Assessing the Usability and Accuracy of a Smartphone-Based Microplate Assay Reader Application

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1. PROJECT DESCRIPTION

Traditional laboratory microplate readers are expensive and bulky pieces of equipment that are used to analyse microplate assays. Due to the machine's cost and size, underfunded and isolated laboratories as well as educational institutions often do not have access to these analysis tools limiting the amount and accuracy of the research they can do. Mobile applications have been developed to complement or provide preliminary analysis but unfortunately the accuracy of the applications is often inadequate and the usability of them has not yet been assessed. Assay plates, as pictured in Figure 1, consist of a number of different wells similar to miniature test tubes that are used to measure the presence of a target entity. The microplate reader, also pictured in Figure 1, is the tool used to analyse the assay plates and utilizes absorbance of light through the wells to determine the concentration of the target entity present.

There are two main aspects to this project, a usability aspect and an image processing aspect. Bellairs et al. developed an algorithm for capturing and processing an image of microplate assays [2015]. His method attained concentration estimates which were within 7% accuracy of commercial microtiter plate readers (MTPRs). The first aim of this project is to improve on the algorithm by using alternative colour and image processing techniques to obtain estimates within 3% of the commercial plate reader's measurement. The second aim is to use user centred design techniques to understand the actual user's desired tasks and pathways in order to produce a usable interface. The ultimate goal of the project is to produce a complete smartphone application for imaging microplate assays with a functional, usable interface and high concentration estimate accuracy.

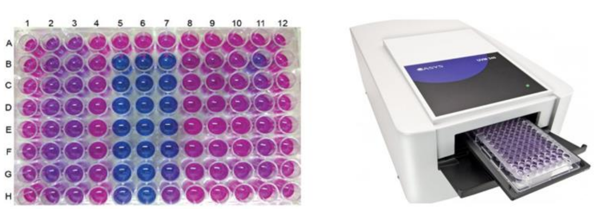

Figure 1. The 96-well assay tray (left) and a laboratory microplate reader (right).

2. PROBLEM STATEMENT

The main aim of this project is to create an Android application for imaging and analysing microplate assays. The application will include a usable, fully featured interface as well as an accurate and efficient imaging and colour estimation algorithm.

There are two main research questions which this project aims to answer. Firstly, can we increase the accuracy of the concentration estimate with alternative colour processing methods? This will be answered by the work done on improving Bellairs et al.'s algorithm [2015]. Secondly, can a user-centred design approach produce an effective and usable microplate reader application? This will be addressed through the design, development and testing of the application interface.

There is a Software Engineering aspect to this project. The application we are developing is targeted at underfunded and/or isolated laboratories as well as students looking to produce preliminarily analyses of microplates. The main focuses of the development are to ensure accurate colour detection and effective usability in order to provide a mobile, cheap and rapid solution for reading microplate assays.

3. RELATED WORK

This project builds on the work previously done by Bellairs et al. [2015]. The techniques that will be used to improve on their work have been found through other similar research outlined here.

Vashist, et al. created a smartphone based colorimetric reader designed to image and analyse microplate assays [2015]. They created a dark box to improve their accuracy. They also made use of a secondary device which was used as an additional light source to illuminate the test tubes from underneath.

They avoided using edge detection because the microplate they used remained constant so the assay wells were always the same distance apart. The dark box they designed ensured that the smartphone was always the same distance away from the microplate. This means the area correlating to the wells in the image will always be in the same place and be the same size.

Vashist et al. used ImageJ to determine pixel intensities [2015, 3]. ImageJ is a Java library designed for image processing [4]. It is capable of reading multiple image file formats, calculating pixel values, editing images and many more advanced features [4]. It is also multithreaded to improve processing performance. The mean intensity of the 40 pixels nearest to the centre of each well was calculated to determine the colour of the well.

The results of this experiment were a coefficient of variance of between 1.7% and 3.7%. In 2 out of the 3 test environments the developed application performed comparatively to a conventional MTPR. These results are exceptional. The paper shows that the extremely expensive and immobile MTPRs can in fact be replaced by smartphones and achieve the same results at a fraction of the cost.

Bellairs et al. followed almost the same method as Vashist, et al. in terms of the dark box used and the secondary source of light [2015]. They showed the value of the dark box in improving the application's accuracy by performing the experiment without the box and recording an error of 30%.

