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# FEASIBILITY OF USING RAMAN-BASED TECHNIQUES FOR BREAST CANCER DETECTION

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FEASIBILITY OF USING RAMAN-BASED TECHNIQUES FOR  
BREAST CANCER DETECTION

Sara Mollamohammada

A THESIS

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# **FEASIBILITY OF USING RAMAN-BASED TECHNIQUES FOR BREAST CANCER DETECTION**

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Breast cancer is one of the common types of cancer among women all over the world. Early diagnosis is an effective way that improve the treatment process and gives the patients a better chance of survival. Many of the patients infected by breast cancer choose breast conservation surgery (BCS). However, some of those will be subjected to mastectomy, and many will have tumor recurrence as there is no precise technique to show the tumor margins. Raman-based methods are powerful techniques with potential to rapidly differentiate normal from tumor tissues and provides a solution to detect tumor margin. This is because the Raman signals provide unique information about a sample and has a molecular fingerprint effect.

This study evaluated Raman Spectroscopy (RS), Surface Enhanced Raman Spectroscopy (SERS), and Coherent Anti Stokes Raman Spectroscopy (CARS) as practical techniques to differentiate normal and cancerous cells and detect breast tumor margin, by using mouse and human tissues as models. Thin excised normal and cancerous tissues from mature mouse and human were sliced and fixed on glass and gold slides, and coated with silver and gold nanoparticles. SERS and CARS spectra, and CARS imaging were detected and analyzed with a Raman and CARS Spectrometer. After that, the results were analyzed using the two-band ratio model, principal component analysis (PCA), and principal

component detrued fluctuation analysis (PC-DFA) to find the difference in Raman and CARS signals of the two groups of tissues. Results from the spectrum/imaging of each type of tissues and comparison of different tissues indicate that the RS, SERS and CARS are viable techniques to differentiate between normal and cancer tissues. Also, the tissues with small thickness (around 5 micron) and smooth surface are more appropriate for all Raman-based techniques. In addition, CARS shows a strong capability to image the tissues and to demonstrate the concentration distribution of lipids, and hence, can be used to distinguish normal and cancer cells. Furthermore, CARS spectroscopy is capable of providing information about the tissues in specific wavelength ranges (e.g. lipids), which makes it an appropriate technique for clinical use to collect information about different stages of cancer from human tissue models within a short time.

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## CHAPTER 1 INTRODUCTION

### 1.1 Background

With nearly a quarter million new cases and over 40,000 cancer deaths each year, breast cancer is the most frequent cancer of women (Al-Hajj, Wicha, Benito-Hernandez, Morrison, & Clarke, 2003). Women with a diagnosis of breast cancer are usually faced to choose between conserving their breast (lumpectomy) versus undergoing a mastectomy (removal of the entire breast). As a result of improved imaging techniques, breast cancers are now diagnosed earlier, leading to increased use of breast conservation surgery (BCS). Breast-conserving surgery removes the tumor and surrounding tissue, but not the entire breast. Once a tumor is removed, a pathology test will be conducted to check if the margins are free of cancer cells. There are a couple of improved assessments of breast tumor margins at the time of BCS, such as intra-operative ultrasonography, specimen radiography, 2-view specimen mammography, and magnetic resonance imaging (MRI) wire localization (Hughes, et al., 2008; Bathla, Harris, Davey, Sharma, & Silva, 2011; Silva, 2012). About 50 to 66% of women in the US undergoing BCS for cancer undergo subsequent mastectomy for positive margins on initial lumpectomy (Bathla, Harris, Davey, Sharma, & Silva, 2011). Delayed definitive diagnosis by histopathology also leads to patients being subjected to multiple surgeries, with increased morbidity and cost. A critical issue is the lack of highly sensitive techniques that can rapidly differentiate normal from cancerous tissues for intraoperative assessment of tumor margins to allow complete

surgical breast tumor margin clearance, reduce the need for mastectomy and repeat surgery, and prevent tumor recurrence.

The long-term goal of this study is to solve this issue by applying Raman-based technologies, such as Raman Spectroscopy (RS), Surface-Enhanced Raman Spectroscopy (SERS), and Coherent Anti-stokes Raman Spectroscopy (CARS). Since each chemical entity has its own unique Raman signature (Schlücker, 2011), the unique biochemical entities in normal versus tumor tissues generate characteristic RS spectra as molecular fingerprints. This is one application of Raman-based methods in intra-operative clinical decision making in breast cancer with potential applications for other cancers and non-cancer diseases.

## **1.2 Objectives**

The objectives of this study are to use mouse and human breast cancer models for preclinical assessment of CARS and SERS from the following perspectives:

- 1) To develop methods to distinguish normal from tumor breast tissue; and
- 2) To evaluate the feasibility of using Raman-based methods to differentiate cancer tissues in different stages.

## **1.3 Thesis Organization**

This thesis contains four chapters. The first chapter is an introduction to the study conducted. It provides a background of the topic, research motivation and objectives. The

second chapter provides a literature review of the existing methods and the research that were conducted before as well as the knowledge gaps and limitations of previous studies. This chapter explains the concept of RS, SERS, and CARS, the mechanism, and application in cancer detection. Chapter three details a series of experiments on the mouse tissue using Micro, Macro, and UV Raman and CARS Spectrometer. This chapter presents the results of experiments conducted using RS, SERS and CARS methods, and the feasibility of Raman-based techniques to differentiate different stages of cancer. A discussion is included in the fourth chapter; detailing the limitations, issues, and feasibility of the applied methods. It also provides the recommendation for future work.





## **CHAPTER 2 RAMAN- BASED TECHNIQUES FOR CANCER DETECTION**

### **2.1 Introduction**

There are wide varieties of techniques that have been proposed by researchers to differentiate tumor and normal cells. To some extent, RS is a technique that appears to have high potential to distinguish normal and tumor cells within a short time. This method classifies the samples into different groups according to their Raman spectra. Furthermore, SERS is another technique that enhances Raman scattering by molecules absorbed on metal surfaces and provides stronger Raman signals. However, none of these techniques provide any information regarding the tissue contents. CARS is a powerful technique that allows vibrational imaging with high sensitivity and speed, and uses lipid, protein, and DNA filters to detect the composition distribution of a sample, and hence, can be used to detect the existence of cancer.

This chapter provides a brief overview of the basic principles of RS, SERS, and CARS, followed by discussion of the application and capability of each of the three aforementioned techniques.

### **2.2 Basic Theory and Principles of RS**

#### **2.2.1 Principles of RS**

RS is a spectroscopic technique that is based on inelastic scattering of monochromatic

light from a laser source. In inelastic scattering, the frequency of photons in monochromatic light changes once it interacts with a sample. Photons of the laser light are absorbed, and then, reemitted from the sample. Frequency of the reemitted photons is shifted up or down in comparison with the original frequency of monochromatic light, which is called the Raman effect (Rosa, 2009) (see Fig. 2.1).

