Introduction

For the following series of experiments we used a TECAN made Freedom EVO® lab robot for high throughput concentration and substance characterization and screening.
We developed a script for the robot to work with during the Azo-Benzene Experiments where we utilized the built in StoreX plate incubator shaker, a plate centrifuge and an Infinite 200 Microplate Reader to conduct hours long experiments with a wide range of parameters all measured and tested at parallel.

In the following experiments we attempted to characterize the interaction of Azo-Benzene(AB) Molecules and E.Coli Bacteria under various conditions, such varying concentrations of AB or Bacteria, shining different wavelengths of light on the samples (which are supposed to transform the AB molecules from "sticky" to "non-sticky") or adding a substance called EDC which is supposed to lower the activation energy of the AB molecules' binding reaction to the cell membrane.

08/10/14

Azo-Benzene(AB) concentration scanning - Run 1

Using the robot scanning plan for placing AB in 4 96-well-plates of varying OD and varying the AB in every plate

We took LB with Top10+1C3 bacteria grown to an OD of: **0.25**

We placed various dilutions of the bacteria in each of the 4 96-well-plates then we centrifuged and extracted only the LB and by placing 175µl of AB we added up with the following starting bacteria OD per plate:

Plate name	Starting OD
plate1	0.05
plate2	0.1
plate3	0.15
plate4	0.2

Following a 1.5hr of incubation we added $23\mu l$ of AB disolved in BA in various concentrations to each of the plates resulting in the following AB concentration in $ng/\mu l$ scheme for all 96 well plates:

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
B	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
C	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
D	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
E	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
F	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
G	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
H	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0

We shone each of the plates with specific wavelength of photons using the Infinity Plate Reader's Fluorescence measurement module for 25 flashes of 460nm for rows A-D and 400nm for rows E-H.

OD measurement:

We measured the OD of each well individually using an Infinity Plate Reader with 10 flashes per well in the following times:

- Once right after incubation
- Once right before adding the AB
- Once right after adding the AB
- Following the addition of AB we measured OD once per 30 minutes for 12 repetitions.

Results:

Upon visual inspection, all wells seemed to be consistently "murky" with no visible sediments.

EDC toxicity to Top10+1C3 bacteria concentration scanning - Run 1

Checking at what levels EDC might be toxic to Top10+1C3 bacteria

We made 12 starters with Top10+1C3 bacteria grown in a 50ml lab tube and added to them varying concentrations of EDC as follows:

Tube #	1	2	3	4	5	6	7	8	9	10	11	12
$\frac{mg}{ml}$	7.40 741	2.46 914	0.82 305	0.27 435	0.09 145	0.03 048	0.01 016	0.00 339	0.00 113	0.00 038	0.00 013	0.00 004

We placed various quantities of the bacteria in each of the 4 96-well-plates then we centrifuged and extracted only the LB and by placing *175µl* of BA we ended up with the following starting bacteria OD per plate:

Plate name	**Starting OD**
plate1	0.05
plate2	0.1
plate3	0.15
plate4	0.2

Following a 1.5hr of incubation we added *23µl* of AB disolved in BA in various concentrations to each of the plates resulting in the following AB concentration in ng/*µl* scheme for all 96 well plates:

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
B	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
C	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
D	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
E	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
F	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
G	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
H	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0

We shone each of the plates with specific wavelength of photons using the Infinity Plate Reader's Fluorescence measurement module for 25 flashes of 460nm for rows A-D and 400nm for rows E-H.

OD measurement:
We measured the OD of each well individually using an Infinity Plate Reader with 10 flashes per well in the following times:

- Once right after incubation
- Once right before adding the AB
- Once right after adding the AB
- Following the addition of AB we measured OD once per 30 minutes for 12 repetitions.

Results:
Upon visual inspection, all wells seemed to be consistently "murky" with no visible sediments.

09/10/14

Azo-Benzene(AB) concentration scanning - Run 2

Using the robot scanning plan for placing AB in 4 96-well-plates of varying OD and varying the AB in every plate

We took LB with Top10+1C3 bacteria grown to an OD of: **0.362**
We placed various quantities of the bacteria in each of the 4 96-well-plates then we centrifuged and extracted only the LB and by placing *175µl* of BA *with EDC disolved in it to a concentration of 0.268mg/ml* we ended up with the following starting bacteria OD per plate:

Plate name	Starting OD
plate1	0.05
plate2	0.1
plate3	0.15
plate4	0.2

Following a 1.5hr of incubation we added *23µl* of AB disolved in BA *with the same concentration of EDC disolved in it* in various concentrations to each of the plates resulting in the following AB concentration in ng/*µl* scheme for all 96 well plates:

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
B	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
C	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
D	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
E	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
F	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
G	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
H	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0

We shone each of the plates with specific wavelength of photons using the Infinity Plate Reader's Fluorescence measurement module for 25 flashes of 460nm for rows A-D and 400nm for rows E-H.