Bellairs et al. created a more versatile application in the sense that it catered to any manufacturer's brand of microplate assay [2015]. This meant the method used by Vashist et al. could not be used as the size of the microplate and the gaps between wells varies from one brand to the next [2015]. In order to detect the position of the wells, Bellairs et al. used the Open CV Circle Hough Transform method [2015]. This method was extremely effective in detecting wells and even performed successfully in the worst case of there being only one well present in the microplate. An additional step was performed in which circles with radii outside two standard-deviations of the mean circle radius were excluded to improve the accuracy by eliminating any detected circles that were not actual wells.

Bellairs et al. also used an area of pixels within the confines of the well in their colour processing but they then compare these to a calibration curve determined by the first row of wells [2015]. The values are then converted to Hue-Saturation values. The well's Hue-Saturation values are linearly interpolated from the calibration Hue curve and the calibration Saturation curve to determine the colour of the well's contents. They only managed to obtain final results within 7% accuracy of commercial MTPRs (Table 1).

Table 1. Results from Bellairs et al. [2015]

Approx red %	% Error in ratio determination		
	Red->Yellow	Red->Green	Red->Blue
90.00	0.66	4.46	5.29
80.00	2.07	6.99	5.81
75.00	2.51	0.94	8.42
65.00	1.60	7.24	7.30
60.00	1.64	4.12	5.86
50.00	3.97	3.42	2.96
40.00	6.98	7.42	1.16
30.00	7.07	25.08	0.67
20.00	5.62	56.18	3.39
10.00	3.63	83.04	7.58
Average	3.57	19.89	4.84
CCC Statistic	0.989	0.252	0.962

Shen, et al. developed a means of measuring pH on paper-based colorimetric immunoassays by quantifying their colours [2012]. They managed to almost perfectly determine the pH values with an R-squared value of 0.9974.

The image processing technique they used involved converting the original RGB images to the CIE 1931 colour space. This was done by obtaining the mean RGB intensities for each region of interest (ROI) and then converting those to chromaticity values. These values could then be used to determine the colour by substituting the values into a calibration plane. The calibration plane was calculated from solutions with known pH levels. The result of the substitution outputs a value on the plane which corresponds to the pH of the test being photographed. This research shows remarkable colorimetric detection by a smartphone and it demonstrates the strengths of the CIE 1931 colour space for detection and colour estimation.

Berg et al. built a highly accurate microplate reader application for a mobile phone [2015]. It uses a 3D printed dark box which houses the smartphone, a 96 well microplate assay, 24 blue LEDs and 96 optical fibres. For the imaging of the microplate the blue light from the LEDs passes through the microplate wells and is collected by the optical fibres which lead to the smartphone camera's lens. Three images at different light intensities are captured. The images are stored on the phone in a RAW 10-bit Digital Negative image format.

The images are sent via the Internet to a cloud server for processing. The images are first converted to tagged file format. The blue channel pixel intensities are then extracted from the image and from this blue channel image the average pixel intensity of each well is calculated using a 15-pixel radius circle around the centre of the wells. The three images from the different intensities are then combined into a high dynamic range image by combining each well's intensity from each image and then normalising. Each well will now have a value between 0 and 1 where 1 represents complete transmittance and 0 represents no transmittance. The final step involves converting the optical density and transmittance values to a quantified index value using a conversion defined by the manufacturer of the assay which is used for the final clinical evaluation. These values are sent back to the phone and displayed to the user in a table.

This application achieved accuracy levels of 99 – 100% for the detection of a number of different illnesses and results were obtained in approximately 1 minute. The accuracy was calculated by comparing their results to those obtained from a commercial MTPR.

Two environments are commonly accepted as appropriate for usability testing. Laboratory testing makes use of a controlled environment where every part of the process is regulated and subjects are recorded. Field studies attempt to simulate the actual environment the tool being tested will be used in including all distractions and interruptions, subjects are also recorded.

In order to understand the motivation for the type of usability environment that will be chosen it is important to understand the differences, strengths and weaknesses between the methodologies. Kjeldskov et al. provides a highly in depth and thorough review of Mobile HCI research and their choice of environment over a number of years [7]. They found without bias that 49% of studies in 2009 used the laboratory approach, 35% of studies opted for the field study approach with the deficit being methodologies that are less prevalent.