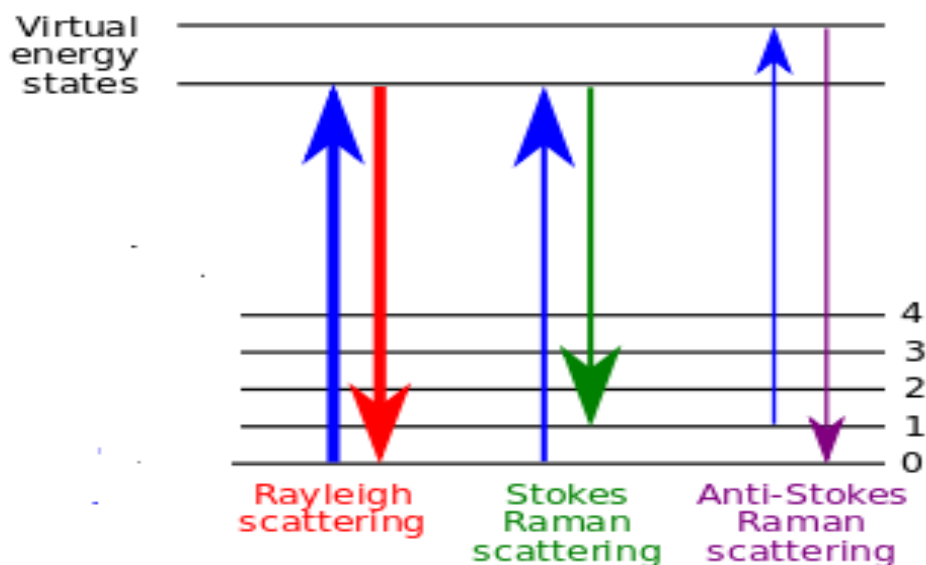


Figure 2.1 Energy- level diagram showing the states involved in Raman scattering process

In Raman effect, the laser beam is considered as an oscillating electromagnetic wave with electrical vector  $E$  that is determined by molecular polarizability  $\alpha$ . Upon interaction with the sample, it induces electric dipole moment  $P = \alpha E$  which causes molecule deformation. Due to the periodical deformation, molecules start vibrating with

a characteristic frequency of  $\nu_m$ . After interaction of the light with the molecule, the light polarizes the cloud of electron to make a virtual state. This is an unstable state, and the photon is rapidly re-radiated. If the electron cloud distortion is the only process involved in scattering, the frequency changes of photons will be very small. This type of scattering is called elastic scattering (Smith & Dent, 2005). However, the nuclear motion may be induced during the scattering process, and in this case, the energy will transfer from the incident photon to the molecule, or from the molecule to the scattered photon. In such a case, the process is called inelastic, and this is Raman scattering. It is generally a weak process, and normally one in every  $10^6$ – $10^8$  scattered photons is Raman scattered (Rosa, 2009). In the inelastic scattering process, the laser beam with the frequency of  $\nu_0$  excites the molecules and changes them to oscillating dipoles, which emit light of three different frequencies (Fig. 2.1) when:

- 1) A molecule without any Raman-active modes absorbs a photon with the frequency  $\nu_0$  and returns back to the same vibrational state and emits light with the same frequency  $\nu_0$ . This type of interaction is an elastic Rayleigh scattering.
- 2) A Raman-active molecule in the basic vibrational state will absorb a photon with frequency  $\nu_0$ . Part of the photon's energy is transferred to the Raman-active mode with frequency  $\nu_m$  and the frequency of scattered light is reduced to  $\nu_0 - \nu_m$ . This Raman frequency is Stokes frequency.
- 3) A Raman-active molecule, which is already in the excited vibrational state, will absorb the photon with frequency  $\nu_0$ . The excessive energy of excited Raman

active mode will be released, and molecule returns to the basic vibrational state, and the frequency of scattered light goes up to  $\nu_0 + \nu_m$ . This Raman frequency is Anti-Stokes frequency.

As shown in Figure 2.1, the Rayleigh process doesn't involve any energy changes, and the light returns to the previous state of energy. Most of the photons scatter following the Rayleigh process. Stokes scattering is promotion from the ground vibrational state of 0 in Fig. 2.1 to the higher state of 1 in Fig. 2.1 that occurs by absorption of energy. However, some molecules may be present in excited state of n state, due to some special conditions, such as thermal energy. Transferring from state of n to ground state of m is called anti-Stokes scattering. In this process, the energy transfers to the scattered photon. Therefore, anti-stokes scattering will be weak compared to stokes scattering, because of lower population of excited state (Rosa, 2009).

Around 99.999% of all incident photons in spontaneous Raman go through elastic Rayleigh scattering, which is not useful for practical purposes of molecular characterization. Only about 0.001% of the incident light produces inelastic Raman signal with frequencies  $\nu_0 \pm \nu_m$  (Rosa, 2009).

The number of molecules in the ground and excited states display the intensities of the stokes and anti-stokes. Boltzmann equation represents this ratio (Smith & Dent, 2005):

$$\frac{N_n}{N_m} = \frac{g_n}{g_m} \exp \left[ \frac{-(E_n - E_m)}{kT} \right] \quad (2.1)$$

where:

$N_n$ : Number of molecules in excited level  $n$ ;

$N_m$ : Number of molecules in ground level  $m$ ;

$g$ : Degeneracy of levels  $m$  and  $n$ ;

$E_n - E_m$ : Difference in energy between levels of  $m$  and  $n$ ;

$k$ : Boltzmann constant ( $1.3807 \times 10^{-23} \text{ JK}^{-1}$ )

$T$ : Temperature (K)

Some vibrations may happen in more than one way, and they have the same energy, even though they occur in different ways. Since the Boltzmann equation must be able to predict all possible states,  $g$  is used to correct all vibrational states. For most cases,  $g$  is equal to 1 and for degenerated vibrations it can be 2 or 3 (Smith & Dent, 2005).

The main parts of a Raman spectrometer include a laser source, a beam splitter, and a detector. The laser source illuminates a spot on the surface under test. Then, the scattered light is collected and sent to a spectrometer and detector (Likodimos, Kontos, & Falaras, 2013). The light used to hit the molecule can be in the range of ultraviolet (UV), visible (Vis) or near infrared (NIR), but the most common choice is Vis. Since peaks due to Raman scattering are sharper than the peaks that detected in the visible region by emission and absorbance, a monochromatic source is the best choice to do Raman measurements (Rosa, 2009).

Figure 2.2 shows the internal part of a micro-Raman spectrometer. As it is demonstrated, the laser light is aligned by mirrors B through the lens (C), and converges

the beam into the pinhole. The light that passes through the pinhole is collimated into a parallel beam by second lens (D). The two mirrors (E and F) reflect the beam onto the notch filter (G). A mirror that is located in the microscope reflects the laser beam to the microscope to illuminate the sample. The position of the lens that focuses on the sample is also adjustable on the software, which can control the width of the laser spot illuminating the sample from 0% to 100%. The sample is visible through the microscope by moving the mirror, using the control (S). A beam splitter is below the mirror, and can be moved by control (T). The beam splitter allows the illumination of the sample with white light so that the sample can be viewed by a microscope. This helps to control the positioning and focusing on the sample (Khan, 2004).