OD measurement:

We measured the OD of each well individually using an Infinity Plate Reader with 10 flashes per well in the following times:

- Once right after incubation
- Once right before adding the AB
- Once right after adding the AB
- Following the addition of AB we measured OD once per 30 minutes for 12 repetitions.

Issues:

During the experiment we were forced to stop the robot and this resulted in the amount of AB placed in plates 3-4 to be uncertain as the aspiration of the BA+AB+EDC solution by the robot and subsequent dispensing into the mentioned plates was unfortunately interrupted.

Results:

Upon visual inspection, all wells seemed to be consistently "murky" with no visible sediments.

10/10/14

Azo-Benzene(AB) concentration scanning - Run 3

Using the robot scanning plan for placing AB in 4 96-well-plates of varying OD and varying the AB in every plate

We took LB with Top10+1C3 bacteria grown to an OD of: **0.2532**
We placed various quantities of the bacteria in each of the 4 96-well-plates then we centrifuged and extracted only the LB and by placing *175µl* of BA we ended up with the following starting bacteria OD per plate:

Plate name	Starting OD
plate1	0.05
plate2	0.1
plate3	0.15
plate4	0.2

Following a 1.5hr of incubation we added *23µl* of AB disolved in BA in various concentrations to each of the plates resulting in the following AB concentration in ng/*µl* scheme for all 96 well plates:

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
B	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
C	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
D	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
E	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
F	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
G	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
H	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0

We shone each of the plates with specific wavelength of photons using the Infinity Plate Reader's Fluorescence measurement module for 25 flashes of 460nm for rows A-D and 400nm for rows E-H.

OD measurement:
We measured the OD of each well individually using an Infinity Plate Reader with 10 flashes per well in the following times:

- Once right after incubation
- Once right before adding the AB
- Once right after adding the AB
- Following the addition of AB we measured OD once per 30 minutes for 16 repetitions.

Results:
Upon visual inspection, all wells seemed to be consistently "murky" with no visible sediments even after an overnight rest on the robots table (no incubation)

11/10/14

Azo-Benzene(AB) concentration scanning - Run 4

Using the robot scanning plan for placing AB in 4 96-well-plates of varying OD and varying the AB in every plate

We took LB with Top10+1C3 bacteria grown to an OD of: **0.19**
We placed various quantities of the bacteria in each of the 4 96-well-plates then we centrifuged and extracted only the LB and by placing *175µl* of BA we ended up with the following starting bacteria OD per plate:

Plate name	Starting OD
plate1	0.05
plate2	0.1
plate3	0.15
plate4	0.19

Following a 1.5hr of incubation we added *23μl* of AB disolved in BA in various concentrations to each of the plates resulting in the following AB concentration in ng/*μl* scheme for all 96 well plates:

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
B	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
C	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
D	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
E	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
F	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
G	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0
H	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.002	0.001	0

We shone each of the plates with specific wavelength of photons using the Infinity Plate Reader's Fluorescence measurement module for 50 flashes×4 locations per well (overall *8 times* more photons than previous runs) of 460nm for rows A-D and 400nm for rows E-H.

OD measurement:

We measured the OD of each well individually using an Infinity Plate Reader with 10 flashes per well in the following times:

- Once right after incubation
- Once right before adding the AB
- Once right after adding the AB
- Following the addition of AB we measured OD once per 30 minutes for 16 repetitions.