Types of usability evaluation techniques must also be described if the choices are to be understood and validated. There are two techniques that are frequently used and were considered for this project. Kjeldskov et al. produced an explorative study of the two approaches namely an expert evaluation (heuristic inspection) and a user-based evaluation (using the think aloud protocol) both conducted from within a laboratory environment [8]. The study found that the heuristic evaluation makes it harder to identify usability problems related to the support for collaborative work and in general fared worse than the think-aloud evaluation. Again these techniques have different advantages and disadvantages and will be discussed later in this proposal.

4. PROCEDURES AND METHODS

4.1 Colour detection

In order to improve on the accuracy of Bellairs et al.'s application two different methods will be implemented and their results compared. Bellairs et al.'s use of a dark box and their use of the Open CV Circle Hough Transform method for well detection will remain unchanged [1].

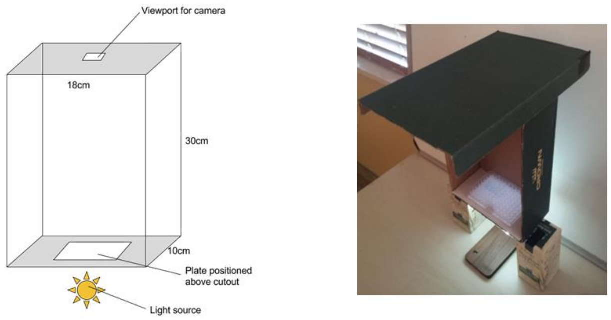

Figure 2. Left: diagram of the box designed to control the microplate lighting and camera. Right: experimental box setup with bottom illumination from a smartphone flash.

The first method for improving the accuracy will be drawing from Vashist et al.'s research [2015]. They used the ImageJ library which offers a large variety of very powerful features which they used very successfully for determining colour.

The second method is one used in Shen et al.'s research [2012]. They converted the RGB images into the CIE 1931 colour space in order to obtain chromaticity values for each colour. These values are then compared to a calibration plane to determine the colour. They achieved very accurate results using this method.

In order to evaluate the accuracy of the results from the two methods they will both be used to image and analyse a picture of a real-world microplate assay prepared by a student in the chemistry department. The same microplate assay will be analysed using a commercial MTPR. The various colour concentration readings will be compared and analysed in order to evaluate the success of the colour detection.

4.2 Usability

To create a usable and effective user interface this project will take on a twofold user centred approach. Firstly, the user's desired tasks and current operations will be investigated using a task analysis and their personas and traits will be identified using a contextual inquiry. Once all the details surrounding the user have been learnt, a prototype design will be mocked up and the second part of the approach will take place. This phase will see the actual users experiment with the design to give initial thoughts into the design and possible improvements. The design will then be iterated on and enhanced with the users constantly being queried for best practices and existing conventions that can be worked into the design.

A laboratory environment will be used with a user based think aloud usability evaluation in order to establish the effectiveness and viability of the application for use in real scenarios.

The laboratory environment will be set up monitoring the user's interaction with the application as they perform the various tasks outlined by the task analysis. Upon completion of the tasks the users will be given the System Usability Scale (SUS) questionnaire and asked to fill in their answers. The SUS questionnaire will be used to evaluate the application's perceived usability and is favoured over other evaluation questionnaires as it is free, short, used in the majority of usability studies and considered highly reliable [9].

After all users have been tested the data will be compiled and analysed in order to produce both quantitative and qualitative results. The quantitative data gathered in the laboratory test session will be used to compute the usability metrics defined in the ISO 9241-11 standard namely effectiveness, efficiency and satisfaction. Qualitative results will also be gathered from the recordings and observations within the session to indicate potential problems experienced. Finally, the SUS questionnaire will be analysed to produce a final usability score.

Findings and possible recommendations based on the results will be discussed and future work outlined.

The final system developed during the course of this project will be a fully functional, usable smartphone microplate assay reader. It will be capable of photographing microplate assays, processing the images and accurately estimating the colours in each well. The interface will be usable and intuitive with appropriate affordances and interface metaphors. The application will effectively deliver and present the results of the image analysis to the user. It will be easy to set up and require little experience to quickly and accurately obtain results.

4.3 Expected Challenges

The challenges we expect to face in this project will be our lack of image processing experience or knowledge. Neither member of the team has ever done any work in image processing. We have very limited chemistry knowledge although the knowledge we require for the project can be quickly and easily learnt from a chemistry student. The image processing technique used must be able to run on a smartphone and not be so resource intensive that it negatively affects the phone's performance. Lastly, the implementation of two separate image processing techniques may prove to be too time consuming and one method may need to be abandoned to ensure no deliverables are late.