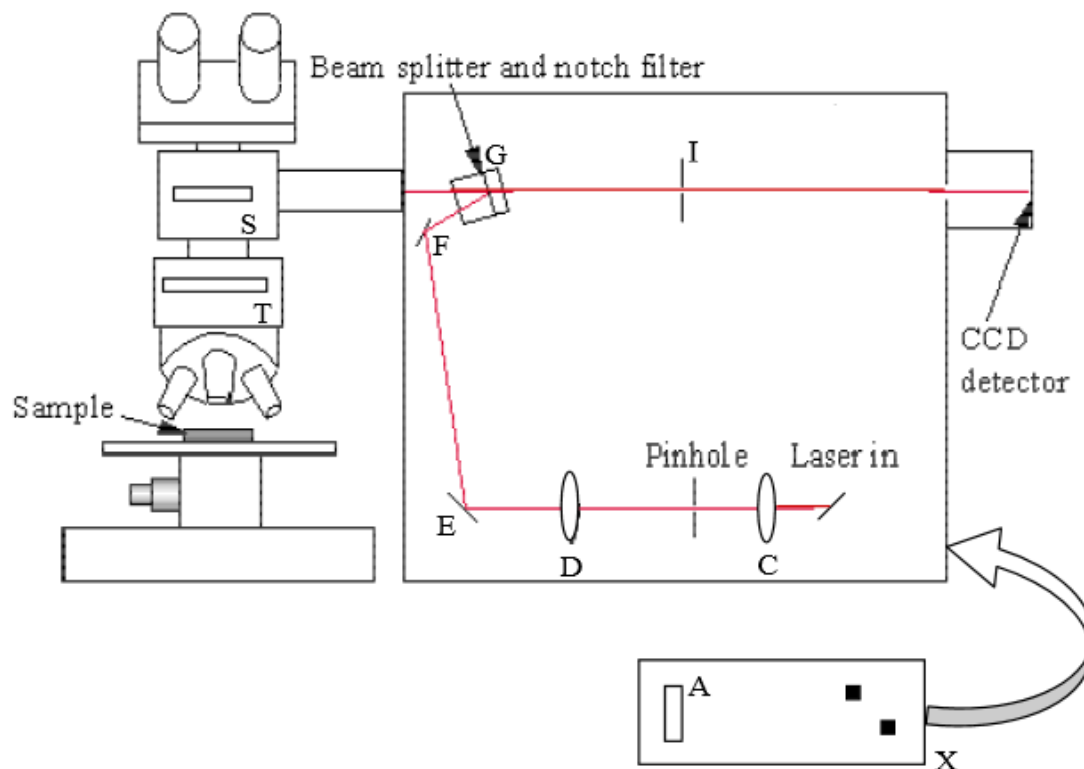


Figure 2.2 Internal Schematic of Renishaw Raman (Likodimos, Kontos, & Falaras, 2013)

### 2.2.2 Applications of RS

RS is proved to be a great analytical method in many fields where chemical analysis is required. RS was basically used in analytical chemistry to examine the organic and inorganic compounds as it was one of the few tools that was capable to detect both elements and molecules. A good example of using RS in chemistry was detection of the purity and physical form of element carbon, sulphur and silicon that was a big achievement in the semiconductor industry (Smith & Dent, 2005)



Researchers have aimed to use RS technique in other majors such as biology to identify chemical compounds, their functional groups and to discover the conformation of complex biomolecules, such as proteins. RS is recognized as a technique which is capable to characterize the composition and structure of a sample, and gives information about the properties of molecules by providing characteristics of their vibrational transitions. Although RS is not an appropriate techniques for all compounds, most of the chemical groups such as S-S, S-H, aromatic rings, -CN, carbonate, and phosphate normally show strong peaks on Raman spectrum. This technique is also used to identify changes in physical form of the components that is applicable to study the structure of peptides and backbone molecules (Smith & Dent, 2005).

Recently, RS has been considered as a powerful technique for biomedical approaches. The reason is that RS is capable to provide detailed chemical information about a tissue sample and represents the changes in chemical content due to appearance or progression of a diseases. The ability of RS to measure several different chemicals shows the importance of this technique in studying cancer because of the heterogeneity of the disease.

A very early look to the concept of using RS for cancer detection was presented by Frank et al. (Frank, McCreery, & Redd, 1995). In this study, the Raman spectrum from the normal and diseased human breast tissue was acquired and compared (Frank, McCreery, & Redd, 1995). Because Raman scattering is generally weak, the comparison was limited to comparing lipid bond that had the strongest peak in Raman spectrum of both types of

tissues. However, with an appropriate system design and using nanoparticles to enhance Raman spectrums, RS can be applied in clinical approaches as SERS.

## **2.3 SERS**

### **2.3.1 Principles of SERS**

The free electrons of a metal can be excited by applying electromagnetic waves (e.g., a laser light) to create plasma oscillations, which is known as plasmonics. Plasmons take the form of surface plasmons and localized surface plasmons. When a surface plasmon is confined to a nanoparticle (NP) of a size comparable to the wavelength of the light, free electrons in the NP participate in the collective oscillation. The optical extinction of the particle has a maximum at the plasmon resonant frequency, which occurs at visible wavelengths for noble metal (e.g., Au, Ag) NPs, and is referred to as a localized surface plasmon resonance (LSPR). Raman spectroscopy uses the principle of plasmons to obtain information on the molecular specificity from their unique vibrational signatures. However, the Raman scattering is inherently weak. The discovery of SERS in the 1970s, particularly the two independent reports in 1997, of the observation of single-molecule emission under SERS conditions, opened the window to using plasmon-active materials for sensing applications. SERS shows a  $10^6$ – $10^{14}$  enhancement when the analyte is bound or close to the surface of noble metal NPs. Since peaks in a SERS spectrum are characteristic of specific molecular vibrations, each chemical entity has its own unique Raman signature. Therefore, SERS can display intrinsic interfacial sensitivity and

selectivity. Furthermore, SERS only observes the material captured by the NPs, eliminating interference from contaminants, reagents, or other components in the solution (Vitol, Z. Friedman, & Gogotsi, 2012).

SERS was reported by Fleischman et al. in 1974 (Kumar, 2012). They observed strong Raman scattering from Pyridine adsorbed onto a silver electrode (Smith & Dent, 2005). Since discovering SERS in 1974, it has become one of the widely-used techniques for different applications as it is able to collect a big amount of information from complex samples adsorbed on metal nanoparticles (Stiufiuc, et al., 2013). Meanwhile, two mechanisms have been proposed to explain the enhancement of SERS: electromagnetic and chemical enhancement.

According to the first SERS theory, the analyte is adsorbed or has been held close to the metal surface, and the interaction occurs between the analyte and plasmon. This phenomena is called the electromagnetic enhancement. Like any other metals, silver surface is covered with electrons. The electron density has distance from the surface, due to the lack of positive charge in the surface. Once the light beam is directed to the surface and interact with those electrons, they start to oscillate across the surface. This oscillation is called surface plasmon. Surface plasmons have a resonance frequency, that makes them to adsorb and scatter light more efficient. This frequency depends on the type of metal and surface. For example, in gold and silver, it occurs in visible region and makes them appropriate for using with visible laser systems that is commonly used in Raman scattering systems. Another important characteristic that must be considered is that the metals are

able to both absorb and scatter the light. The ratio between the absorbed and scattered light depends on the type of metal. For silver, the scattering is dominant (Smith & Dent, 2005). The basic principles of SERS electromagnetic enhancement is shown in Figure 2.3.