Results:

Upon visual inspection, **there were visible sediments in most of the wells of certain plates!**

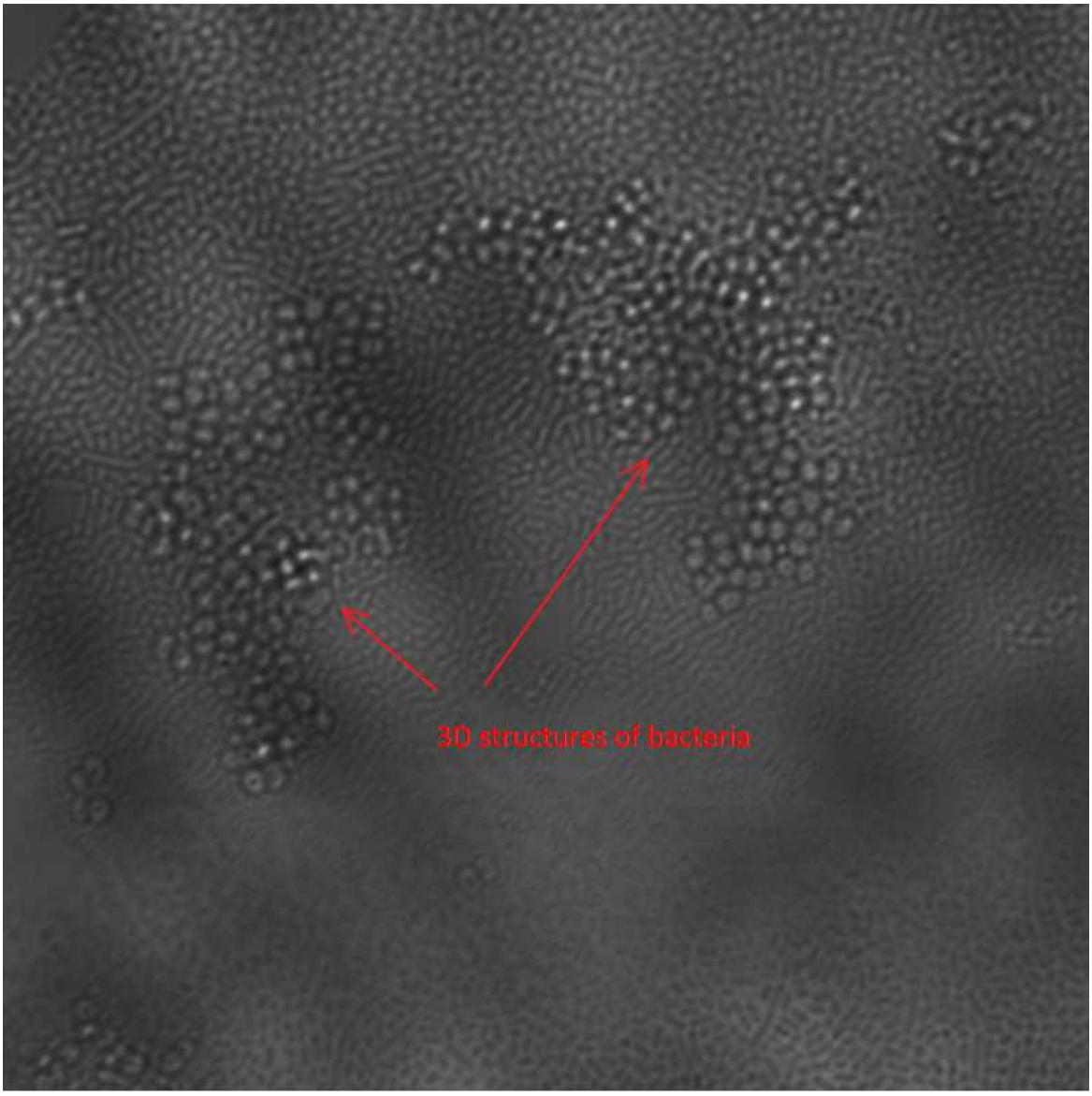
Attached is the email sent to all of the participants of the experiment:

> The 4th run of our experiment just finished and upon visual inspection of the plates I can very clearly see some kind of bacterial sediment at the bottom of in nearly all of the wells of the **first plate**, the largest sediments appear to be in the final columns on each end of the plate, that is 1,2,10,11,12.
> There perhaps some sediments in the other plates as well. In those plates I'm also seeing something reminiscent of the sediments shown in **plate1** but they appear to be around the edges of the well, so they are hard to distinguish from shadows.
> They seem to be larger at the bottom(400nm) of the plate on the columns with the highest concentrations of AB (well H1 seems to be sediment heavy, the rest