5. EHICAL, PROFESSIONAL & LEGAL ISSUES

There are limited ethical and legal factors affecting this project however one main concern will be acquiring ethical clearance for conducting usability tests on the Chemistry students. The process can be kept within the Computer Science department and will be processed swiftly given the relevant forms are filled out correctly. There are no legal or ethical considerations in the medical aspect of this project as this research makes use of anonymized assay plate samples provided by the Chemistry department and does not put anyone at risk.

6. ANTICIPATED OUTCOMES

6.1 System

What we aim to get out of this project is a fully functional Android microplate reader application that caters for all the intended user's tasks and desired pathways. The application aims to be within an appropriate accuracy threshold and an improvement on the work from which this application is based. The final product also aims to be usable and effective for users as a user centred design process will be followed.

6.2 Expected project impact

Upon completion of this project we aim to have produced a commercially viable product that can complement traditional microplate readers and more importantly increase the research capabilities of underfunded and/or isolated laboratories as the developed mobile solution will be useful for preliminary assay plate analysis. Additionally, this product could provide a tool for educational institutions to demonstrate certain life sciences operations.

6.3 Key success factors

- Accuracy will have to be within a certain threshold (3%) which will depend on leveraging the correct tools and utilizing the correct techniques
- Effective task analysis, contextual inquiry and resulting interface design will have to be done if the application is to be considered usable. The total average SUS score of the final usability test is the metric that will be used to determine this project's success. An above average score of over 64 will indicate the application is widely considered as usable and effective.

6.4 Commercialization

The best outcome for this project involves the final product being a commercially viable application. This application could be released on the Android Play store enabling laboratories, students and educational institutes to easily download the application. This availability will provide these users with enhanced research capabilities and a tool that demonstrates an essential life sciences tool.

7. PROJECT PLAN

7.1 Risks

There are a number of potential risks with the majority revolving around time and tight deadlines. Acquiring chemistry students for contextual inquires and usability testing could take an extended amount of time as will be obtaining microplate readings and samples from the chemistry department. The above dependencies and risks will be mitigated by maintaining frequent communication with contacts within the Chemistry department. Refer to Appendix B for a more detailed risk matrix.

7.2 Timeline

The timeline is displayed as a Gantt Chart in Appendix A

7.3 Resources Required

In order to enable us to get within the targeted accuracy range we will require two Google Nexus 5Xs. These will provide us with high quality 12MP cameras with enhanced light sensitivity that will boost the chances of success. The phones are also running Android OS which provides us with improved flexibility and control over the phones sensors and interfaces. As this project is building on previous research we will also utilize the previously used DIY dark box to improve accuracy and make use of the OpenCV and ImageJ libraries for the image processing. In order to develop the application Android Studio IDE and the Android SDK will be used.

7.4 Deliverables

- Prototype of application user interface utilizing existing SDK
- Functioning, accurate image processing software
- Fully implemented and working interface
- Final complete application with all features and image processing integration

The first deliverable we will be producing for the project is an initial prototype. The prototype will include the first iteration of the application's interface integrated with the first stage of the backend implementation which will have a skeleton code base consisting of dummy method calls for all the intended features. This version will be used for the initial software feasibility demonstration.

The second deliverable will be an alpha stage version of the application. It will have a revised interface based on an evaluation of the initial design. The back end will have the well detection algorithm implemented and the better performing colour detection algorithm.

After the first round of testing, the beta stage version of the application will be produced. This will include an improved user interface based on the test results and will be almost identical to the final version. The back-end will be modified according to the test results. This version will undergo another round of testing.

The final software deliverable will be the completed, tested application including any final tweaks required as a result of the second round of testing.

Two fully fledged research papers will be produced outlining the process undertaken by each member of the project team and their respective results. Other deliverables include a poster describing the project and a web page.

7.5 Milestones

- Complete knowledge of current microplate reading process and user's desired pathways
- Initial application design with required features
- Complete development and testing of two colour processing algorithms
- Initial interface design evaluated
- Integration of interface and colour detection complete
- Final testing complete
- Final adjustments done and application complete

7.6 Work Allocation

Shaun Maxwell will be responsible for developing the back end software for the application. He will develop the image processing software, the colour estimation algorithm and any other features that may become necessary during the course of the project.

James Foster will be responsible for designing and developing the front end for the application. He will create and test the user interface and any other visual requirements for the application as well as conduct usability tests.

