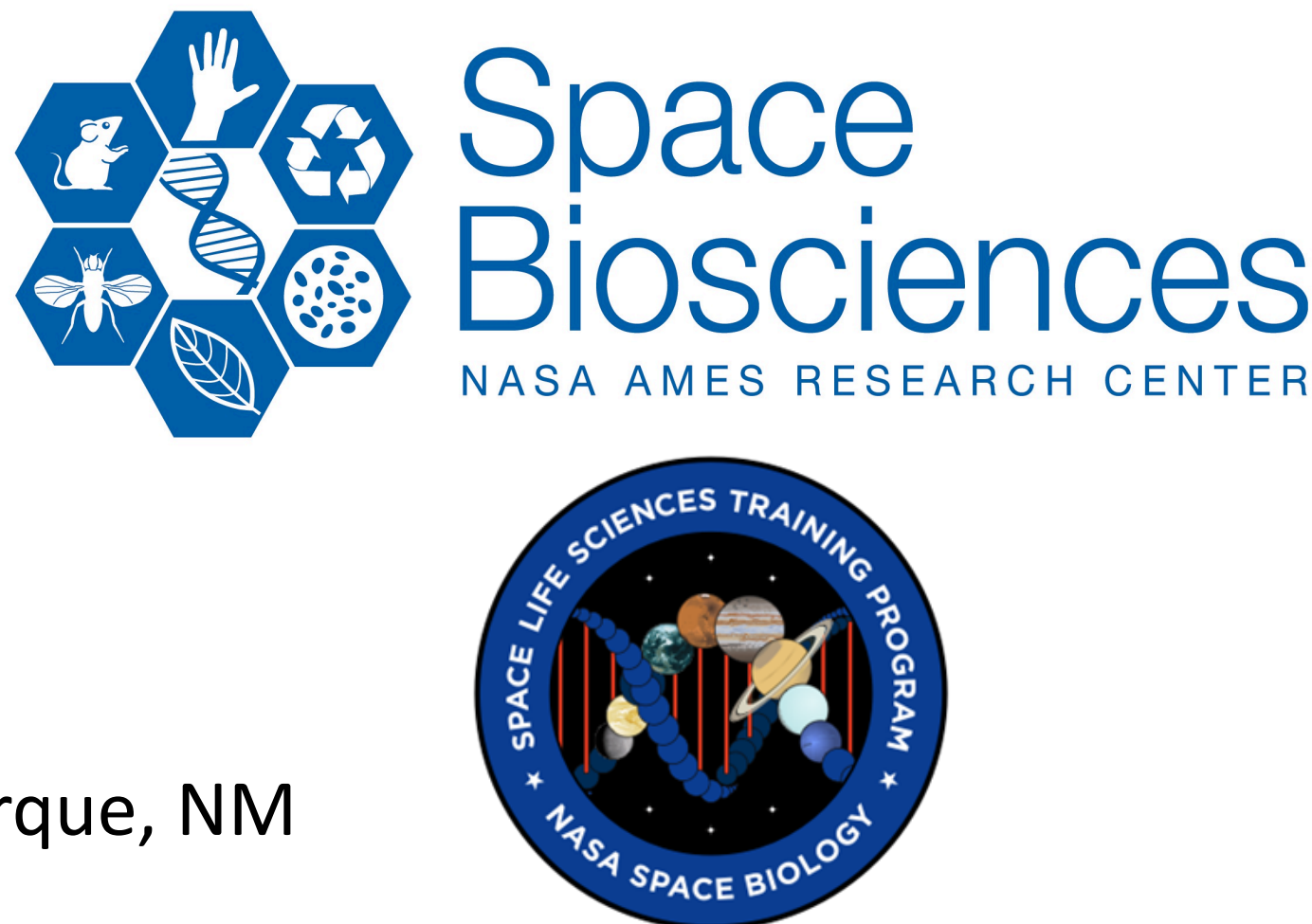
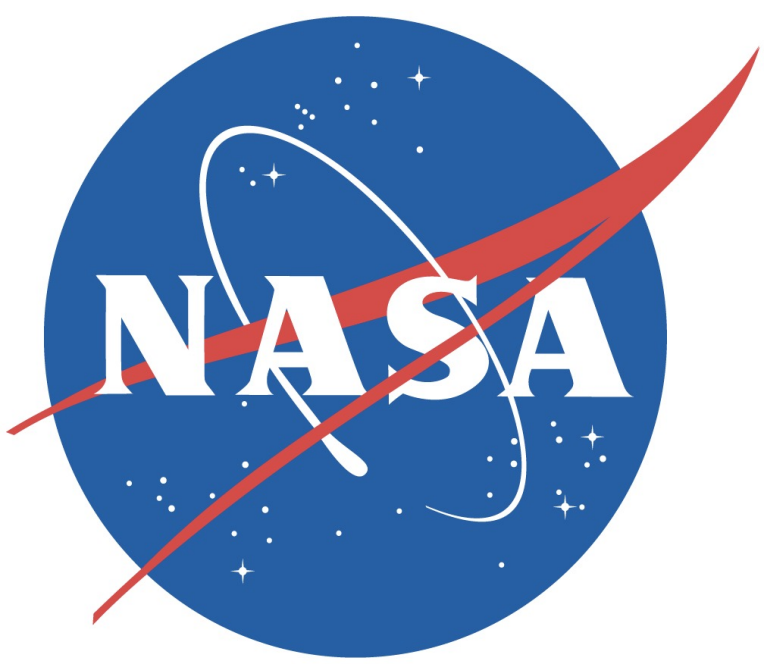