According to the second mechanism, the adsorbate has chemical bonds to the surface, and excitation happens once electrons from the metal surface transfer to the molecule, and back to the metal. This process is named as charge transfer or chemical enhancement. According to these two theories, chemical enhancement can only happen from the first layer of analyte which is attached to the metal surface. However, the electromagnetic enhancement is likely to occur in the subsequent layers (Smith & Dent, 2005).

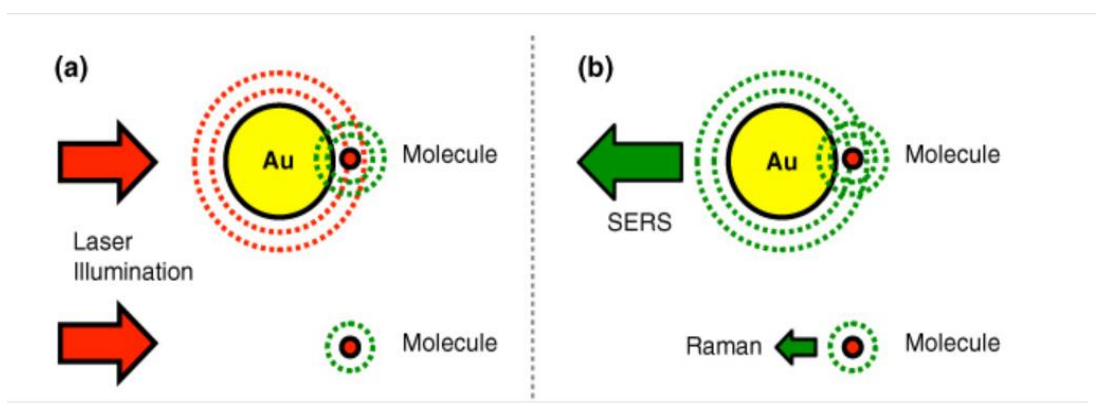


Figure 2.3 Basic SERS electromagnetic enhancement. A gold nanoparticle will enhance both (a) the incident field and (b) the scattered field, significantly enhancing the Raman signal from a proximate molecule. Since the intensity is enhanced twice, i.e. the nanoparticle acts as both a receiver and a transmitter, the SERS signal is proportional to the intensity of the incident field squared (Lindquist, Nagpal, McPeak, Norris, & Oh, 2012)

The easiest way to describe the electromagnetic SERS can be based on a metallic sphere model (Schrader, 2008). This approximation is appropriate if the metal particles are single without any aggregation. Once an electric field is applied to a small metal sphere, the field at the surface can be described as:

$$E_r = E_0 \cos\theta + g \left( \frac{a^3}{r^3} \right) E_0 \cos\theta \quad (2.2)$$

where:

$E_r$ : The total electric field at the distance of  $r$  from sphere metal (V/m)

$E_0$ : The incident electromagnetic wave (V/m)

$a$ : The radius of the sphere model (m)

$\theta$ : The angle relative to the direction of electric field (degree)

$g$ : Related to the dielectric constant:  $g = \frac{\epsilon_1(\vartheta_L) - \epsilon_0}{\epsilon_1(\vartheta_L) + 2\epsilon_0}$

where  $\epsilon_0$  &  $\epsilon_1$  dielectric constants of the medium are surrounded metal sphere (F/m), and  $\vartheta_L$  is the frequency of incident light beam (Hz). Once the value of  $g$  is maximum, the value of  $\epsilon_0$  is close to 1. At this frequency, the excitation of the surface plasmon largely increases the local field that is experienced by the absorbed molecule on the surface of sphere metal. In these conditions, the molecule is immersed in freely moving electron cloud, and those movements increase the polarization of the surface electron. The electrons of the analyte that is absorbed on the metal surface will interact with the cloud and make bigger polarization around the molecule. It is also known that once a small particle adsorbed on a surface, the strongest enhancement happens from interaction between particles. Once the

laser is directed to the surface, some parts of the surface got active and some others remain inactive. The active parts are called hot spots, which are mostly between the particles. The frequency of plasmon decreases once the particle size increases. The point which two particles touch each other, will produce a strong electric field, and thus, the contact point will become SERS active. Other parameters may also affect the SERS effect. The frequency chosen, particle shape and size, and particle aggregation will affect SERS enhancement if the electromagnetic enhancement is the dominant phenomenon (Smith & Dent, 2005).

The second theory of the SERS process, charge transfer, involves forming a chemical bond between the analyte and the surface of the metal, which generates a surface species, including the analyte and some atoms of metal. Then, the electrons can freely transfer from metal surface to the analyte. Formation of this surface species will increase the polarizability of the molecule, due to the interaction with electrons of the metal. Consequently, the enhancement will occur with new electronic states due to the bond between analyte and metal surface. Charge transfer occurs only from the molecules that are directly attached to the surface, and basically in the first layer (Smith & Dent, 2005).

It is very difficult to predict which of these two mechanisms will be applied on a SERS process. Generally, it is believed that electromagnetic enhancement may have a bigger effect than charge transfer, but this is still the topic of many studies (Smith & Dent, 2005).

In order to get strong enhancement, the tested molecule structure must be in vicinity of nanoparticles from several points. The nanoparticles used for SERS can be made in different shapes such as spherical, rod, triangular, hexagonal, and many others types. (Schatz, Young, & Van Duyne, 2002). The magnetic and optical properties of nanoparticles are closely related to their size, shape, size-distribution, and surrounding environment. Therefore, to SERS effectively, very strict control over average particle size in a specific size range and uniform particle morphology is required which still remains a challenge (Quester, Avalos-Borja, Vilchis-Nestor, Camacho-López, & Castro-Longoria, 2013). As it was mentioned, several surfaces have been tested as SERS substrate including Ag, Au, Cu, and Li; but the best results were acquired using gold and silver nanoparticles. Silver offers more enhancement effect than gold, and it also can be excited in a wider range, from UV to the Infrared (IR) while gold is limited to the IR region (Stiufiuc, et al., 2013; Murphy, Lucht, Schmidt, & Kronfeldt, 2000). Therefore, the type and shape of nanoparticles have a very important effect on SERS results because it directly affects the formation of hot spots.

### **2.3.2 Types of SERS Nanosensors and their Applications**

Usually, SERS has been used as SERS nanosensors for different applications. Here, we classify plasmon-active SERS nanosensors into three types as described below.

Type 1–Nanosensors based on colloidal NPs in test solutions. Usually, NPs can be made via metal (e.g.,  $\text{AgNO}_3$ ) precipitation in different solutions (e.g., sodium citrate)

without any subsequent treatment. Because of the large variations of the SERS enhancement factor on the surface, only molecules located at the positions of highest enhancement, i.e., hot spots (often  $< 1\%$ ) can be detected at the single-molecule level (Fang, Seong, & Dlott, 2008). To increase the enhancement factor and/or hot spots, a variety of NPs with different shapes (e.g., nanorods, nanoprisms, nanocubes), structures (e.g., core-shell), and metals (Ag, Au, Cu) have been developed.

Type 2–Nanosensors with NPs with corners, shape tips, or thin shells on substrate.

Considerable effort has been made toward designing highly plasmon-active substrates (ranging from highly roughened metal films or electrodes to metallic structures) using coinage metal NPs (e.g., Au, Ag, Cu) with different shapes (e.g., spheres, cubes, stars, triangles, bipyramids, crescents). These substrates have been fabricated using techniques based on self-assembly, notably nanosphere lithography (Fang, Seong, & Dlott, 2008), and on standard photolithography and electron beam lithography (Laurent et al., 2005).

