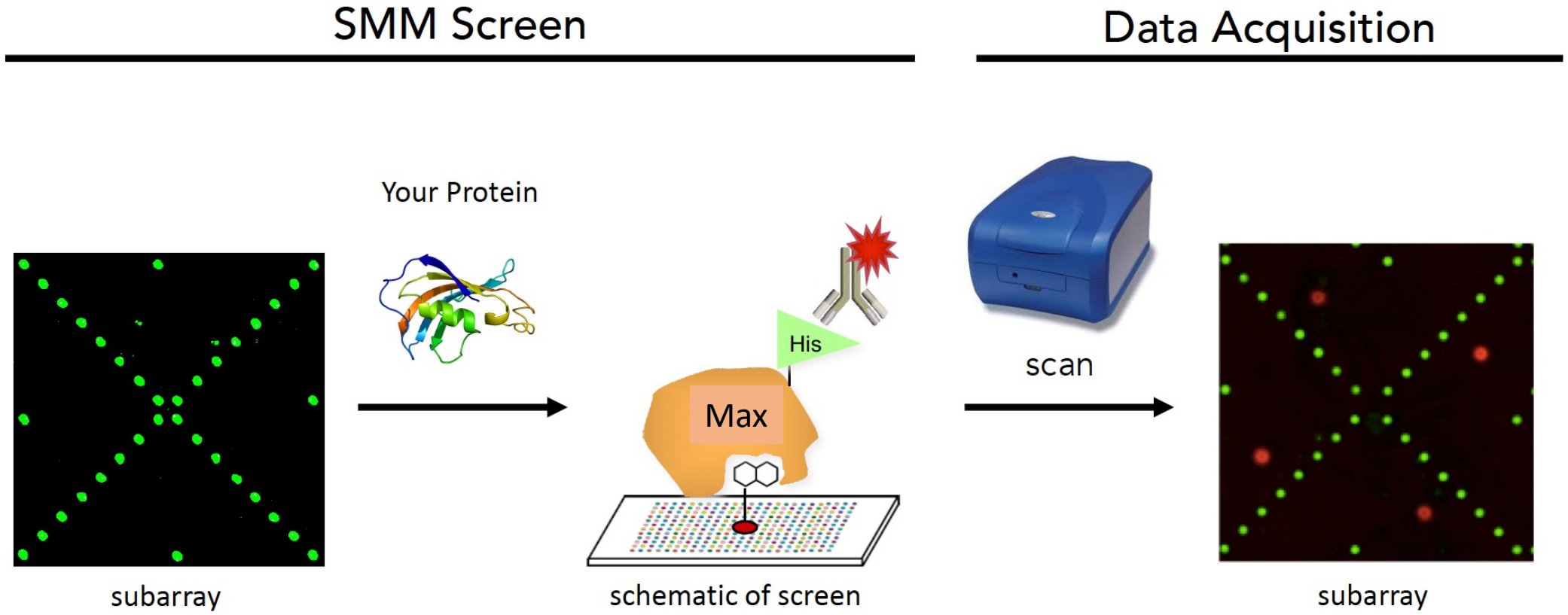
Last lab:
Prepared SMM slides

Instructors scanned slides and prepared computational analysis

This lab:
SMM analysis to identify putative binders



SMM workflow



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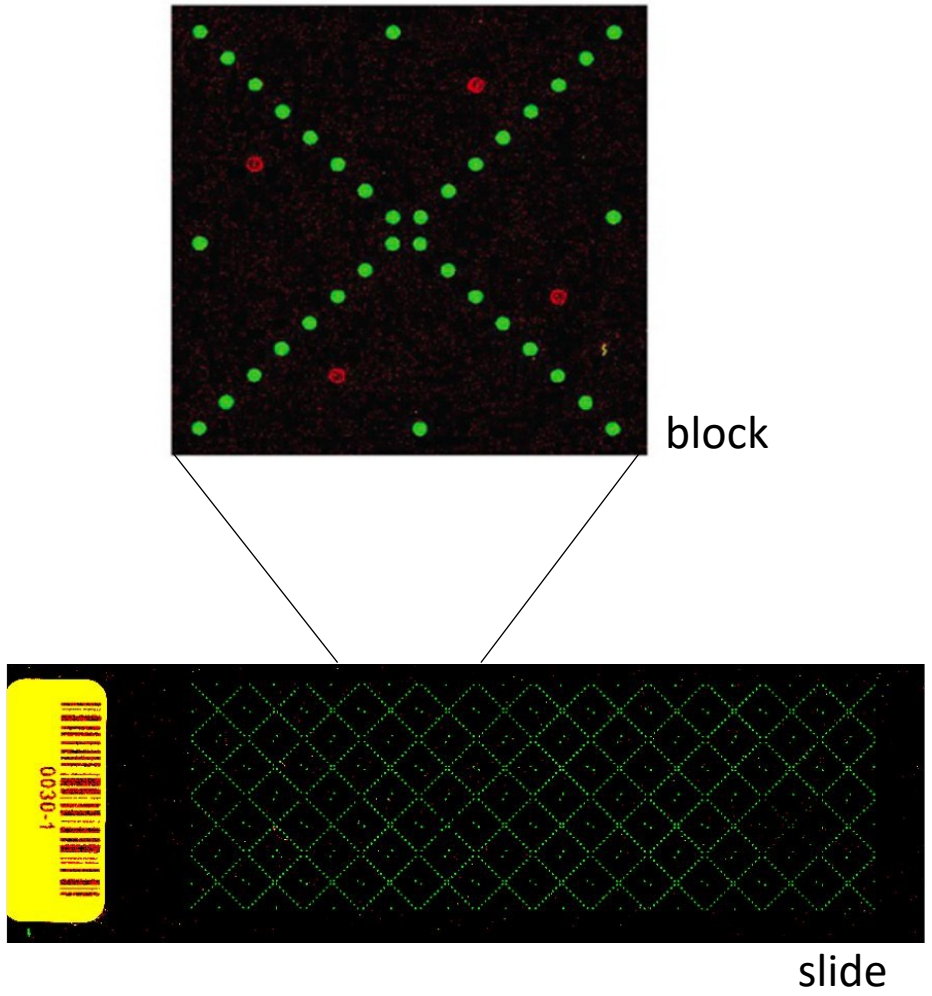