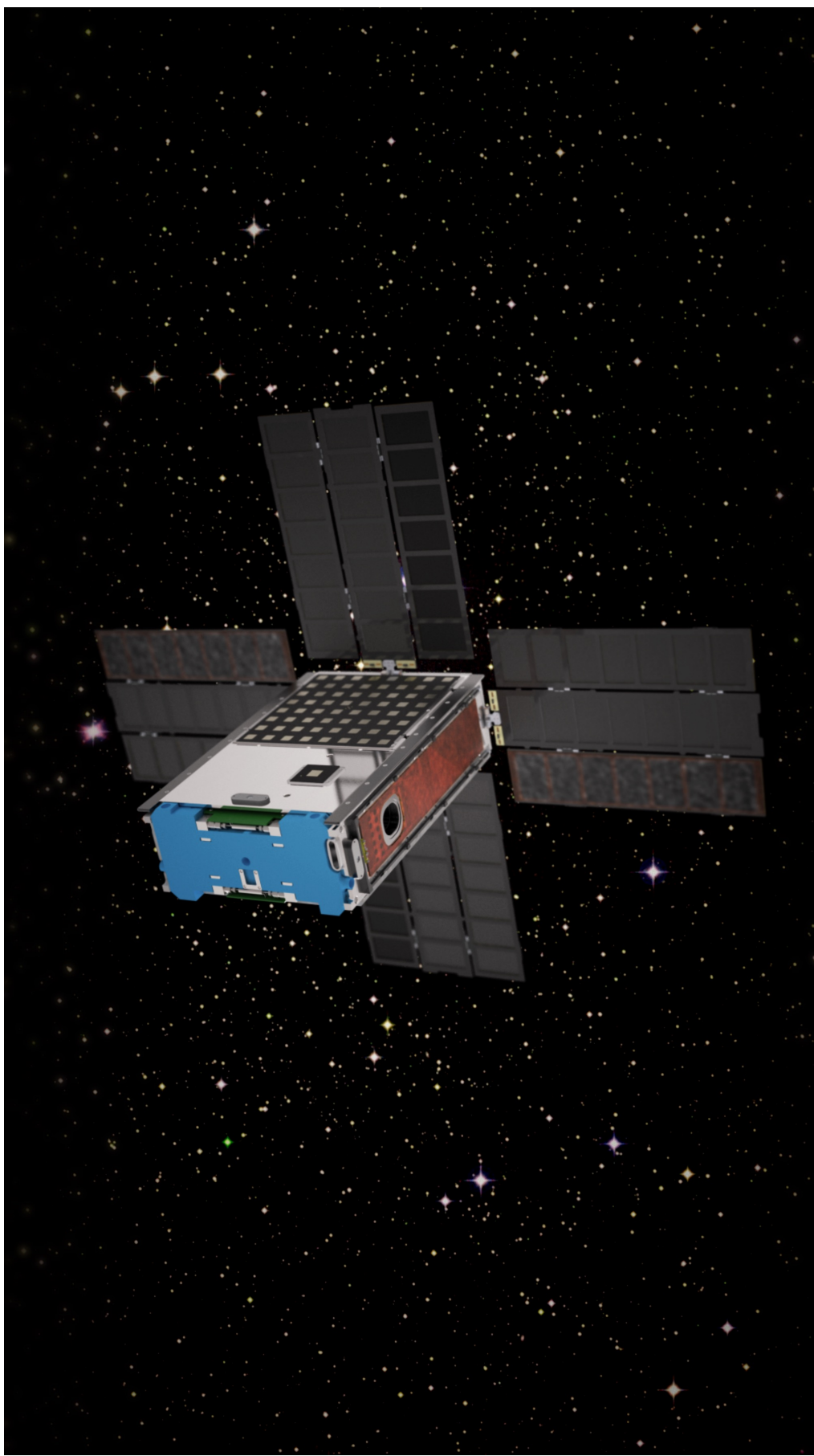
Improving Data Analyses for a Biological Mission to Deep Interplanetary Space