Type 3–Nanosensors with fixed and controlled configurations. Fiber-optic or glass-pipette tips with SERS functionality (enabled by silver film or immobilized metal colloids) are currently being used in studies of single-cells (e.g., intracellular pH measurement). The diameters of these kind of nanopipettes are about 100–500 nm, smaller than conventional glass pipettes (e.g., 1–3  $\mu\text{m}$ ). These nanopipettes are largely suitable for probing a cell nucleus and cytoplasm. Carbon nanotube-based nanopipettes can be functionalized by Au NPs for single-cell studies; but for SERS applications, the main obstacle in designing very small probes is the size limitation on metal particles. Arrays of



NPs having diameters of 20–100 nm would yield the best Raman enhancement, while NPs smaller than 10 nm would provide insufficient SERS enhancement (Pahlow, et al., 2012). TERS combines SERS with some form of tip-base microscopy, e.g., scanning tunneling microscopy (STM) and atomic force microscopy (AFM), and allows superior spatial resolution of 10–30 nm or even  $< 10$  nm (Vitol, Z. Friedman, & Gogotsi, 2012). TERS is a technique that truly measures the properties of a single spot and allows us to control the position (and characteristics) of the hot spot.

Both Type 1 and 2 nanosensors have been used for detection of nucleic acids (e.g., DNA), pathogens, cells and tissues, and target molecules in complex samples (e.g., body fluids), among other. They have also been used with microfluidic lab-on-a-chip devices and TERS for in vivo and in vitro diagnostics (Vitol, Z. Friedman, & Gogotsi, 2012)

The methods of using colloidal nanosensors have evolved from ultralow concentrations, to the technique of Langmuir-Blodgett film, and to bi-analyte methods<sup>[8]</sup>. With bi-analyte methods, it is possible to work at a relatively larger concentration, while retaining the ability to identify single-molecule events. The performance of colloidal nanosensors can be further improved if: a) the NPs are coated with a bilayer of a chemical (e.g., cetyltrimethylammonium bromide (CTAB)) such that only the hot spots (e.g., tips) of the NPs are available for adsorption of the target analyte (Stuart, Biggs, & Van Duyne, 2006), or b) TERS is used (Vitol E. O., 2012). Currently, colloidal nanosensors can be made for label-free or target-specific (so called SERS labels or nanotags) detection.

Currently, considerable research on Type 2 nanosensors has been focused on: a) using the sensors to monitor the binding of molecules onto the surface-bound species of the sensors, e.g., antibody-antigen, DNA-DNA, and DNA-protein interactions, and b) sensitivity of NP shapes, structures, and functionalization to enhancement factors. For example, nano- or micro-line arrays fabricated with microfluidic channels and then attached to either glass or gold surfaces have been used in LSPR detection (Lee, Coodrick, & Corn, 2001). LSPR nanosensors have been used for detection of a stimulant of the chemical warfare agent mustard gas (Stuart, Biggs, & Van Duyne, 2006).

Type 3 SERS sensors have been used in many biomedical applications, such as sensing pH in a single living cell, protein and lipid analysis, detection of characteristic spectral signatures of a cell nucleus and cytoplasm, and in situ analysis of living cell functions (e.g., protein expression in response to a change in cell osmolarity) (Vitol, Z. Friedman, & Gogotsi, 2012).

### **2.3.3 Application of SERS for Biomedical Purposes**

SERS is capable to show important information regarding the molecular contents of different materials (Smith & Dent, 2005). In order to use SERS technique effectively, the type of nanoparticles used for enhancement should be carefully selected. To the date, silver and gold nanoparticles have shown the most effective enhancement. Any molecule of sample that is close to hot spots will provide enhancement for Raman spectrum of the sample under test. However, colloidal nanoparticles in spherical form have shown be more useful as they have more contact points with the solid sample and provide hot spots to enhance Raman signals (Kumar, et al., 2007).

Applying SERS for biomedical purposes has been studied before. SERS has been used as a non-invasive technique for cancer imaging, histological analysis of biopsy samples, and also in-vivo and ex-vivo tumor detection.

A couple of ex-vivo studies were conducted using SERS for cancer detection. In a study by Aydin et al. (2009), the SERS spectrum of freezing tissues from human brain was collected by mixing it with silver colloidal nanoparticles. The SERS spectrum from different types of brain cancer were obtained and categorized into normal and tumor group. (Aydin, Altaş, Kahraman, Bayrak, & Çulha, 2009).

SERS was also used as an imaging and mapping tool for in-vivo applications. One of the examples of using SERS for in-vivo cancer imaging was developed by using gold nanotags. The gold nanoparticles that were composed of nanotags covered with organic reporters for cancer imaging. Several mice were used as models, and the colloidal nanotags

were injected to the tail vein to detect tumors in head and neck of the mice (Vendrell, Maiti, Dhaliwal, & Chang, 2013).

In one of the most recent studies, imaging of small living subjects was shown by Gambhir group. In this study, the Gambhir group provided a noninvasive deep-tissue molecular images in mouse liver, by applying SERS imaging. The gold nanoparticles coated with different types of Raman reporter and silica layer and single-wall carbon nanotubes were used to show Raman imaging from the whole-body of a mouse, and in vivo tumor targeting (Keren, et al., Noninvasive molecular imaging of small living subjects using Raman spectroscopy., 2008). The Gambhir group continued working on SERS imaging and developed another method to detect brain tumor margin. In this method, they used a new triple modality MRI-Photoacoustic-Raman nanoparticle and combined MRI and Raman imaging to detect the margin of tumor. That triple-modality magnetic resonance imaging-photoacoustic imaging-Raman imaging nanoparticles (or MPR) was composed of a 60-nm gold core covered with a Raman reporter and silica shell and  $Gd^{3+}$ . Those MPRs were injected intravenously into a mouse body. The MPRs couldn't accumulate in healthy part of brain because of the different shape of vasculatures in normal and healthy part. This method was helpful to separate normal and cancer part of brain and the tumor margin was distinguished by combining Raman and MRI imaging (Kircher et al., 2012).

Most of the SERS experiments in medical science are conducted using gold or silver nanoparticles. Regardless of the big potential to utilize gold and silver nanoparticles for medical purposes and in-vivo studies, the nanoparticle toxicity must be investigated

before any in vivo applications. Silver nanoparticles usually perform with very large enhancement effect but they are not appropriate for in-vivo studies. Gold nanoparticles are preferable choice for in-vivo studies, but their enhancement effect is not as much as silver nanoparticles (Kumar et al., 2007). Most toxicology studies show that the surface chemistry and dimensions of gold nanoparticles directly affect the toxicity of particles. According to the previous studies, The Polyethylene glycol (PEG) coated gold have the minimum toxicity effect in in-vivo studies and especially for drug delivery purposes (Zhang et al., 2011).

## **2.4 CARS**

SERS has shown some success in tissue characterization and imaging, but it is a long imaging process, which is not a good approach for clinical purposes. To solve this problem, Coherent anti-Stokes *Raman* scattering (CARS) was introduced as a fast and powerful imaging technique. The principles and applications of CARS are described as follows.