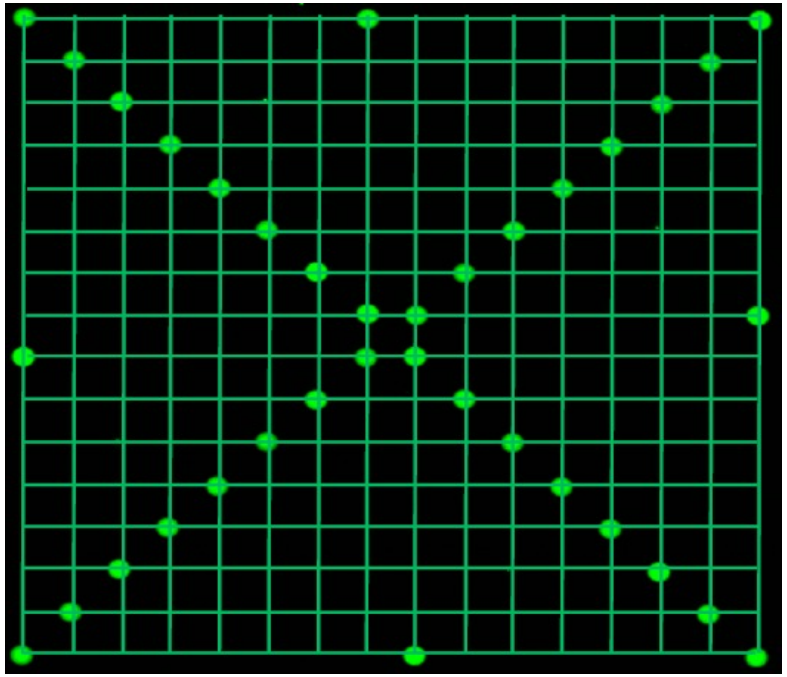
Workflow for SMM data analysis

1. Align spots using fluorescence on 532 nm channel (sentinel spots)
2. Quantify fluorescence on 635 nm channel
3. Identify ‘hits’ with improbably high fluorescence
4. Complete ‘by eye’ analysis of putative hits to manually remove false positives