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Abstract

Onboard the Artemis 1 rocket, NASA plans to launch the first deep space bioscience mission past low Earth orbit (LEO) since 1972, BioSentinel, a 6-Unit (6U) biological CubeSat. BioSentinel's goals are to assess the effect of deep space ionizing radiation (IR) on DNA and cell damage response, using *Saccharomyces cerevisiae*, or budding yeast, as a model organism. BioSentinel accomplishes this by measuring Optical Density (OD) in addition to metabolic activity using the redox dye alamarBlue, both of which will be read through light emitting diode (LED) lights of differing wavelengths. With the largest and most sophisticated energy-providing solar panels utilized on a biological CubeSat to date, BioSentinel will be equipped with an IR dosimeter to identify what doses of radiation the yeast is exposed to at any point in time, in addition to a transponder to send such data back to NASA Ames' Multi Mission Operations Center (MMOC). Biology computational programs and data processing scripts are necessary to analyze this complex data once it is received, including automated subroutines to analyze duplication rate, alamarBlue reduction, and time periods when paired against numerous other variables. Overall, we have implemented several VisualBasic biocomputational programs to analyze such data in an organized and efficient manner so that conclusions can be made rapidly. Furthermore, we showcase why efficient analysis of particular parameters is important to better understand the risks that deep space IR poses to astronauts, especially when considering the upcoming Artemis missions.



First Parameter: Cell Duplication Rate

BioSentinel is responsible for identifying several variables, all of which is does with the usage of LED lights and florescence. One of these variables, absorbance, or Optical Density (OD), is observed with an 850-nanometer infrared LED light. The BioSentinel team more-or-less only possessed absorbance curves to tell the story of growth. This data arose from experiments performed at Brookhaven National Laboratory. This proved to be an insufficient variable for the real mission when standing alone, and thus I was responsible for creating an automated subroutine program to capture cell duplication rate, or in other words the amount of time it take for a well of budding yeast to double in concentration. Overall, duplication rate tells much more of the story of growth, especially in relation to metabolism. Luckily, this summer a fully- fledged program ready for BioSentinel data once it arrives to Earth via transponder past LEO, was successfully created and implemented.