8. REFERENCES

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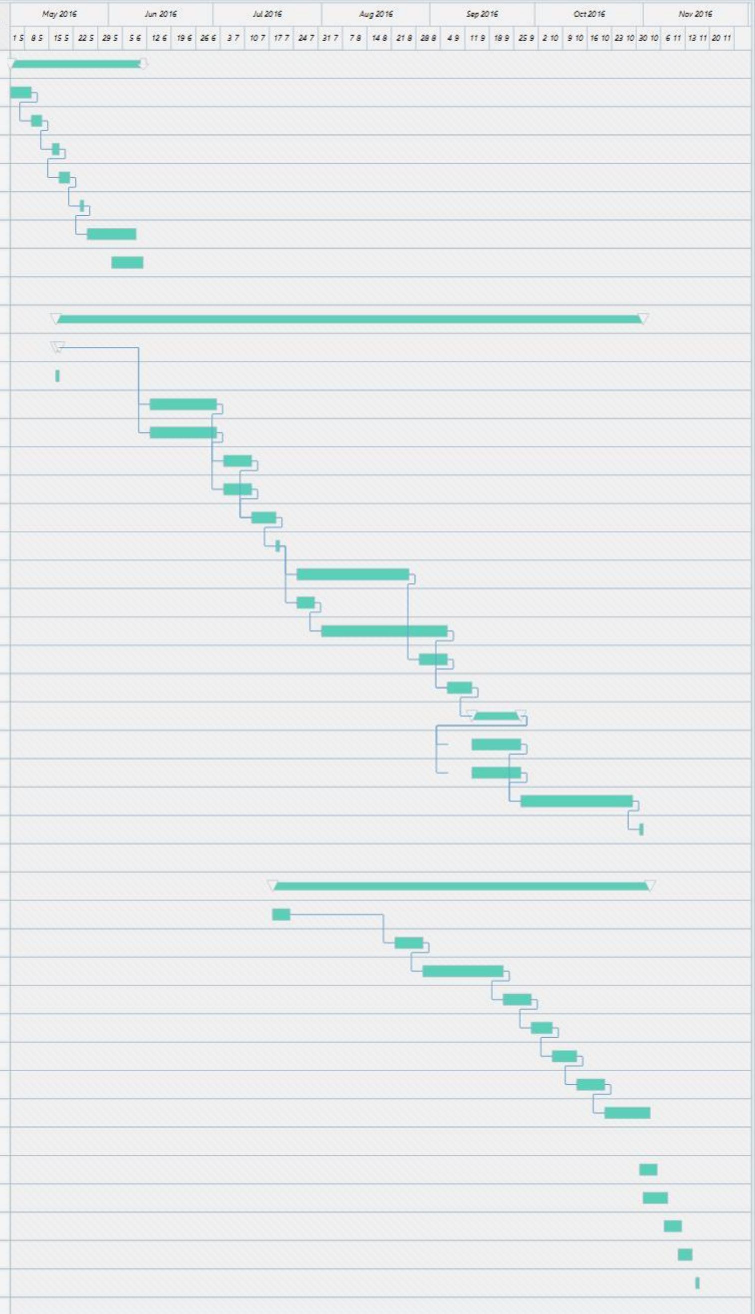
Appendix A