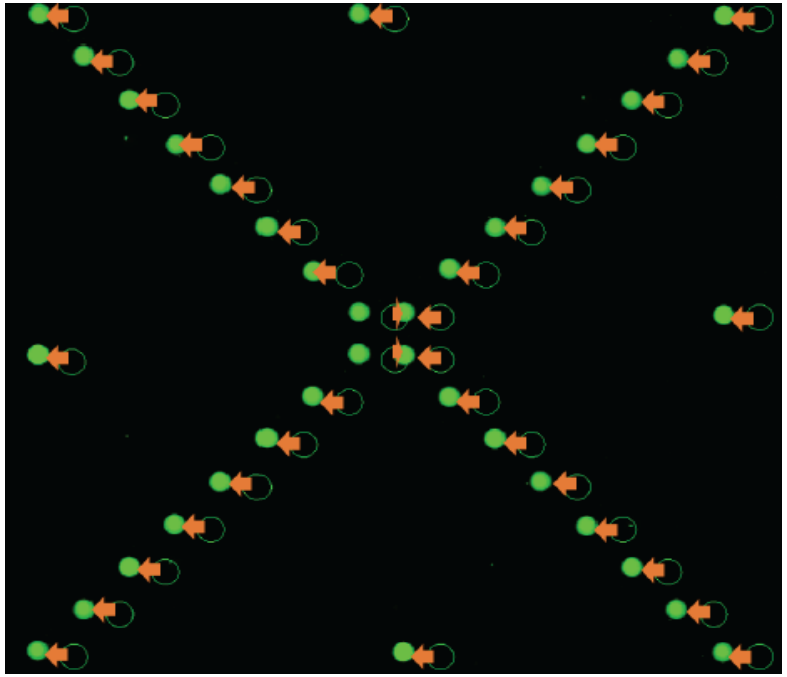


Align SMM using sentinel spots

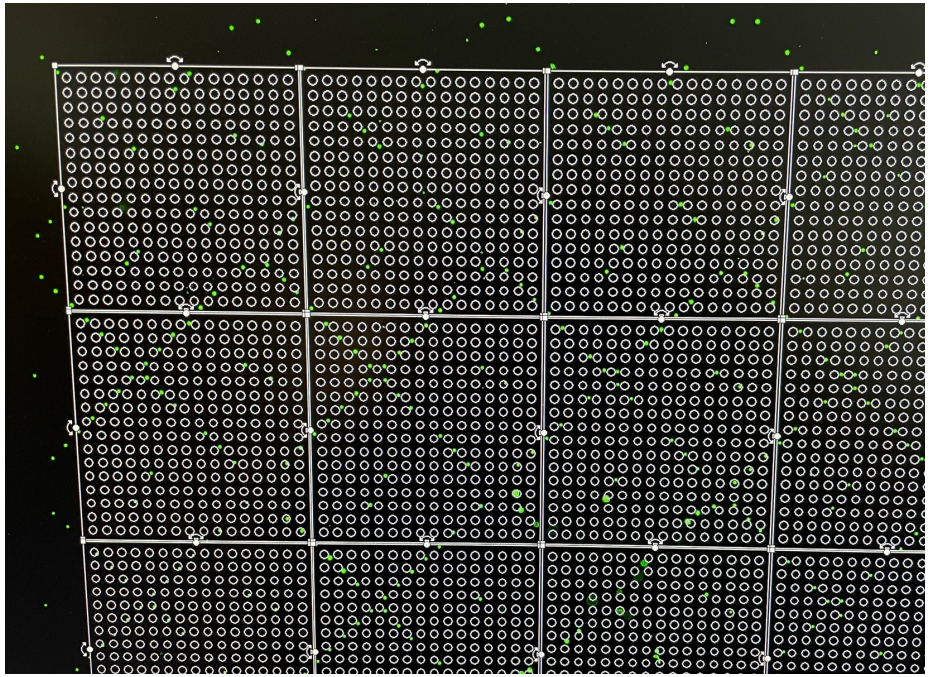
- Slides are printed in block patterns (16 rows x 16 columns)
- Each ligand spot is identifiable via intersecting lines between sentinels



