Surveillance of dairy animals using a smartphone-based system

DESIGN DOCUMENT

DEC1616
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1 Introduction

1.1 PROJECT STATEMENT

We want to use the smart phone to precede the fluorescence polarization assay kit. Fluorescence polarization techniques are particularly useful for inter-molecular interactions. This method is direct and immediate detection of the bound tracer molecules / free rate. Considering smart phone is popular in these years. Our project will make a new device to replace traditional spectroscopic instruments. We want use CCD camera in smart phone to replace the detector (PMT) to assay the result. In order to reach the purpose for our project. We must to design the new equipment. Different software will be used to design the project. For the first part: Xin Tong studies the X-Code to development the APP for the iPhone, which can provide the picture data for the fit. For the second part: Tianqi Luo learns about Fluorescence polarization assay and Lei Wei and Di Zhao design the optical structure.

1.2 PURPOSE

Fluorescence polarization plays an increasingly important role in many areas of life science. Most hospital use FP assay C-reactive protein to discover disease and follow up the disease. Fluorescence polarization is more safe and reliable, which does not pose a threat to the researchers, it does not produce radioactive waste is difficult to handle. In addition, FP required less sample, high sensitivity, good reproducibility and easy operation. In our project we want use advantage of fluorescence polarization without the inconvenient traditional spectroscopic instruments. Considering a lot of factors. We want to use the smart phone to replace detector (PMT). Because smart phone is popular today and CCD camera can achieve the same function as photomultiplier. Our project uses the advantage of fluorescence polarization and convenience of smart phone, which will be more easily to make medicine assay, blood assay and many areas. So our project will make a big effect in many areas of life science.

1.3 GOALS

For this senior design project has different parts. For the final goal we want to design a device and application for iPhone. Our new device can use iPhone to make a fluorescence polarization assay. So our project will be more easily to make medicine

assay, blood assay and many areas than traditional spectroscopic instruments. As a list for our parts' goals:

(1) Designing the correct optical model to allow the laser attach the destination by polarization and reflection.

(2) Using Solidwork to design the structure to hold the optical units and smartphones.

(3) Developing an application for iPhone and use iPhone's camera to read the data.

(4) Using the achieved data to analyze by fluorescence polarization assay.

2 Deliverables

The advantage of our project is that uses CCD camera to replace the traditional detector (PMT). In order to finish our project, we must make a new device. There is our to-do list:

(1) Using the Code V to design the optical simulation in the computer. If the optical simulation can success.

(2) We will use the Solidwork to design the structure to hold the optical component and iPhone. Then we will send our Solidwork file to 3D print and get real structure.

(3) Developing app for iPhone will provide necessary data from sample for people to assay.

(4) Assaying data by fluorescence polarization method.

So the optical design, app development, Solidwork design and fluorescence polarization assay are all the most important parts for our project. Just because every part is important and independent. So every goals are necessary for our project. We only finish every goals step by step which will accomplish the final goals to make a perfect device and app for medicine assay, blood assay and other life sciences.

3 Design

Include any/all possible methods of approach to solving the problem. Discuss what you have done so far. What have you tried/implemented/tested etc. We want to know what you have done.

3.1 SYSTEM SPECIFICATIONS

3.1.1 Non-functional

a. We need to adjust the lens and mirrors to get the images. We not only need to know how the camera which in the smart phone worked, but also need to know how to operate which is the exact same way for changing the attributes of an image.

b. We need to know what polarization is, and make sure that we can let others know what it is. We have three EE students, and one of us will explain to others clearly about what the polarization is so clients would know what effect such analysis can create.

c. People need to provide their email addresses, and then we can send the analysis information of the image to them. The analysis data could only be collected by sending emails; otherwise, when starting the analysis, the previous one will be deleted.

3.1.2 Functional

a. First part is the mechanical part. We want to use the camera and lens, which are in our smartphone. The phone's camera can collect the image accurately, which the essence is to hold the position of the phone well.

b. Second part is embedded optical part; we can see the reflection dimension of the object clearly from the smartphone camera. The dimension of the object is reflected by two general glasses mirror, and we need to add some lens to collect the lights.

c. Another part is application part, during scanning. We can analyze the polarization intensity from the line-chart by clicking on any two spot of the image. Polarization is calculated by the special formula, we programmed it into the app.

d. When analyzing the image, we can see the object's horizontal and side image, as the original object is 3-dimensional. Since the screen is a 2-dimensional, we need to decompose the 3-dimensional real object into 2-dimensional

3.2 PROPOSED DESIGN/METHOD

We hope that we can analyze the data by using our mobile phone. We can analyze their wavelength and bands. Through repeated comparison and testing, we can collect the accurate data. We believe that our people can use their phones to test their blood samples in the future. Humans can detect their blood samples by using any mobile device.