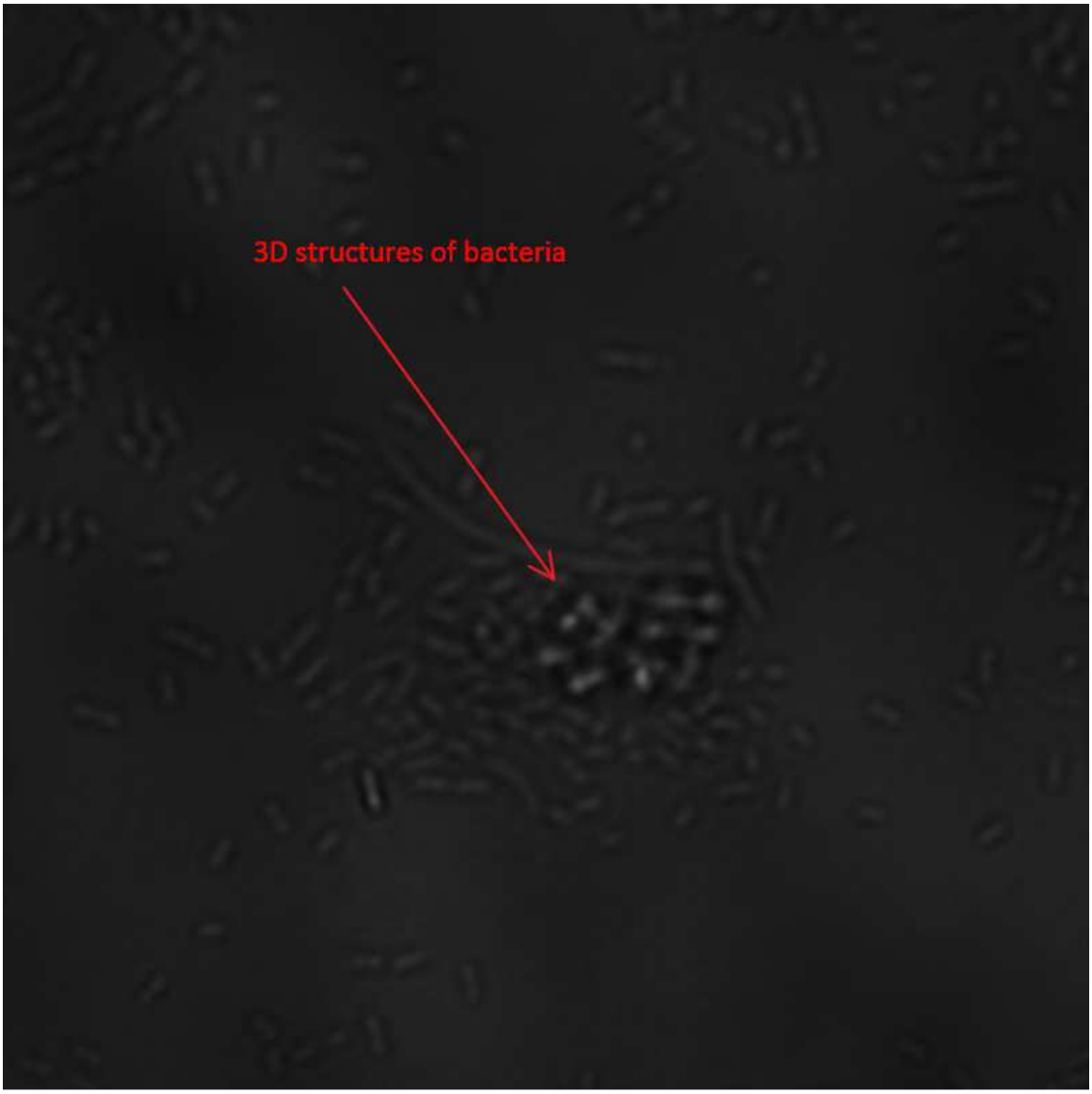
have smaller clumps, and from column 3 onward they seem more oblong, almost line like).
The top of the plate however has a sediment almost as large as H1 in A1 (same AB concentration, different light) but the range A12:C12 has the largest sediments by far with A10:C12 smaller, but still very visible.
The other plates have some clearly visible sediments, but the only noteworthy ones are A12 on plate 3 and A12,B12,H11,H12 on plate 4 as I see it. Again there are other wells that seem to have some kind of sediment, but it's at the edge and not very well distinguished from the shadows cast by the edges, so i do not consider the "noteworthy".
These results were not seen in any of the other runs, but the other runs' plates were checked a few hours after leaving the storeX incubator shaker as opposed to now, where i checked them almost immediately after.
Another change that was made was the lighting time was increased 8-fold from 25 flashes to 50 flashes in 4 different spots on the wells.
Now, these sediments could very well be left over from the centrifuge, although from my experience the sediments after the centrifuge do not look like these and the plates were shaken intermediately for several hours since then.
I honestly do not know what to make of these results, and am lacking a proper way to take a picture of them, but I'm trying to document as much as I can nonetheless.