Concept



Process

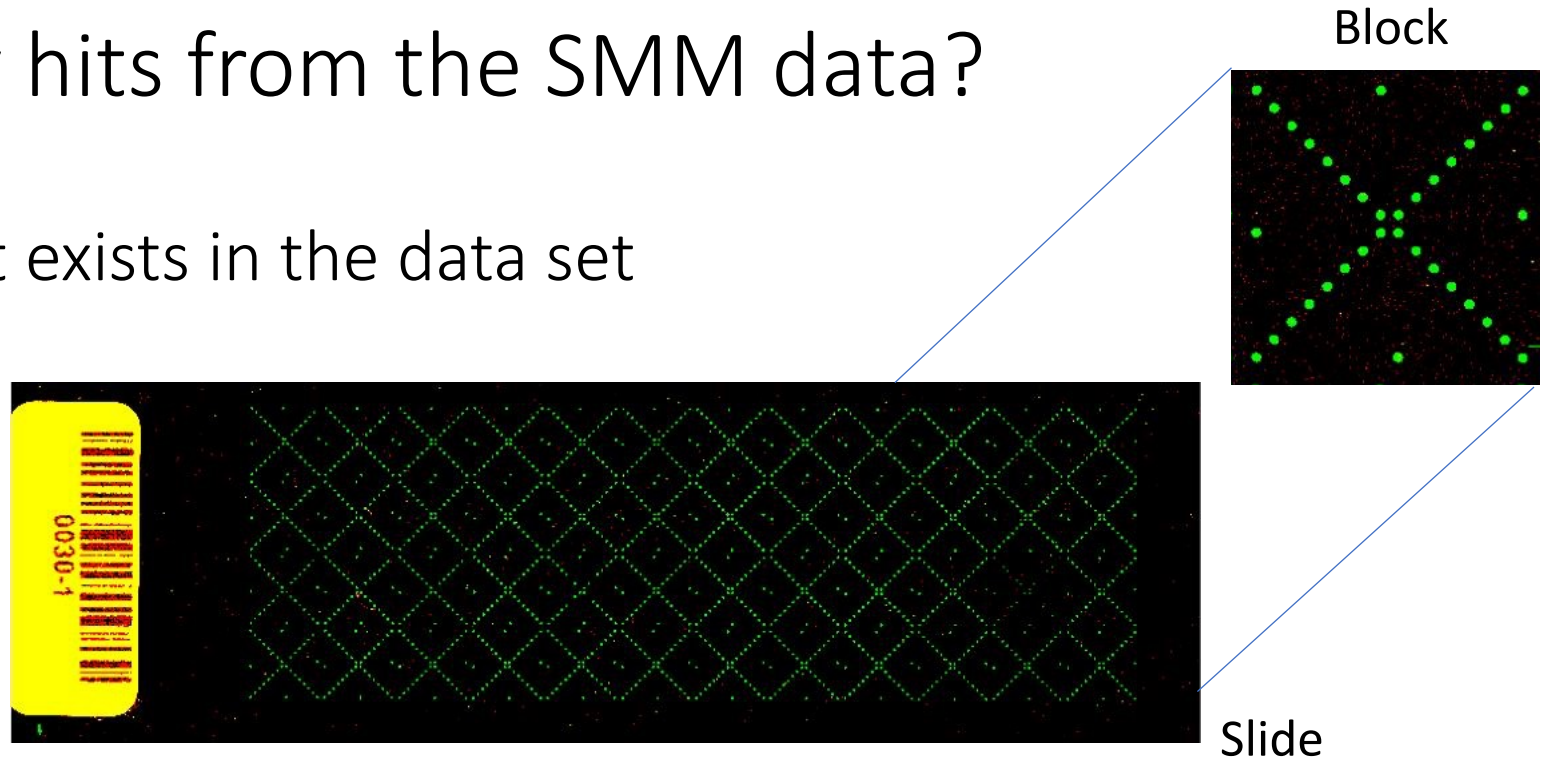


Real life

How do you identify hits from the SMM data?