Methods

Currently, data possessed by NASA Ames meant to imitate future BioSentinel data performed at Brookhaven National Laboratory is contained within Microsoft Excel; furthermore, it is expected that real BioSentinel data will be transferred to excel format. The main tool utilized to write this program was VisualBasicCode, the main programming language applied for Microsoft excel data. In the program, several features such as for loops, the excel solver, and other mechanisms were frequently utilized.

Results

The results of this program ended with success, meaning the cell duplication rate was correctly outputted in the format desired. Specifically, the program synthesized is able to take OD data from any number of wells and analyze them all entirely in mere seconds to output not only their duplication rate, but other values as well including miu value, lag time, overall growth rate, and maximum growth rate. These computed values represent an expansion of the original task.

4.394332	WT YB571-1 0 cGy	4.310177	WT YB571-1 0 cGy
0.482041	-0.25131	0.04561	0.088164
5.828624	0	0.070391	0.004846
1.609438	0	0.108118	0.011689
0.183538	0.105361	0.165429	0.003608
	0.200671	0.251339	0.002567
	0.367725	0.377877	0.00103
	0.510826	0.559517	0.002371
	0.847298	0.810843	0.001329
	1.203973	1.141457	0.003908
	1.609438	1.54862	0.003699
	2.036882	2.011221	0.000659
	2.494123	2.491044	9.48E-06
	2.908721	2.943929	0.00124
	3.291713	3.334883	0.001864
	3.619887	3.6472	0.000746
	3.859559	3.881603	0.000486
	4.054679	4.049421	2.77E-05
	4.1778	4.165548	0.00015
	4.284353	4.244028	0.001626
	4.310053	4.296221	0.000191
	4.317488	4.330563	0.000171
	4.318968	4.352999	0.001158
	4.316006	4.367591	0.002661
	4.310053	4.377051	0.004489
	4.321923	4.383174	0.003752
	4.324868	4.38713	0.003877
	4.33511	4.389686	0.002979
	4.343805	4.391335	0.002259
	4.342361	4.392399	0.002604
	4.353856	4.393085	0.001539
	4.360973	4.393528	0.00106
	4.36804	4.393814	0.000664
	4.376456	4.393998	0.000308
	4.383415	4.394117	0.000115
	4.390325	4.394193	1.5E-05

Figure 1: An example of two specific wells analyzed from the program and their respective outputs. Value highlighted in blue is duplication rate of that particular well in hours. Both wells are Wild Type and exposed to 0 Gy while also possessing similar duplication rates, implying that the program runs as intended. Other values present include miu value, lag time, overall growth rate, and maximum growth rate.

To elaborate, a program to efficiently and quickly identify duplication rate is necessary because it provides light on both the story of growth within wells, but more importantly to identify differences in growth between the Wild Type Budding Yeast and Rad51 Budding yeast, which is impaired in its ability to double DNA strand repair. As we can see from figure 2 below, the duplication rate for a Rad51 budding yeast well is far longer than that of the Wild type. The conclusion, while seemingly expected, is completely necessary for when analyzing data sent back from BioSentinel. Indeed, there is no better way to showcase the effect of IR on DNA double strand repair than through duplication rate.

3.92745	WT YB571-2 60 cGy	4.57766	rad51 YB572-1 60 cGy
0.4199	-0.08	0.04136	0.01474
5.94613	0	0.06307	0.00398
1.65075	0.1431	0.09591	0.00223
0.04611	0.1431	0.1452	4.4E-06
	0.26826	0.21839	0.00249
	0.32542	0.32528	2.1E-08
	0.43078	0.47775	0.00221
	0.69315	0.68804	2.6E-05
	0.96141	0.965	1.3E-05
	1.32687	1.30841	0.00034
	1.72551	1.70375	0.00047
	2.13553	2.12177	0.00019
	2.52265	2.52586	1E-05
		2.86	2.88399
		3.15208	3.17777
		3.37259	3.40385
		3.5641	3.56944

Figure 2: Left: Wild type budding yeast well with 60 Gy. Right: rad51 budding yeast well with 60 Gy. Duplication rate for rad51 budding yeast is far larger than that of wild type, even though both are exposed to the same amount of radiation.