The sediments remained intact even after an overnight stay on the table(no incubation).

Using a ×60 magnification light microscope we managed to take t
he following image from plate4, well H1:



And the following image from plate1 well H12



It's worth noting that clusters of the size shown in well H1 for plate 4 or 3D structures as shone in both wells are highly unusual for this type of bacteria from previous experience

14/10/14

Azo-Benzene(AB) with\without EDC comparison - Run 1

Using the robot scanning plan for placing AB in 2 96-well-plates of similar OD and similar the AB in every plate to check the effects of EDC on the reaction

We took LB with Top10+1C3 bacteria grown to an OD of: **0.1468**
We placed *199.6µl* of bacteria+LB in both 96-well-plates then we centrifuged and extracted only the LB and by placing *106µl* of BA(in the second plate *with EDC*

disolved in it to a concentration of 0.268mg/ml, in the first without any EDC) we ended up with the following starting bacteria OD per plate:

Plate name	Starting OD
plate1	0.2765
plate2	0.2765

Following a 1.5hr of incubation we added *92µl* of AB disolved in BA in various concentrations to each of the plates resulting in the following AB concentration in ng/*µl* scheme for all 96 well plates:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	4	2	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039
B	0	4	2	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039
C	0	4	2	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039
D	0	4	2	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039
E	0	4	2	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039
F	0	4	2	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039
G	0	4	2	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039
H	0	4	2	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039

We shone each of the plates with specific wavelength of photons using the Infinity Plate Reader's Fluorescence measurement module for 60 flashes×4 locations per well (one fifth more light than the previous run on 11/10/14) of 460nm for rows A-D and 400nm for rows E-H.

OD measurement:

We measured the OD of each well individually using an Infinity Plate Reader with 1 flash×16 locations in the following times:

- Once right after incubation
- Once right before adding the AB
- Once right after adding the AB
- Following the addition of AB we measured OD once per 30 minutes for 16 repetitions.

Results:

Upon visual inspection, both plates showed elongated, almost line-like sediments at their bottom, with a tendency for the edge wells to have larger sediments.

15/10/14

Azo-Benzene(AB)/Blank bacteria with BA comparison - Run 1

Using the robot scanning plan for placing AB in 2 96-well-plates of similar OD and similar the AB in every plate to check the effects of EDC on the reaction

We took LB with Top10+1C3 bacteria grown to an OD of: **0.4256**

We placed *65.66µl* of bacteria+LB in both 96-well-plates then we centrifuged and extracted only the LB placing *106µl* of BA(in the second plate *with EDC disolved in it to a concentration of 0.268* mg/ml, in t without any EDC) we ended up with the following starting bacteria OD per plate:

Plate name	Starting OD
plate1	0.2765
plate2	0.2765

Following a 1.5hr of incubation we added *92µl* of BA to the first plate, and shone upon it with specific wavelength of photons using the Infinity Plate Reader's Fluorescence measurement module for 60 flas locations per well of 460nm for rows A-D and 400nm for rows E-H.

The plate was returned to incubation and the second plate extracted to the table.

~After that the program encountered an error and the experiment was not resumed.

OD measurement:

We measured the OD of each well individually using an Infinity Plate Reader with 1 flash×16 locations in the following times:

- Once right after incubation
- Once right before adding the extra BA - only for the 1st plate
- Once right after adding the extra BA - only for the 1st plate

Results:

Upon visual inspection after a night of incubation , the first plate (the one in incubation/shake overnight) showed round sediments at the bottom of most wells, and the smallest ones were around the middle of the plate.
The second plate(sitting outside all night) had sediments all around the plate except for a clear circle in the middle of each well. This was hypothesized to have been caused by the dispersion of bacteria during pipetting.