### **2.4.1 Principals of CARS**

Raman imaging needs a high average power due to the small cross section of Raman scattering. Accordingly, it takes several hours to get a Raman image of cells and tissues. This long exposure time limits using Raman microscopy to the study of dynamic living systems. These issues in Raman microscopy can be avoided by multi-photon vibrational microscopy based on CARS (Peltier et al., 2002). CARS is another form of spectroscopy

for stronger vibrational signals which was first reported by Maker and Terhune at the Ford Motor Company in 1965 (Pawley & Masters, 1996). CARS is sensitive to the nuclear vibrations of chemical bonds, which allows vibrational imaging with high sensitivity and speed. The combination of optical spectroscopy and microscopy provides a non-invasive method to visualize different types of molecules in living cells (Cheng & Xie, 2004).

CARS is a third-order nonlinear process that is used to enhance the weak Raman signals. CARS includes a pump beam at frequency of  $\omega_p$  and a Stokes beam at frequency of  $\omega_s$ , which interact with a sample via a wave-mixing process. Once the beat frequency  $\omega_p - \omega_s$  matches the frequency of a Raman active molecular vibration, the resonant oscillators are coherently driven by the excitation fields and generate a strong anti-Stokes signal at  $\omega_{as} = 2\omega_p - \omega_s$  as shown in Figure 2.4.

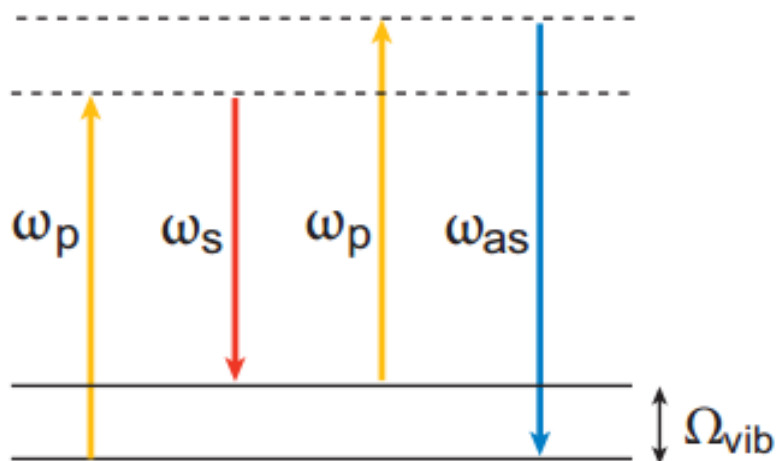


Figure 2.4 Energy diagram of CARS. When the difference between the pump and Stokes frequencies ( $\omega_p - \omega_s$ ) matches the molecular vibrational frequency,  $\Omega_{vib}$ , the anti-Stokes signal is generated at a frequency  $\omega_{as} = 2\omega_p - \omega_s$  (Evans & Xie, 2008)

Cell biology and tissue imaging are the two fields that have had a big progress after developing of CARS. CARS is able to perform chemically specific, label-free imaging at the micron scale. Stimulated excitation of vibrational transitions in coherent signal accumulation in CARS produces a strong directional signal, making Coherent Raman Imaging much more sensitive than conventional vibrational microscopy. Consequently, CARS requires only moderately average powers that are easily tolerable by living biological samples. Also, the nonlinear CARS signals are generated only at the focus where the excitation intensities are the highest. This leads to 3D sectioning capability, which is essential for imaging tissues or cell structures.

#### **2.4.2 Applications of CARS**

The first CARS which was used by Reintjes group at the Naval Research Laboratory (Evans & Xie, 2008). It was used as an improved mechanism for microscopy, which used to visualize living cells with different vibrational modes, including the phosphate stretch vibration (DNA), amide I vibration (protein), OH stretching vibration (water), and the CH group of stretching vibrations (lipids). CARS is a powerful imaging tool that it is able to take tissue imaging in a very short time. The CARS microscope has already shown an important impact in the area of lipid imaging (Cheng, Jia, Zheng, & Xie, 2002). As an example, the resonant CARS signal is able to detect single lipid bilayers or single cellular membrane from the symmetric CH<sub>2</sub> stretching mode. The CARS

microscope is also able to differentiate between saturated and unsaturated lipids, and it can reveal information about the density of lipid membranes. The CARS microscope is highly sensitive to pick up signals from single phospholipids membranes, which is an advanced step to study membrane biophysics, vesicle transport and organelle mapping. Most significantly, the strong CARS signal from lipid has revealed several advanced steps to study biomedical approaches and in the progression and treatment of neurodegenerate diseases (Le, Yue, & Cheng, 2010; Cheng & Xie, 2004).

Another important vibrational response in biomedical studies is detection of proteins. Although the CARS microscope is unable to conclusively distinguish different types of proteins, it is still able to generate maps of protein that is an advanced step to study tissues and cells. The spatial distribution of protein density is usually an important sign of diseased tissue (Le, Yue, & Cheng, 2010).

Besides CARS microscopy, there are several non-linear optical processes for cell imaging, such as second-harmonic generation (SHG), sum frequency generation (SFG), and third harmonic generation (THG). SHG and THG are more practical, as they use one laser beam and can be implemented on a two photon fluorescence microscope (Zoumi, Yeh, & Tromberg, 2002). Compare all of these imaging methods, CARS microscopy is more informative than SHG and THG microscopy as it is able to provide rich information about molecular vibration. SFG microscopy is also capable to provide vibrational contrast but it is mostly surface sensitive rather than bulk-sensitive (Cheng & Xie, 2004).



Developments of CARS over years have enabled its application in chemical, materials, biological, and medical fields. Several studies in chemistry have been conducted on lipid vesicles, lipid layers, and the ordering of lipid domains. CARS imaging is useful for in vivo and in situ studies, especially where the use of selective labels might be impossible. Compared to other techniques such as magnetic resonance imaging (MRI), CARS doesn't have a large penetration depth; instead, it provides subcellular spatial resolution and high time resolution. An in vivo application of CARS imaging was first shown on the skin of a mouse. In this research, CARS microscopy was used to visualize the lipid structures throughout the 120  $\mu\text{m}$  depth of mouse ear skin (Evans et al., 2005).

Recent studies also focused to visualize the delivery of retinol, a drug that is used to stimulate collagen growth in skin. The conjugated polyene structure of the drug gives a strong vibrational band that can be used for specific imaging with CARS (Pudney, Mélot, Caspers, Van Der, 2007).

In another in-vivo study, CARS microscopy has been applied to visualize the structure of excised mouse lungs. Lung tissue is composed of small air sacs called alveoli that are covered with a lipid surfactant. CARS images of lung tissue show these alveoli all along with several lipid-rich cells, most likely surfactant cells, Clara cells, and macrophages (Evans & Xie, 2008) (see Fig 2.5).