Second Parameter: Creation of Graphing program for Growth Variables/Parameters

As mentioned prior, BioSentinel captures growth through OD readings, which are then turned into graphs depicting growth over time of any well. Though to properly understand the difference in growth between the wild type and rad51 budding yeast, an automated program comparing the two is necessary. Thus, as an SLSTP Research Associate, I've created an automated program that can take any set of wells and graph in a bar chart format so that differences in growth are far more visible. Specifically, the parameters observed here are miu, lag time, and duplication rate, all of which pertain to the story of growth within the wells.

Methods

Methods are identical to that of Parameter/Task #1, except in this code graphing functions were utilized.

Results

Utilizing code from parameter 1 and adding code that would turn certain pieces of data in graph format, the results of the program were a success. As depicted in figure 3, utilizing the code created during the program, one is able to take any set of Wild Type and/or Rad51 budding yeast well and chart out miu, lag, and duplication values to determine the difference regarding difference doses of radiation and budding yeast type.

Analysis of Dup Rate, Growth Rate, and Lag Time in regards to budding Yeast replicates when exposed to Gamma Radiation

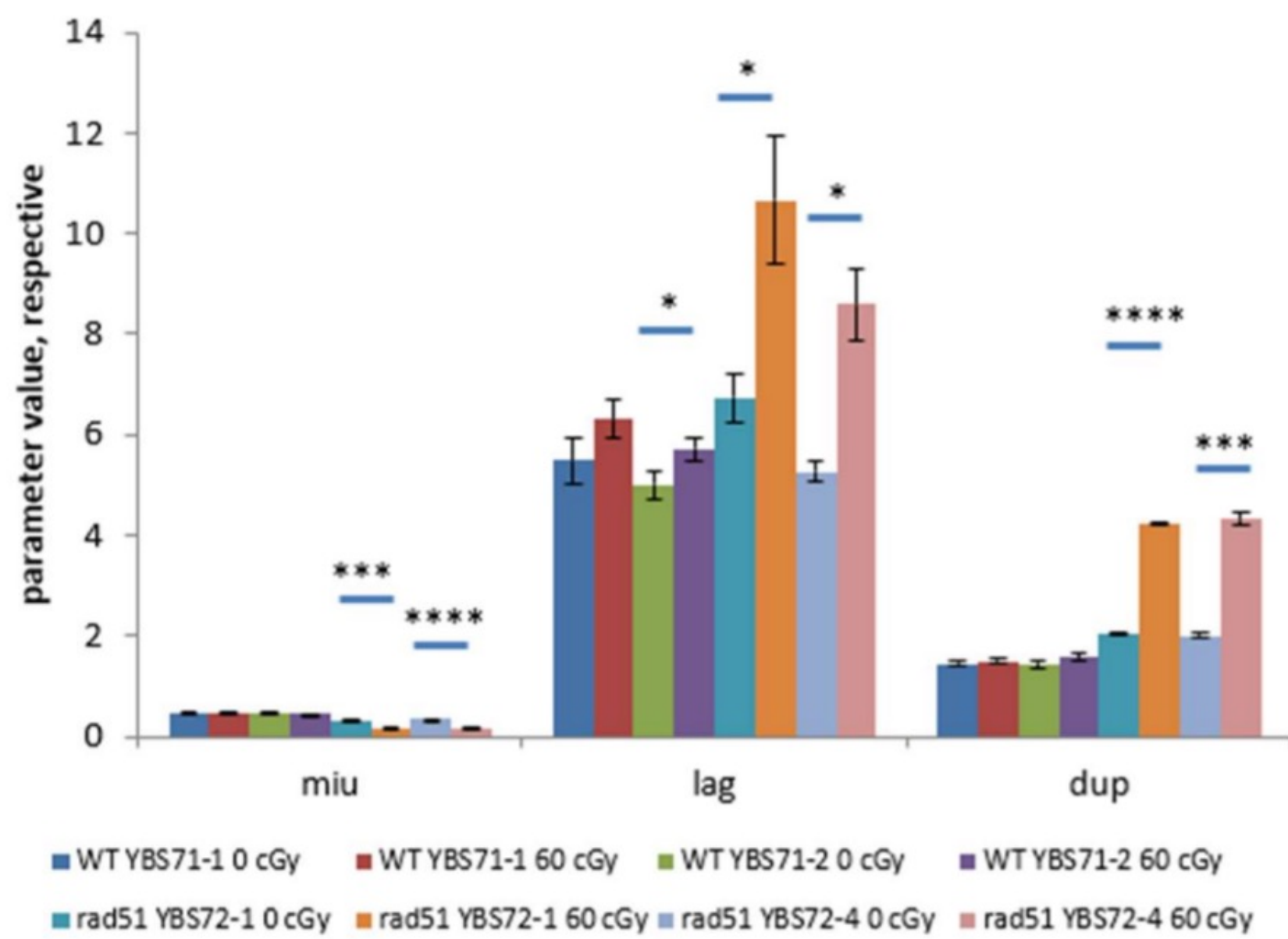


Figure 3: Four wild type wells and four rad51 wells are applied here. Each well represents the average of 3 replicates.