First, consider bias that exists in the data set

- Across all slides
- Within each block
- Within each slide



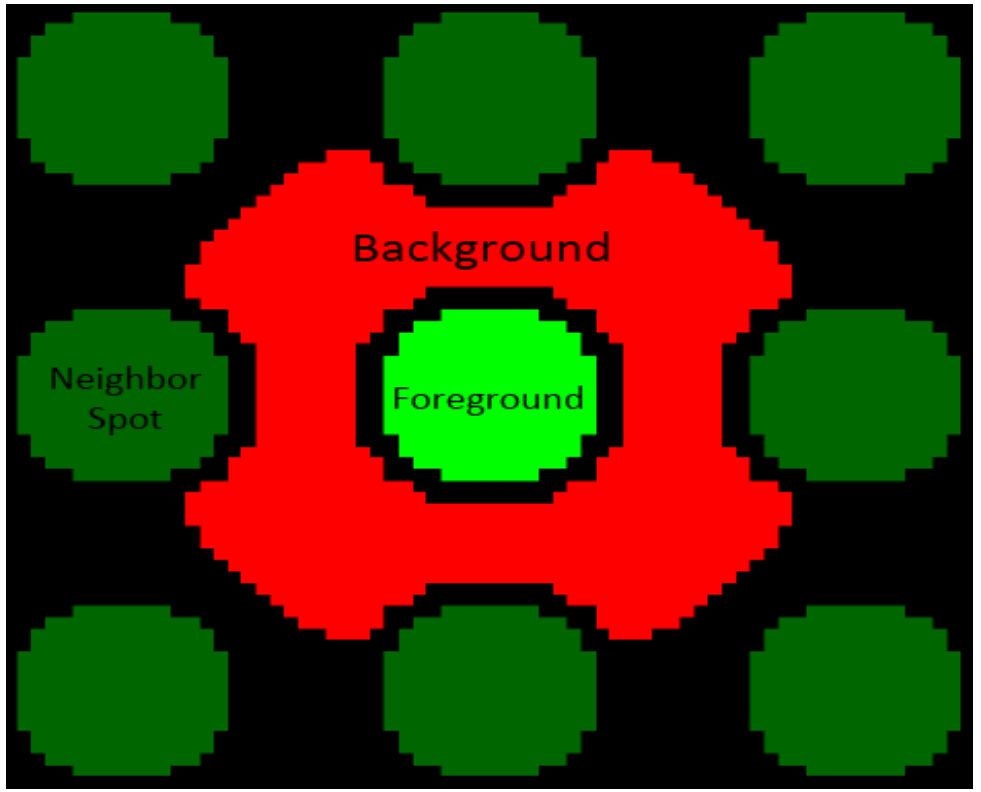
Then, identify hits with significantly higher fluorescence over background

Lastly, manually confirm hits to eliminate false positives

Fluorescence represented by an array of numerical values which are used to calculate z score

4	3	4	4	3	2	3	4	3	5	4	6	3	3	3	2	3	2	2
3	5	4	3	3	3	5	6	7	8	5	6	4	4	4	3	3	3	3
3	3	3	3	4	8	12	92	275	311	256	61	11	6	3	3	3	3	4
4	3	3	4	8	173	625	818	823	856	815	831	568	136	9	5	4	4	3
5	3	4	8	273	830	814	835	873	890	836	857	818	771	201	9	6	2	2
3	4	7	175	780	805	877	941	936	920	973	921	842	819	714	125	6	3	2
4	4	29	568	868	867	905	909	936	994	954	931	963	875	813	490	15	5	4
4	5	131	754	852	906	958	920	963	923	917	904	951	930	851	716	95	6	3
4	5	229	796	879	924	934	923	962	961	993	993	945	989	867	780	162	6	4
3	7	254	827	879	965	949	960	982	926	918	955	927	984	872	765	204	7	3
4	5	175	808	883	996	951	998	935	976	971	940	922	961	872	804	132	4	4
4	4	57	666	859	968	999	947	977	985	916	928	960	974	841	678	62	4	4
4	3	11	406	839	897	915	930	946	993	914	911	977	900	830	359	10	3	4
3	2	5	60	624	830	890	973	903	921	912	930	881	850	613	54	6	3	3
3	4	4	7	92	602	873	856	882	913	887	885	842	589	82	7	4	3	3
3	4	3	4	5	23	266	697	838	828	837	667	261	21	5	4	4	5	4
3	3	4	4	4	6	9	12	27	49	28	11	9	7	5	3	3	4	3
3	5	3	5	4	4	7	4	4	6	6	3	5	3	3	3	3	4	4