The whole design is divided into four parts. The first part is the theory, we studied many papers to determine use which kind of method to excite fluorescent molecules in our sample. The second part is the optical design, with the guidance of our professor, we try a variety of optical design. We try to make the model as small as possible. After we finished optical design, we began the third part the 3D printer. We can make our model can match our smart phone or other device. The last part is the development of APP. It allows mobile devices to detect a variety of samples.

A fluorescence anisotropy measurement system holder can be designed and printed with 3D printing technique. In the holder setup, a laser is used as the light source and it emits light to the sample to produce fluorescence. A polarizer is right next to the laser from which the light can be fully polarized in one direction. A cuvette that contains sample is placed towards laser behind the polarizer in a slot and a convex lens that works to collimate light to improve light efficiency is placed on the side of the sample slot. A beam cube splitter is exploited to split the light polarization in two opposed directions as well as two mirrors are used to obtain the resulting light after polarization. In addition, a filter is placed at another side of the holder to get rid of the excess light from the laser. 3D printing is a perfect method to possibly fabricate such an imaginary item that is one of the core parts of this research. The material used to print is a kind of strong & flexible plastic and it was blackening afterwards.

3.3 DESIGN ANALYSIS

Here, we implement a system that is capable of measuring the full emission spectra of any light emitter (chemical fluorophore or quantum dot) and is thus capable of differentiating a broad range of tags. We demonstrate a simple interface to a conventional smartphone that enables its internal camera to function as a high resolution and sensitive fluorescence spectrometer. By placing a transmission diffraction grating directly in front of the camera, along with optics for collimating light emitted from a liquid fluorescent sample onto the grating, an emission spectrum is distributed across the pixels of a complementary metal-oxide semiconductor (CMOS) image sensor with a single-pixel wavelength increment of 0.338 nm/pixel. We demonstrate that the smartphone fluorimeter is capable of performing a sensitive molecular- beacon FRET assay for a specific microRNA sequence with performance that is better than a conventional laboratory fluorimeter, with a detection limit, the lowest measured concentration that has an intensity value greater than three standard deviations above the negative control value, of 10 pM. Our results show that smartphone-based spectroscopic fluorimeters is a route toward portable bimolecular assays for viral/bacterial pathogens, disease biomarkers, and toxins, and this general-purpose approach may be extended to other photon-producing assay platforms such as FP, chemo luminescence, and fluorescent-tagged sandwich assays.

4 Testing/Development

Briefly to say, this app is going to measure the RGB values and calculate the intensity collected from the camera view of the two samples. The value is dynamically changed when touching and move.

Based on the studying of the disadvantages of the old version app, we have known the rectangle based capture tool is not convenience in user case. More over, the capture tool bounds the edge of the view, so sometimes the samples are not within the capture

tool. To fix this, we create the new version of app that uses two cross capture tool. The mid of the cross is the measure spot, so we take the RGB values within the spot.

5 Results

For the whole summer and this semester Tianqi Luo test the kit from MRC also test from our set up, then compare these two data, we can see two different spots in the sensor, and for this semester he uses the different concentration kit to test.

Le Wei is design the whole optical model. It also has three generations for his design, Le Wei uses the Code V to design the model and have already to make the four different versions.

According to the Wei Le's data, Di Zhao made her structure on Solidworks well, she has three generations of holder design, we also use the 3D printer to printed out every generations holder.

Xin has successfully run the code onto his device, but the modification task is just starting. Based on the speed of development. But his APP develop coding has some problem, so he should make more process for the coding.

6 Conclusions

Continuing from last semester, we are successfully to do the project expect the APP develop, at the beginning of this project we all think that it is more challenge for us, and we pay more effort on every week's tasks and make sure every concept in our project we totally understand. From this project we learned new knowledge, we used more software to solve the new problem and we actually solve the problem, we knew that what is the it is pretty cool for us to do this project, our advisor Dr. Meng Lv and our group leader helps us a lot, if we met some problems that during the process, we will send the email to our group mentor or advisor. The meeting time with advisor holds on Monday and Sunday every week, our team meeting holds on three times each week. After finished the meeting we should write the TO-DO list and send the advisor to check.