16/10/14

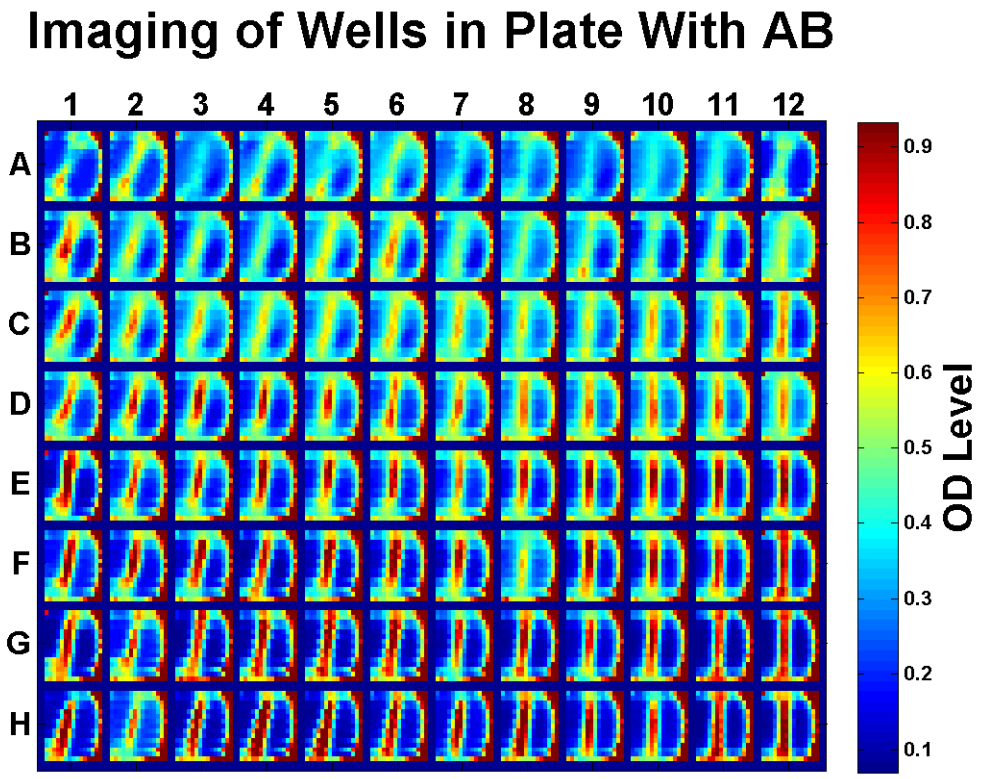
Plate Imaging - Run 1

Using the platereader to "take a photo" of wells' sediments

We took the 4 plates from the last 2 experiments(With\Without EDC (we shall call it AB/EDC) and Blan
\AB (B/AB)) and measured the OD of each well as a 15×15 grid with 1 flash per measurement and the
MATLAB to present all wells in a single graph, essentially "taking a picture" of the Microplate using the
Reader

First Picture:

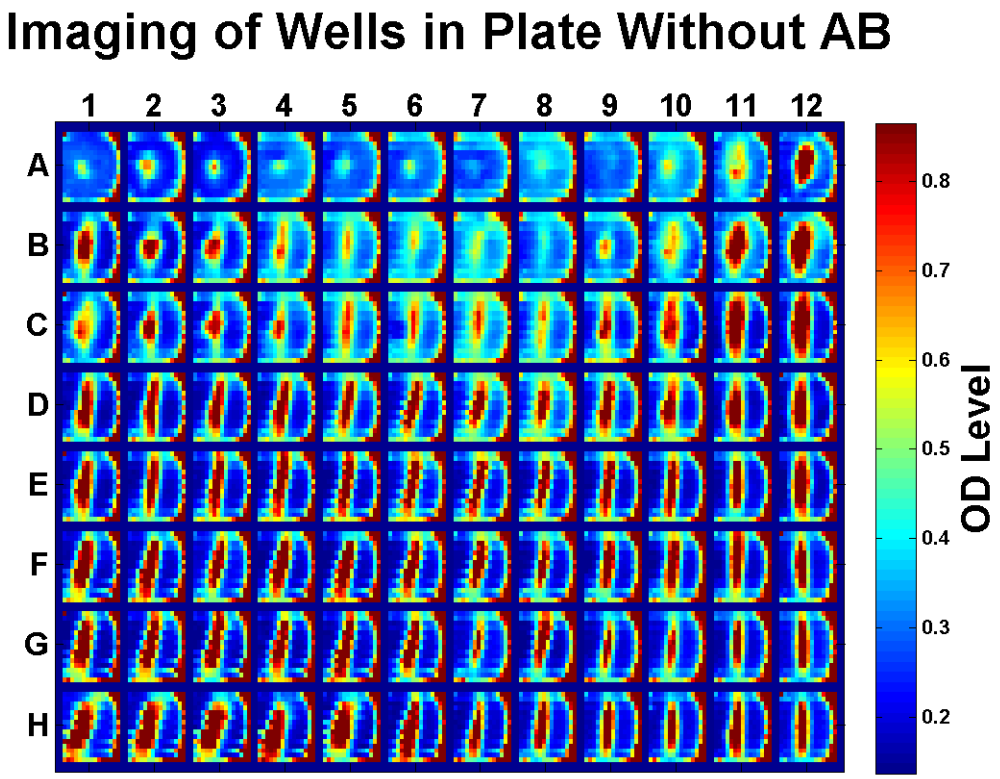
We started out by measuring the AB/EDC experiment plate with the EDC and AB:



The thing that became immediately apparent with this plate was the shape of the sediments being tha
single, or sometimes double, line (the resolution of 15×15 does not allow to see the parallel double li
structures) which does not seem to be random at all.

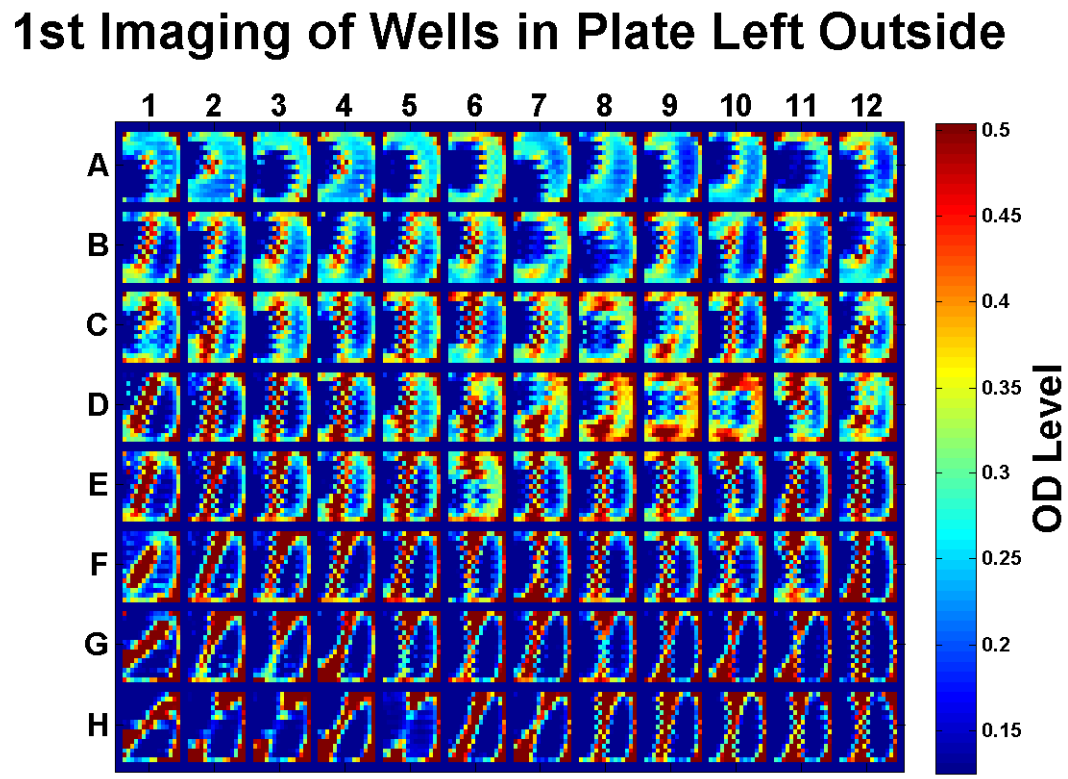
Second picture:

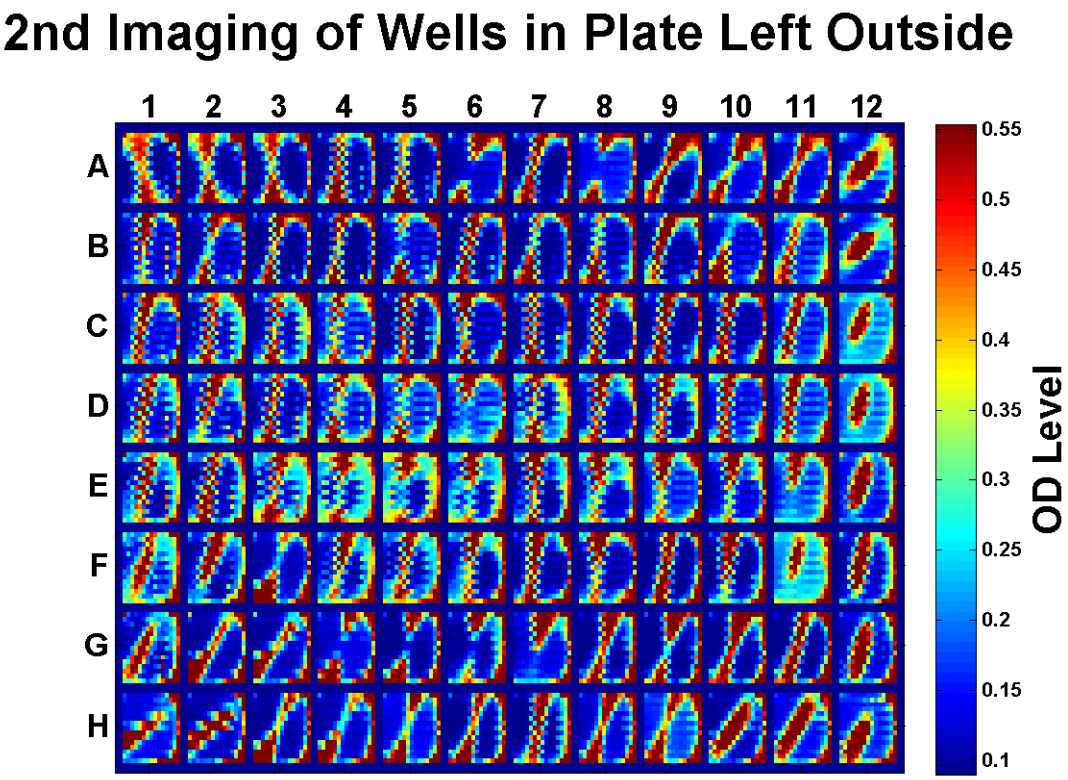
We then went on to take a picture of the control plate from the B/AB experiment, which had round sediments as opposed to the linear ones shown in the AB plates.



This result is surprising, we have round sediments at the top and distinctly linear ones at the bottom.
We were even more puzzled as all of the plate's sediments looked to be circular when inspected by the naked eye before the Imaging process with the Plate Reader.

Pictures 3&4:
We then turned to the plate from the B/AB experiment that had been left outside of the shaker\incubator overnight without AB and had uniform sediments all over the well except for a circular "hole" at the center and attempted to image the plate:





For the 1st image we can clearly see that the first few wells (Rows A:C) seem as previously described, uniform with a hole in the middle, whereas the further we head down the plate, we see more and more the linear pattern discussed earlier.

We should remind once more that this is the plate that not only didn't have AB in it, but wasn't even incubated!
In the 2nd image we see that the whole plate has gone to a linear pattern, as was observed by our naked eye.
It should also be mentioned that these plates sat for hours on the workbench without a change to their sediment structure.

Analysis:
We observed that the way the Platereader goes through the plate is from A1 to A12 to B12 to B1 to C1 and so on…
What was interesting about it was the fact that the whole process of imaging one plate took around 17 minutes, meaning the time gap between plates is substantial, and since the sediments started to restructure mid-imaging we ended up having what could be considered a rough time-lapse of the sediments' restructuring in the Platereader.

Conclusion:
Since we started off with sediments of varying shapes and structures and ended up with the same type of structure for all plates, and ended up having all of the plates show the same structure after being in the plate reader, and lacking previous evidence that OD measurement(at 595nm) causes bacteria cluster in these formations or at all for that matter, we are left with the only reasonable explanation - the plates changed shape after being contaminated with AB, but where?
The first plate to spend 17 minutes was one with lots of AB in it, and these findings led us to hypothesis that perhaps Azo-Benzene has a volatile property when dissolved in water/BA and so the the plate reader became saturated with AB molecules and thus contaminated the plates during the reading.

What's next?
For our next experiment we plan to aerate the lab and equipment as best as we can and run the same protocol of experiment as the last 2 experiments only with no AB in the lab at all.
This experiment will not be included in this document or our Wiki page as we will not have the results in time.