- Each pixel is represented by a number that indicates intensity of the signal



Signal-to-noise ratio

$$(SNR) = \frac{\mu_{foreground} - \mu_{background}}{\sigma_{background}}$$

Identifying hits with significant fluorescence

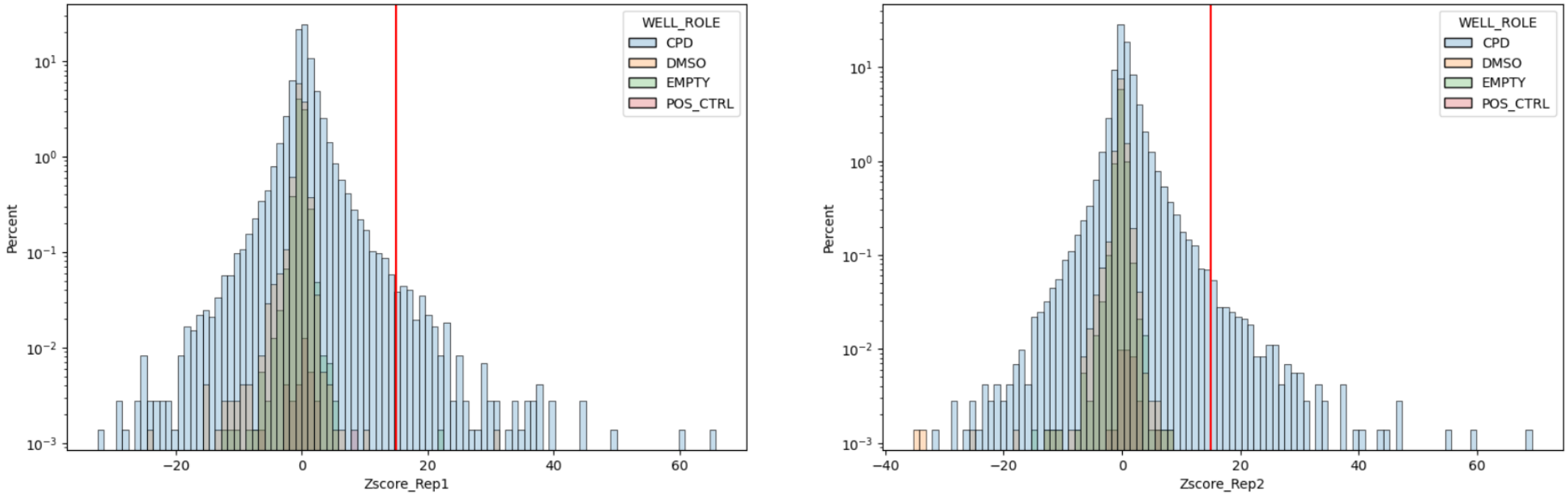
median absolute deviation (MAD)

$$\text{Robust Z-score} = \frac{SNR_i - \text{median}(SNR)}{\text{median}(|SNR_i - \text{median}(SNR)|) * 1.48}$$