The beneficial of this project in the future that is the APP will be appear on the market, it should be make contribution to the field of medicine and life science because through the APP it can easily to get the data to analyze, small molecules substances such as drugs content in the sample.

From our undergraduate senior design, we learned a lot, we are very successfully to finished it, for these senior we realized that the book study it different than research, work with perseverance it is most important in the research, we had a lot fun during our undergraduate senior design process.

7 References

List any references used in the document.

Reference for paper:
1>. (*Smartphone Fluorescence Spectroscopy*)
2>. (*Pierce® Fluorescent Protease Assay Kit*)
3>. (*Fluorescence Polarization in Life Science*)
References for videos (YouTube)
4>. *SolidWorks Tutorials*
http://www.youtube.com/watch?v=cy3ExIAcI2Y (part 1/3)
https://www.youtube.com/watch?v=ll_9D6J2yTo (part 2/3)
https://www.youtube.com/watch?v=ofYL-lCrEv4 (part 3/3)
5> **Code V Study:**
Code V Optical Design Software:
https://www.youtube.com/watch?v=6-wIkoiwvXo
https://optics.synopsys.com/codev/?gclid=CICXjtu6hMsCFQgxaQod01ILJg
https://optics.synopsys.com/learn/learn-student-license.html
6> **optical design**
https://lightmachinery.com/optical-design-center/
http://www.zemax.com/
http://www.atmos-software.it/Atmos.html
7> **Fluorescence Polarization assay kit**
https://www.youtube.com/watch?v=HiG6KWkkrV4
https://www.youtube.com/watch?v=OdBNVrPvJMY

8 Appendices

Appendix I

Materials and reagents

Rhodamine 6G (R6G) and Coumarin 540A were purchased from Exciting, Inc.. Both R6G and Coumarin 540 were dissolved in methanol and mixed with glycerol (Thermos Fisher Scientific Inc.). Reagents of PGE2 FPIA kit was purchased from Enzo Life Sciences, Inc. (Catalog # ADI-920-001). The kit contains PGE2 FPIA Antibody (Monoclonal antibody to PGE2), Assay Buffer 1 Concentrate (Tries buffered saline containing proteins and sodium aside as preservative), PGE2 FPIA Conjugate Concentrate (Fluorescein conjugate to PGE2) and PGE2 Standard (1,000,000pg/ml PGE2).

FP detection apparatus

A fluorescence anisotropy measurement system holder can be designed and printed with 3D printing technique. In the holder setup, a laser is used as the light source and it emits light to the sample to produce fluorescence. A polarizer is right next to the laser from which the light can be fully polarized in one direction. A cuvette that contains sample is placed towards laser behind the polarizer in a slot and a convex lens that works to collimate light to improve light efficiency is placed on the side of the sample slot. A beam cube splitter is exploited to split the light polarization in two opposed directions as well as two mirrors are used to obtain the resulting light after polarization. In addition, a filter is placed at another side of the holder to get rid of the excess light from the laser. 3D printing is a perfect method to possibly fabricate such an imaginary item that is one of the core parts of this research. The material used to print is a kind of strong & flexible plastic and it was blackening afterwards.

Fluorescence anisotropic tests

In fluorescence, it is already known that a molecule will get excited to a higher energy state due to the absorption of a photon and get down to a lower state by emitting some photon and losing some energy as heat. Thus, a necessary condition of excitation would be having the electric field oriented in a particular axis about the molecule from the light. The polarization from the emitted photon is unique due to this reason.

Preparation of FPIA for PGE2

Prostaglandin E2: One of the prostaglandins, a group of hormone-like substances that participate in a wide range of body functions such as the contraction and relaxation of smooth muscle, the dilation and constriction of blood vessels, control of blood pressure, and modulation of inflammation. Prostaglandin E2 (PGE-2) is released by blood vessel walls in response to infection or inflammation that acts on the brain to induce fever. Thus, it has been studied as a potential disease biomarker, and an efficient and simple assessment method of miR-21 would be useful for diagnostics and identifying therapeutic targets.

Prostaglandin E2 (PGE2) is formed in a variety of cells from PGH2, which itself is synthesized from arachidonic acid by the enzyme prostaglandin synthetase1-4. It has been proven that the PGE2 has many biological actions such as vasodilation, both anti- and

action, modulation of sleep/wake cycles, and facilitation of the replication of human immunodeficiency virus. In addition, it increases CAMP levels, stimulates bone resorption, and has thermoregulatory effects as well. It has been shown to be a regulator of sodium excretion and renal hemodynamics. By combining the antibody with the PGE2, with the size of molecule of the sample will getting larger, its rotation speed is decreasing, consequently, the polarization will be significantly different than before.