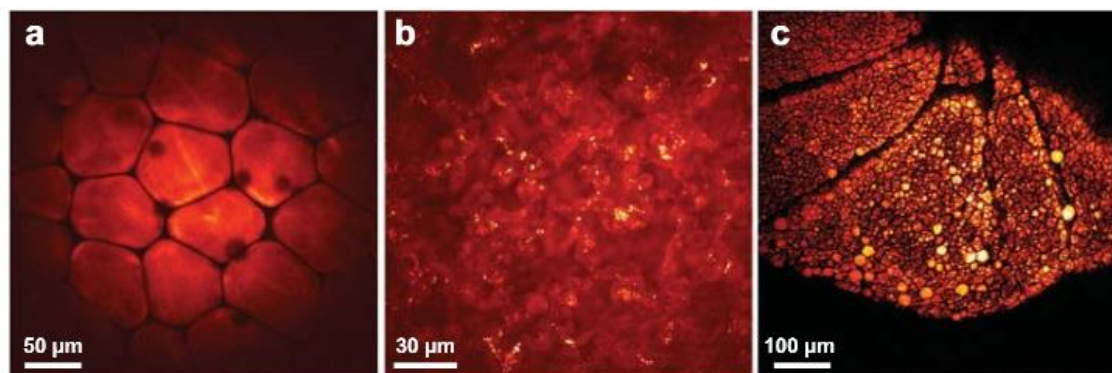


Figure 2.5 CARS imaging of several tissues with CH<sub>2</sub> contrast. (a) CARS image of white adipose tissue of mouse omentum majus. The adipose cells are packed with fatty acids and give strong CARS signals. (b) CARS microscopy of mouse lung tissue, showing the individual (Evans & Xie, 2008)

## 2.5 Summary

Raman-based technologies, including RS, SERS, and CARS, are among the most powerful techniques for biomedical studies owing to their sensitivity and molecular fingerprint effect. The ability of Raman-based techniques to detect different chemicals shows the importance of these techniques in cancer studies, because of the heterogeneity of the cancer tissue. RS and SERS work best to differentiate various types of tissues, while CARS is preferred in identifying the components of the tissues, which can be used as an indicator of cancer progression.

## **CHAPTER 3 APPLYING RAMAN-BASED TECHNIQUES FOR BREAST CANCER DETECTION**

### **3.1 Introduction**

In this chapter, we investigate the applicability of using RS, SERS and CARS techniques to distinguish between normal and tumor tissues, and to evaluate the feasibility of using these Raman-based techniques for detection of cancer in different stages.

The study was performed in three phases. At the first phase, different types of normal tissues (fresh, frozen, and fixed) from mouse models were collected, and Raman based techniques (Macro/ Micro Raman, CARS) were tested to determine the optimum test conditions required to acquire the strongest Raman shift. The results of the first part was used to design the test conditions of the second phase. In the second phase, the Raman based techniques were used to differentiate tumor and normal tissues in mouse models. The results of the second phase were used in the third phase to evaluate the feasibility of using CARS and RS techniques for cancer detection in different stages, using human breast tissue as model.

### **3.2 Materials and Methods**

Table 3.1 shows the tests that were conducted using RS, SERS, and CARS techniques in this study. Details about the tests conditions in each phase are described below.

Table 3.1 Summary of performed tests under different conditions

Sample code	Sample Type	Conditions		Wavelength (nm)	Enhancement	Results Shown in figure:
		Sample	Test			
Phase I: Optimum Test conditions						
SERS-T1	mouse mammary gland- normal	Fixed	Macro Raman	785	Silver NPs	3.11
SERS-T2	mouse mammary gland- normal	Fresh	Macro Raman	785	Silver NPs	3.12
SERS-T3	mouse mammary gland- normal	frozen	Macro Raman	785	Silver NPs	3.13
SERS-T4	mouse mammary gland- normal	fresh	Micro Raman	785	Gold NPs	3.14
SERS-T5	mouse mammary gland- normal	fresh	Micro Raman	785	Silver NPs	3.15
SERS-T6	mouse lung- normal	fresh	Micro Raman	514	Silver NPs	3.16
SERS-T7	mouse mammary gland- normal	fresh	Micro Raman	514	Silver NPs	3.17
Phase II: Differentiation of Normal and Tumor Tissues Using Mouse Models						
SERS-T8	mouse mammary gland- normal & tumor	fixed	Micro Raman	785	Gold surface	3.19
RI-T1	mouse mammary gland- normal	fixed	Micro Raman	785	NA	3.23
RI-T2	mouse liver- normal	fixed	Micro Raman	785	NA	3.24
CARS-IMG-T1	mouse mammary gland- normal	fixed	CARS	~ 300	NA	3.25
CARS-IMG-T2	mouse mammary gland- tumor	fixed	CARS	~ 300	NA	3.26
CARS-SPT-T1	mouse mammary gland- normal	fixed	CARS	~ 300	NA	3.27
CARS-SPT-T2	mouse mammary gland- tumor	fixed	CARS	~ 300	NA	3.29
Phase III: Cancer stages identification in human						
RS-UV-T1	human breast tissue- benign and stages 1-3 of tumor	fixed	UV-Raman	244	NA	3.31
CARS-SPT-T3	human breast tissue- benign and stages 1-3 of tumor	fixed	CARS	~ 300	NA	3.34

### **3.2.1 Mouse Mammary Gland Normal and Tumor Tissues**

The mouse models were used for the tests of the first and second phases. Mouse mammary gland tissue virus (MMTV)-Wnt-1 mice have overexpression of the Wnt-1 ligand under the MMTV promoter within the epithelial cells of the mammary gland. Mammary glands from virgin hemizygous female mice carrying the (Wnt1)<sup>1Hev</sup> transgene resemble hormonally-stimulated glands from pregnant mice (Li, Hively, & Varmus, 2000).

B6SJL-Tg (Wnt-1)<sup>1Hev</sup>/J mice were purchased from The Jackson Laboratory (Sacramento, CA, US) and housed according to IUCAC guidelines for humane treatment of animals. Upon the development of a mammary tumor ~ 2 cm in diameter, mice were euthanized via CO<sub>2</sub> asphyxiation and subsequent cervical dislocation according to the approved IUCAC protocol. Mammary tumors and the adjacent normal mammary tissue were harvested, and used as a fresh or fixed tissue.

### **3.2.2 Preparation of Normal and Tumor Histological Sections (Fixed Tissues)**

The standard tissue processing included a series of steps so that the soft tissue was supported in a medium for sectioning. The first step in preparation is dehydration that removes the water from the tissue by immersing it in ethanol. To minimize tissue distortion, the specimens are dehydrated in a graded ethanol series from water through 10%–20%–50%– 95%–100% ethanol and immersed for around 10–15 mins in each of them. In the next step, Histology- grade xylene (100%) is used to remove the extra ethanol from the tissues. The slides were immersed in xylene for about 5 mins. Finally, the tissues

were embedded using paraffin block that allows very thin sectioning. A microtome (RM2235, Leica Biosystems, Buffalo Grove, IL, US) was used to cut the tissues in 5- $\mu\text{m}$  thickness. After that, the tissue ribbons were transferred to a warm water bath ( $\sim 42\text{ }^{\circ}\text{C}$ ) that allowed them to float on the surface, and could be adhered onto a slide placed in hot water. Slides were allowed to dry in the air for a few hours to gradually melt the excess paraffin wax (Wick., Mills, & Brix, 2008).

For the first phase of the tests, the normal tissues from Liver and mammary glands of mouse models were collected. Some were used as fresh and frozen, and some were sliced and fixed on glass slides using the histology method.