scale factor for the normal distribution

Robust Z-scores help eliminate the influence of outliers

Robust Z-score calculated for all compounds



Histogram of robust z score of wells in both replicates

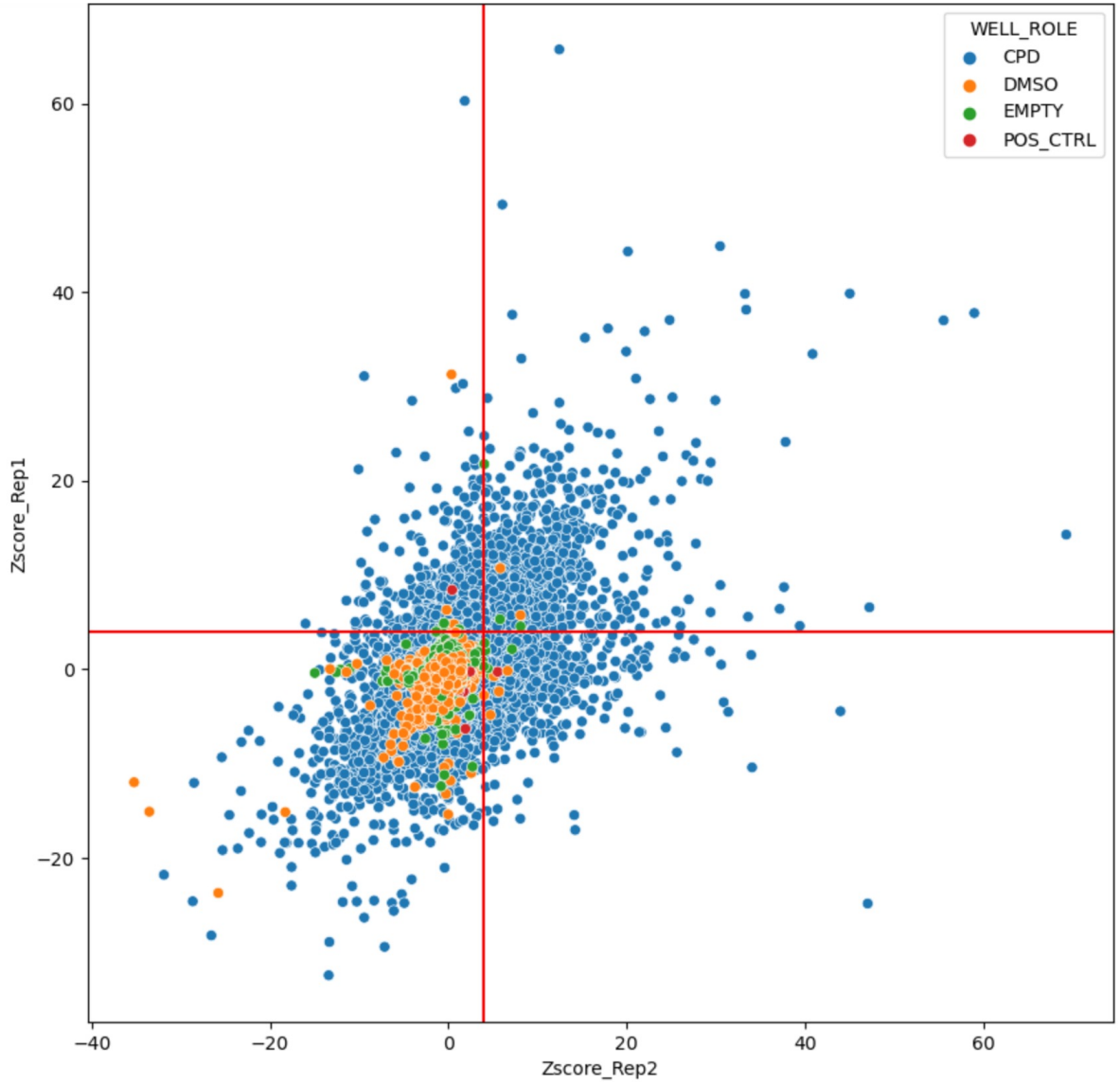
- Why are the empty wells clustered around zero?
- Where do we expect to see the putative binders represented?

Use a scatterplot to compare consistency of replicates

- Expect to see a linear relationship between replicate z scores

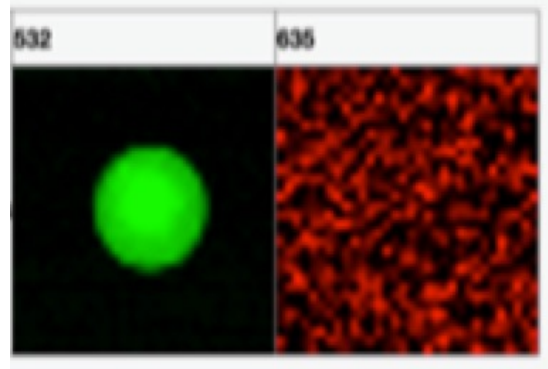
- What does it mean if there are replicates that do not have a linear relationship?