Image processing

When an ordinary digital photo is taken, the original image is divided into a grid as it is captured by the CMOS sensor. Pixels (the smallest resolvable grid spacing) are normally arranged in a two-dimensional grid, and each pixel of an image is a composite of three subpixels representing the red, green, and blue primary colors. The color information on each pixel is stored in 3 different multilayers of RGB color space. This results in a 2592 × 1936 × 3 matrix whose elements are real numbers ranging from 0 to 255 (8-bit number) for each digital color photo. However, when the camera is operated as a photodetector, we are only concerned with the total intensity gathered by all the subpixels because the hue information is spread over the screen as a color spectrum. Transformation from primary RGB colors to a HSV (Hue- Saturation-Value) color map was performed to provide a photon intensity of each pixel. HSV is a cylindrical coordinate representation of points in a RGB color space, where the V axis represents brightness of a corresponding color determined by V = Max (R, G, B). The values from our RGB image were converted into a V image in a 2592 × 1936 × 1 matrix of pixels.

Appendix II

Version 1 as the fig.1 below Version 2 as the fig.2 show, and the final Version as the fig.3 Shows.

Comparison:

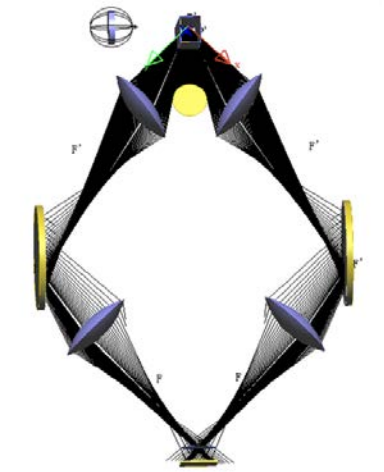
Compare to our first generation we add a prism there to separate the lights but the whole system is still too complex. Thus, in our third generation, we delete all the lens after the prism. We just use a small convex lens to gathering the lights before the prism the whole system will looks very simple and clear.

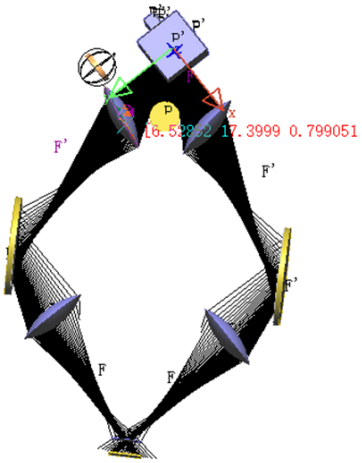
Parameters:

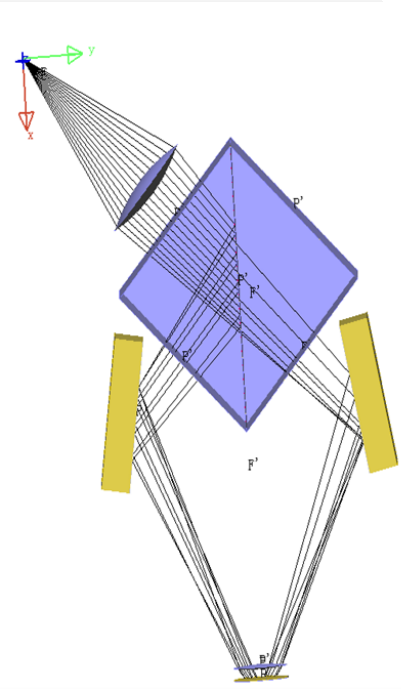
lens: diameter is 12.5mm. focal length is 16mm.

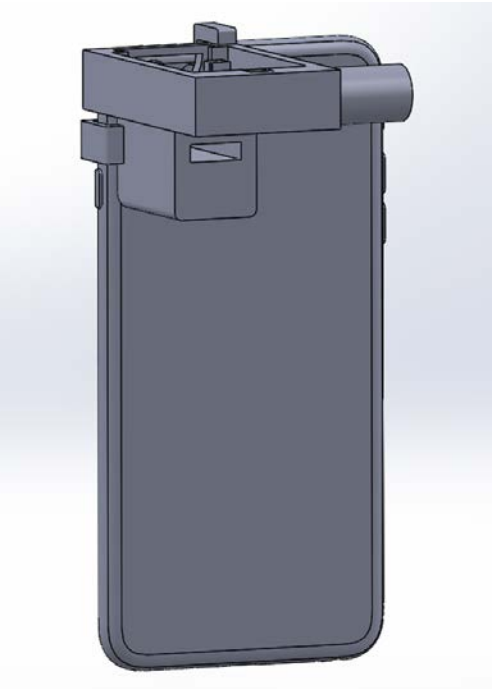
sample: 5mm width and 20mm height.