Programs such as these are vital for analyzing BioSentinel data. The main goal of BioSentinel is to determine the effect of IR on budding yeast, to which witnessing it in graph form in an understandable and publishable manner is important. As is obvious from figure 3, the lag and duplication rates for Rad51 wells are far larger than that of wild type, implying that the programs work as intended and will work when BioSentinel data arrives via transponder.

Third Parameter: Time vs alamarBlue Reduction

As mentioned prior, the dye used to identify levels of metabolism in BioSentinel is alamarBlue dye, which does not stain the cells, but is instead mixed with growth media. While blue at first, metabolic activity causes this blue dye to turn pink, then clear, through chemical reduction. Data on metabolic activity through alamarblue dye is detected through two LED lights, one red one at 630 nm to measure blue, and a green LED light at 570nm to measure pink. Metabolic activity is one of the prime indicators of functioning, healthy cells and is thus an important parameter to be recorded in bulk aboard BioSentinel. While simple readings of blue and pink color are present from prior experimentation for testing, prior there was no program present that would efficiently answer the question of how much time it'd take for a certain percentage of blue to be chemical reduced to pink, and from pink to clear. This summer, utilizing data reformatting and features of excel, a program has been created for that specific purpose.

Methods

While VisualBasicCode was not utilized for this task, the Microsoft Excel match and index functions in addition to data reformatting were heavily utilized.

Results

Like the tasks above, the program was a success, allowing one to take any number of wells and showcase how long it would take for alamarBlue to be reduced to a certain point. This is showcased in graph form in figure 4.