ID	Task Name	Start	Finish	Duration	Actual Duration
1	**Project Proposal**	**04 May 2016**	**10 Jun 2016**	**28d**	**10d**
2	Scaffold	04 May 2016	09 May 2016	4d	1d
3	First Draft	10 May 2016	12 May 2016	3d	1d
4	Final Handin	16 May 2016	17 May 2016	2d	1d
5	Proposal Slideshow	18 May 2016	20 May 2016	3d	1d
6	Presentation	24 May 2016	24 May 2016	1d	1d
7	Revise Project Proposal	26 May 2016	08 Jun 2016	10d	1d
8	Web Presence	02 Jun 2016	10 Jun 2016	7d	1d
9					
10	**Application Development**	**17 May 2016**	**31 Oct 2016**	**120d**	**1d**
11	**Microplate Reader Demonstration**	**17 May 2016**	**17 May 2016**	**1d**	**1d**
12	Contextual Inquiry & Task Analysis	17 May 2016	17 May 2016	1d	1d
13	Compile and Investigate Existing Code	13 Jun 2016	01 Jul 2016	15d	1d
14	Initial Interface Design	13 Jun 2016	01 Jul 2016	15d	1d
15	Create Dummy Method Calls	04 Jul 2016	11 Jul 2016	6d	1d
16	Build Initial Interface	04 Jul 2016	11 Jul 2016	6d	1d
17	Integrate Dummy Methods with Interface	12 Jul 2016	18 Jul 2016	5d	1d
18	Initial Software Feasibility Demonstration	19 Jul 2016	19 Jul 2016	1d	1d
19	Develop Colour Processing Algorithms	25 Jul 2016	25 Aug 2016	24d	1d
20	Evaluate Initial Interface Design	25 Jul 2016	29 Jul 2016	5d	1d
21	Implement Second Interface Iteration	01 Aug 2016	05 Sep 2016	26d	1d
22	Test Colour Processing and Select Best Performer	29 Aug 2016	05 Sep 2016	6d	1d
23	Integrate Interface with Colour Processing	06 Sep 2016	12 Sep 2016	5d	1d
24	**Test Full Application**	**13 Sep 2016**	**26 Sep 2016**	**10d**	**1d**
25	Test Interface	13 Sep 2016	26 Sep 2016	10d	1d
26	Test Accuracy	13 Sep 2016	26 Sep 2016	10d	1d
27	Implement Any Necessary Changes	27 Sep 2016	28 Oct 2016	24d	1d
28	Final Code Handin	31 Oct 2016	31 Oct 2016	1d	1d
29					
30	**Project Report**	**18 Jul 2016**	**02 Nov 2016**	**78d**	**1d**
31	Background/Theory Section	18 Jul 2016	22 Jul 2016	5d	1d
32	Scaffold	22 Aug 2016	29 Aug 2016	6d	1d
33	First Implementation/Experiment/ Performance Test + Writeup	30 Aug 2016	21 Sep 2016	17d	1d
34	Final Prototype/Experiment/ Performance Test + Writeup	22 Sep 2016	29 Sep 2016	6d	1d
35	Implementation and Testing completed.	30 Sep 2016	05 Oct 2016	4d	1d
36	Outline of complete paper	06 Oct 2016	12 Oct 2016	5d	1d
37	Final Draft	13 Oct 2016	20 Oct 2016	6d	1d
38	Final Paper Handin	21 Oct 2016	02 Nov 2016	9d	1d
39					
40	Final Project Demonstration	31 Oct 2016	04 Nov 2016	5d	1d
41	Poster	01 Nov 2016	07 Nov 2016	5d	1d
42	Web Page	07 Nov 2016	11 Nov 2016	5d	1d
43	Reflection Paper	11 Nov 2016	14 Nov 2016	2d	1d
44	Open Evening	16 Nov 2016	16 Nov 2016	1d	1d
45					



Appendix B

Number	Risk	Consequence	Impact (1-10)	Probability (1-10)	Mitigation
1	Limited participation of chemistry students	Insufficient testing leading to an ineffective interface and limited understanding of features required	7	6	Frequent communication with the chemistry department and supervisor providing the partcipating students
2	Unable to aquire sample plates	Unrealistic colour detection tests	3	3	Frequent communication with the department and supervisor providing the samples
3	Team member drops out	Scheduled milestones and deadlines could be missed and dependencies will mean project may not be able to be fully completed	8	2	Reduction of dependencies using the previous years work as a substitute for the missing team members section.
4	Internal conflict between team members	Communication breakdown resulting in incorrect features being implemented and an increased workload	3	4	Respecting the other team member and value their contribution to the project
5	Underestimting time needed to complete tasks	Tasks take too long and deadlines are missed leading to an incomplete project or missing functionality	7	4	Correct and accurate time estimation during the planning phases and frequent checks to ensure project is on track.
6	Gold plating	Actual necessary functionality will not be able to be implemented	8	3	Ensure core features are implemented first and additional features are added only if time allows
7	Misunderstanding regarding types of features desired by users	Developed application does not solve the problem faced by the user rendering the soluition inappropriate	7	2	Ensure a full and thourough task analysis is done. Further tests utalizing the users will also make sure the correct features are developed
8	Scope creep	Enlarged scope results in inability to complete the core of the project leading to inaccurate tests and a partially implemented final solution	6	4	Regular meetings with team members and project supervisors to maintain a common project vision and appropriate scope
9	Cant improve colour accuracy unit	Inaccurate final product reducing potential for use In real life scenarios	7	4	Previous years project will be available for a substitute but otherwise there is no solution