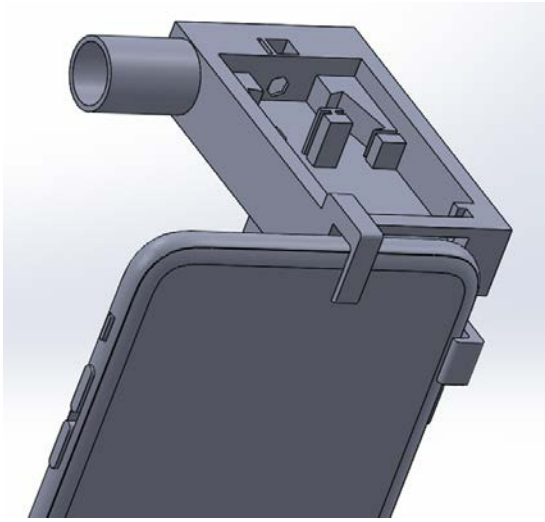
Whole device: 60mm width and 80mm length.




16.538 17.3999 0.799051

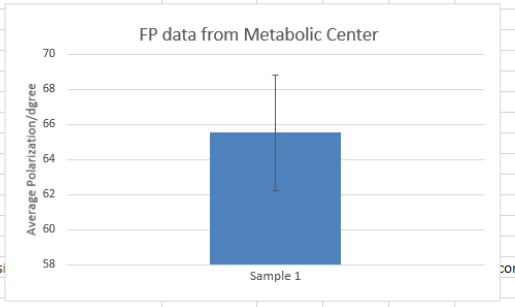


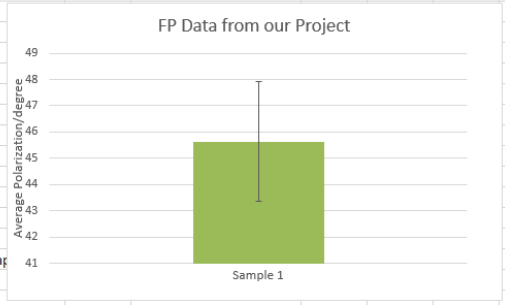




FP Test			1st TEST	2nd Test	3rd Test	4th Test	Average
Sample 1	Whole R6G	Sample 1	-1.63282	-0.71586	0.76097	2.00697	0.104815
Sample 2	R6G : Glycerol 4:1	Sample 2	4.72059	7.54502	9.95417	6.24277	7.115638
Sample 3	R6G : Glycerol 1:1	Sample 3	13.54107	23.80695	22.88187	28.12515	22.08876
Sample 4	R6G : Glycerol 1:2	Sample 4	62.79327	64.8936	65.74705	68.64647	65.5201
		Sample 4	65.5201				
		Sample 4	45.63				

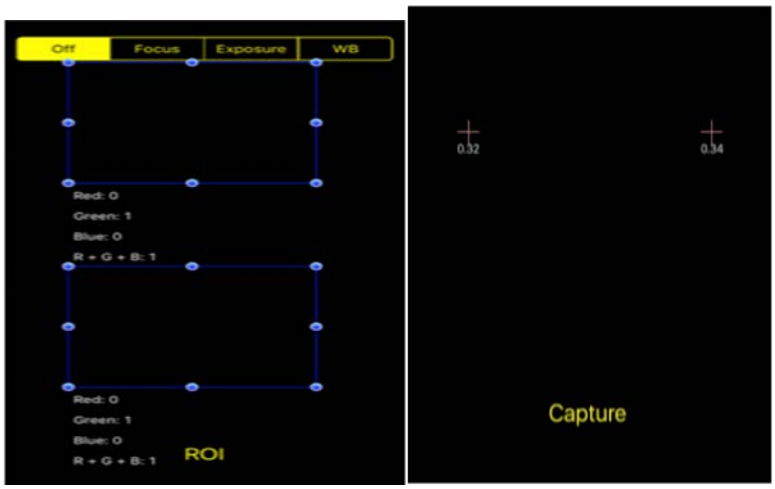
Parallel Intensity	1978	2052	2045	2037
Parallel Intensity	1890	1959	1980	1931
Parallel Intensity	497	521	500	520
Parallel Intensity	1838	1821	1709	1710
Perpendicular Intensity	1984.47	2054.94	2041.89	2028.84
Perpendicular Intensity	1872.24	1929.66	1940.97	1907.04
Perpendicular Intensity	483.72	496.77	477.63	491.55
Perpendicular Intensity	1620.81	1599.06	1498.14	1490.31