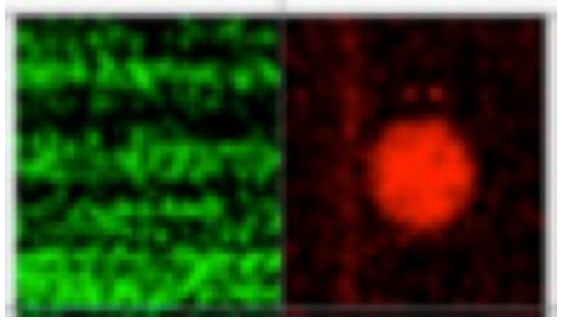
- Where do we expect to see our putative binders in this graph?



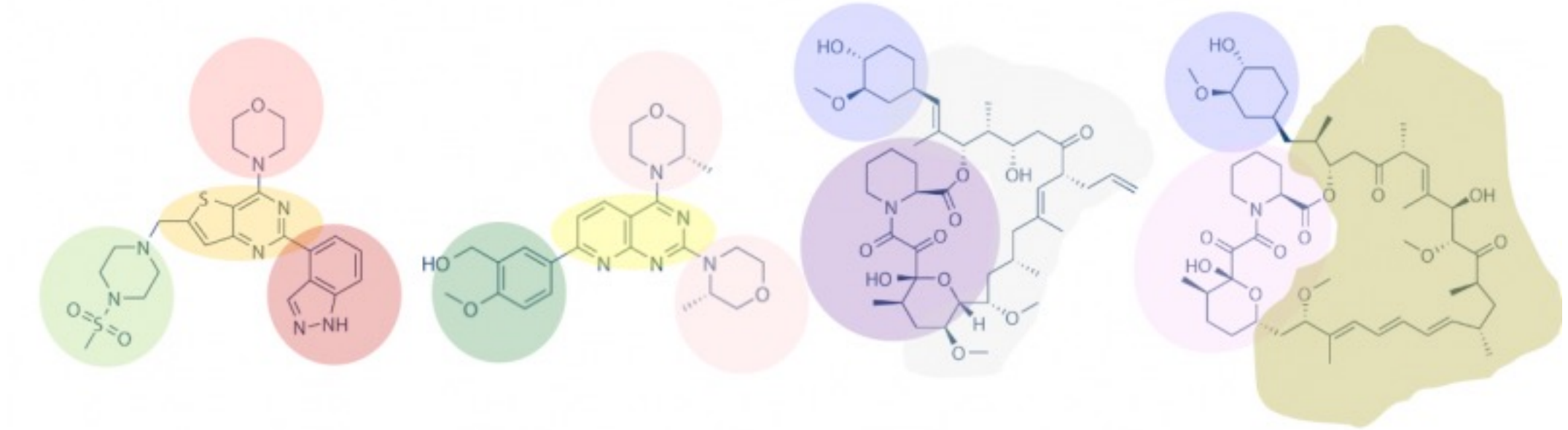
How do you validate hits manually?

Internal_ID	Zscore_Rep1	Zscore_Rep2	Slide_Rep1	Slide_Rep2	Loc1(block, row, col)	Loc2(block, row, col)
KI10167	37.029230	24.797054	50033718.0	50033720.0	2,2,3	2,7,11
KI10451	38.126523	33.380424	50033718.0	50033720.0	42,12,8	42,15,14
KI10796	16.684962	18.340951	50033718.0	50033720.0	45,10,16	45,12,4
KI10776	19.640085	18.046225	50033718.0	50033720.0	41,1,6	41,8,10
KI11103	16.929258	19.408047	50033718.0	50033720.0	42,1,12	42,6,8
KI11145	18.763623	15.206143	50033718.0	50033720.0	8,10,14	8,14,16
KI12064	25.653114	15.651485	50033718.0	50033720.0	41,11,4	41,15,5
KI20071	22.544108	24.043693	50033689.0	50033693.0	26,12,11	26,5,4
KI20165	25.236351	23.586604	50033689.0	50033693.0	4,2,3	4,7,11
KI20173	36.988660	55.511073	50033689.0	50033693.0	4,16,2	4,6,9





How will you identify common structures?



For Today

- Work through SMM analysis procedure
- Evaluate chemical structures of identified hits
- Work on homework and begin thinking about Data Summary

For M1D8

- Answer the wiki questions in the Homework section to begin work on the Data Summary Implications and Future Works section

- With your Lab Partner, incorporate the feedback on your methods homework and revise to include experiments from M1D4-M1D5