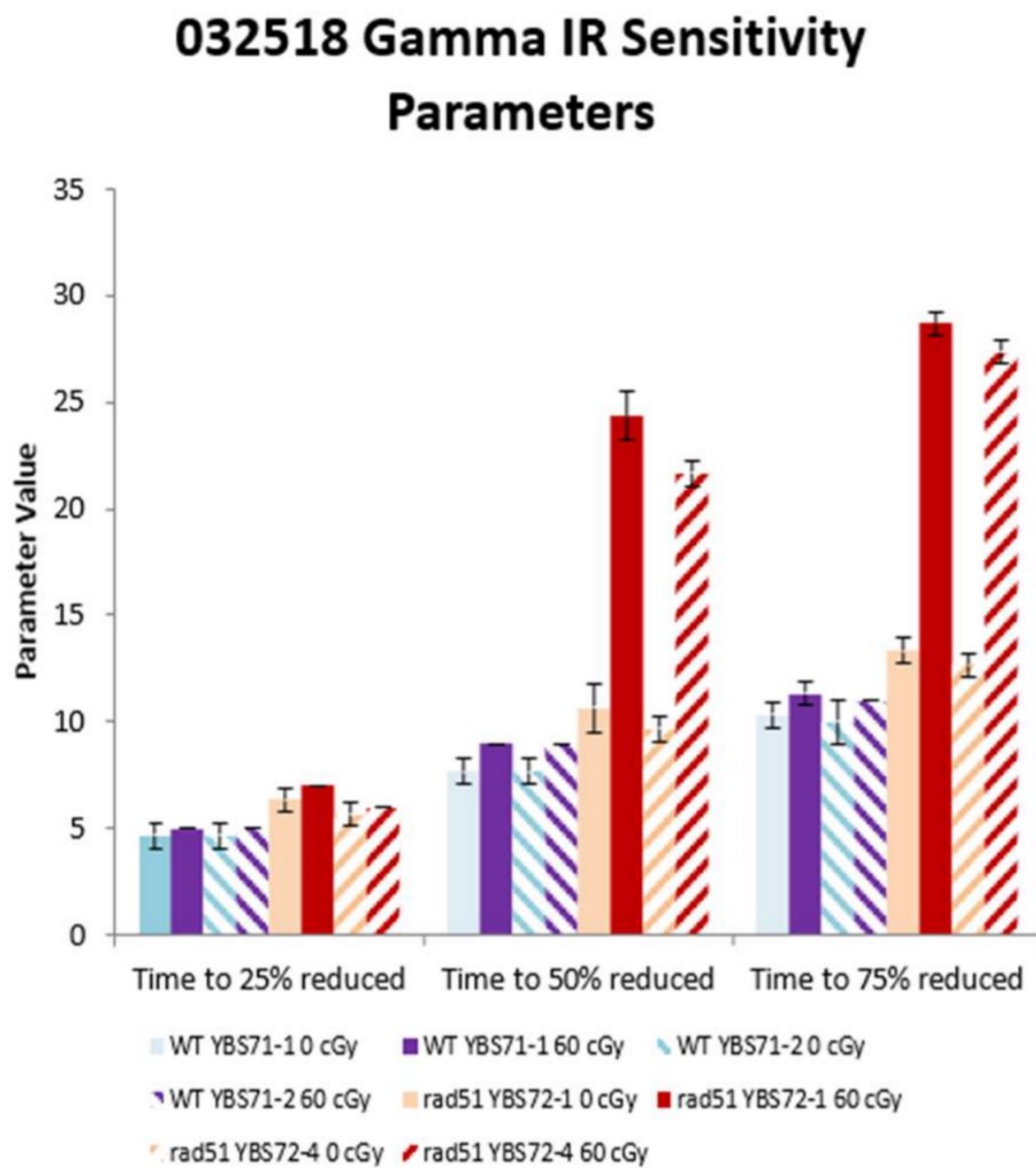


Figure 4: The amount of time is takes for alamarBlue to be 25, 50, and 75 percent reduced to pink form is present above regarding both wild type and rad51 budding yeast wells. Any desired percentage can be calculated with an input into the Excel program. Program from pink to clear is also available, though not shown in figure.

Knowing how long it takes for alamarBlue takes to be reduced to a particular percentage is an important parameter because it gives further information on not just the growth of cells, but their viability as well. After all, even if cells are growing, that does not necessarily mean that their functionality is viable. Furthermore, as is present in figure 4, rad51 duration to reduction are far longer than wild type, thus showcasing that their impairment is indeed present, IR has affected them more negatively, and most of all that the program works successfully as intended.

Conclusion

BioSentinel serves as the first biological experiment performed past LEO since 1972; furthermore, as one of the few secondary payloads aboard Artemis 1, great value is placed on the ability to receive useful data in a timely manner. For this data to be analyzed properly and advance U.S. interests, automated programs, coded and non-coded, are necessary to complete this. Prior to my time as an SLSTP Research Associate, these programs were not present, but now with my skills and resources from the BioSentinel team led by Dr. Sergio Santa Maria, these automated programs that can output large amounts of clear and comprehensive information of scientific value have now been created and successfully implemented, ensuring they will be of great use for NASA and the United States as a whole.

Acknowledgements

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