To program the calculation, we iterate through all the RGB values within the measure spot. Then we can figure out intensity of light parallel and perpendicular. Finally, we use the theorem from FP to calculate the polarization.

Indicators from the old version app that uses resizable rectangle. Below is the new version of the app that uses cross indicators.



Appendix III

Real time testing rigorousness: we test the device at ECPE lab. The process to fill in sample liquid to application measurement has to be very careful. Wearing gloves and take slight action to fill the liquid is the fundamental to be in a research lab. This is what I have learned that would affect my future.

Continuous design issue: in real time design, we have to engage infinite issues. Even though we could update our scenario to make design comparatively better, new issues will appear as well. For example: to change the indicators from resizable rectangle to cross indicators, we know this plan will ease for the users and gain more precise data, but the process to change, which in developer area, is to break the codes and add the new one, will take a long time.

Update knowledge in lifetime: as we have mention on presentation, FP is kept updating currently, so as developers, keep learning and updating the knowledge, questing and discovering new stuff is very important for preventing self from culling out in the future.

Appendix IV

```
//changing shape, TODO:
- (void)drawRect:(CGRect)rect {
    CGContextRef context = UIGraphicsGetCurrentContext();
    CGContextSaveGState(context);

    // (1) Draw the bounding box.
    CGContextSetLineWidth(context, 1.0);
    CGContextSetStrokeColorWithColor(context, [UIColor blueColor].CGColor);
    CGContextAddRect(context, CGRectInset(self.bounds, kSPUserResizableViewInteractiveBorderSize/2,
        kSPUserResizableViewInteractiveBorderSize/2));
    CGContextStrokePath(context);

    // (2) Calculate the bounding boxes for each of the anchor points.
    CGRect upperLeft = CGRectMake(0.0, 0.0, kSPUserResizableViewInteractiveBorderSize,
        kSPUserResizableViewInteractiveBorderSize);
    CGRect upperRight = CGRectMake(self.bounds.size.width - kSPUserResizableViewInteractiveBorderSize, 0.0,
        kSPUserResizableViewInteractiveBorderSize, kSPUserResizableViewInteractiveBorderSize);
    CGRect lowerRight = CGRectMake(self.bounds.size.width - kSPUserResizableViewInteractiveBorderSize, self.bounds.size.
        height - kSPUserResizableViewInteractiveBorderSize, kSPUserResizableViewInteractiveBorderSize,
        kSPUserResizableViewInteractiveBorderSize);
    CGRect lowerLeft = CGRectMake(0.0, self.bounds.size.height - kSPUserResizableViewInteractiveBorderSize,
        kSPUserResizableViewInteractiveBorderSize, kSPUserResizableViewInteractiveBorderSize);
    CGRect upperMiddle = CGRectMake((self.bounds.size.width - kSPUserResizableViewInteractiveBorderSize)/2, 0.0,
        kSPUserResizableViewInteractiveBorderSize, kSPUserResizableViewInteractiveBorderSize);
    CGRect lowerMiddle = CGRectMake((self.bounds.size.width - kSPUserResizableViewInteractiveBorderSize)/2, self.bounds.
        size.height - kSPUserResizableViewInteractiveBorderSize, kSPUserResizableViewInteractiveBorderSize,
        kSPUserResizableViewInteractiveBorderSize);
    CGRect middleLeft = CGRectMake(0.0, (self.bounds.size.height - kSPUserResizableViewInteractiveBorderSize)/2,
        kSPUserResizableViewInteractiveBorderSize, kSPUserResizableViewInteractiveBorderSize);
    CGRect middleRight = CGRectMake(self.bounds.size.width - kSPUserResizableViewInteractiveBorderSize, (self.bounds.size.
        height - kSPUserResizableViewInteractiveBorderSize)/2, kSPUserResizableViewInteractiveBorderSize,
        kSPUserResizableViewInteractiveBorderSize);

    // (3) Create the gradient to paint the anchor points.
    CGFloat colors [] = {
```