### **3.2.3 Preparation of Silver and Gold Nanoparticles**

Silver nanoparticle solution was prepared by the method reported by Lee and Meisel (1982). Silver nitrate ( $\text{AgNO}_3$ –99.5%) and sodium citrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ –99%) were purchased from Science Fisher (Pittsburgh, PA, US). Deionized (DI) water with a resistivity  $\sim 18\text{ M}\Omega\cdot\text{cm}$  from a Reverse Osmosis (RO) system (VP-17-4010, Aries Filter works, West Berlin, NJ, US) was used to prepare all aqueous solutions.

According to Lee and Meisel (1982), 90 mg of silver nitrate was added to 500 mL water. This solution was heated to the boiling point and stirred for 5 minutes at 800 rpm with a vortex mixer (11-100-49SH, Fisher Scientific, Pittsburgh, PA). A 10 mL aliquot of 1% sodium citrate was added into the solution drop by drop at the boiling point, and heating continued until the color changed to yellow. The nanoparticle solution was cooled down to

the room temperature ( $22 \pm 1$  °C), and transferred to a brown bottle to keep away from direct light.

The gold nanoparticles were made using the classical method introduced by Turkevich in 1951 (Turkevich, Stevenson, & Hillier, 1951). Chloroauric acid ( $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$ ) and sodium citrate ( $\text{C}_6\text{H}_5\text{O}_7\text{Na}_3$ ) were purchased from Sigma Aldrich (St. Louis, MO, US), and DI water from the RO system (VP-17-4010, Aries filter works, West Berlin, NJ, US) was used to prepare all aqueous solutions. A 100 mL aliquot of 0.01% chloroauric acid solution was prepared, and 5 mL of 1% sodium citrate solution was added to it at the boiling point. The color of the solution changed to red that indicated the formation of gold nanoparticles in the solution.

The SEM images of silver nanoparticles and colloidal silver and gold nanoparticles are shown in Figures 3.1 and 3.2. The average diameter of silver nanoparticles was estimated around 70 nm. The size distribution of gold nanoparticles, measured with “Zetasizer Nano-S” instrument (Malvern Instruments Ltd., Malvern, UK), was estimated around 137 nm (Figure 3.3).

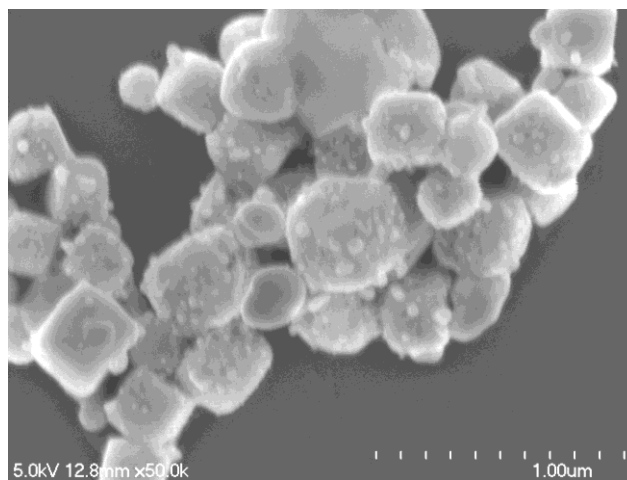


Figure 3.1 SEM image of silver nanoparticles



Figure 3.2 Colloidal gold (right) and silver (left) nanoparticles



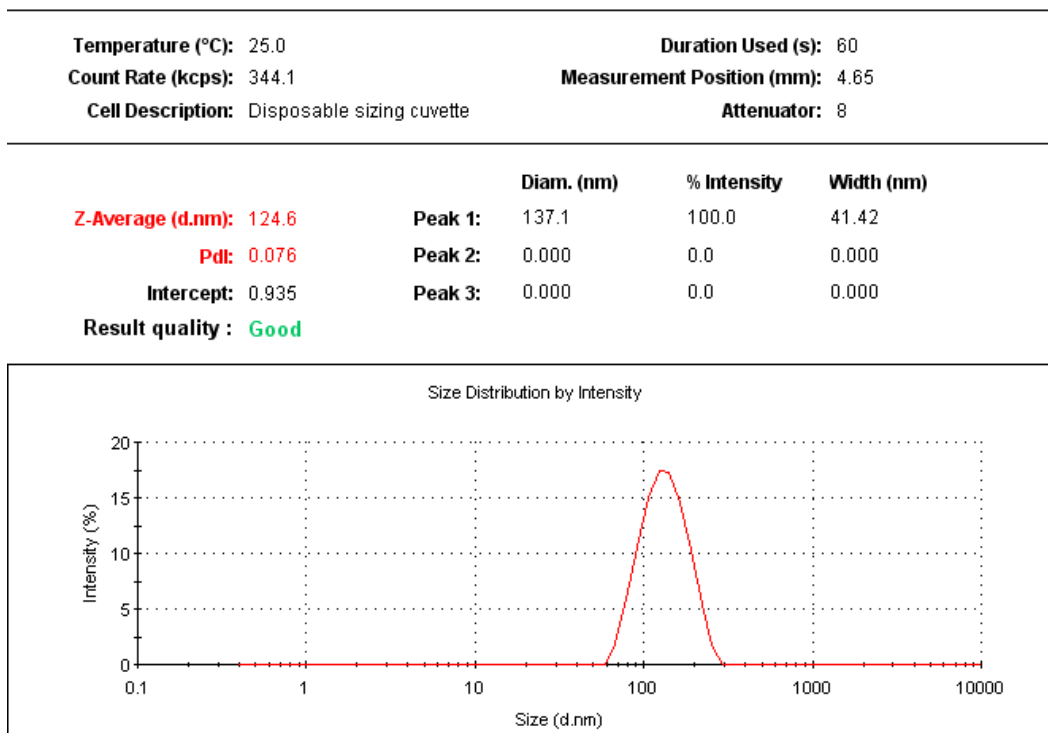


Figure 3.3 Size distribution of gold nanoparticles

### 3.2.4 Raman Spectrometer Set up

The RS, SERS, and CARS measurements were performed using the Renishaw InVia Reflex Raman microscopy system (Renishaw PLC., New Mills, Wotton-under-Edge, UK), Macro-Raman Spectrometer (customer-made by Dr. Alan Gift, Department of Chemistry, University of Nebraska- Omaha), Deep UV-Raman Spectrometer (Renishaw PLC., New Mills, Wotton-under-Edge, UK), and CARS microscope (customer-made by Dr. Yongfeng Lu, Department of Electrical Engineering, University of Nebraska- Lincoln), respectively. Different laser wavelengths can be illuminated to the sample. The Micro Raman Spectrometer is capable to provide laser in three different wavelengths, 530, 633, and 785

nm. The measurement process in Macro-Raman Spectrometer is similar to Micro-Raman, but the Macro-Raman focuses on a larger area of the tissue ( $\text{mm}^2$ ), and it is limited to single laser wavelength (785 nm). UV-Raman Spectrometer provides laser in the wavelength of 244 nm. For the CARS, the laser powers for the pump and the broadband Stokes lasers before entering the microscope are 30 and 55 mW, respectively. Due to the nonlinear process, the laser overlap spot is estimated to be around 300 nm in diameter. Figures 3.4, 3.5 and 3.6 show a Reinshaw Micro-Raman Spectrometer, customer-made Macro-Raman Spectrometer, and a CARS.



Figure 3.4 Reinshaw Micro- Raman spectrometer

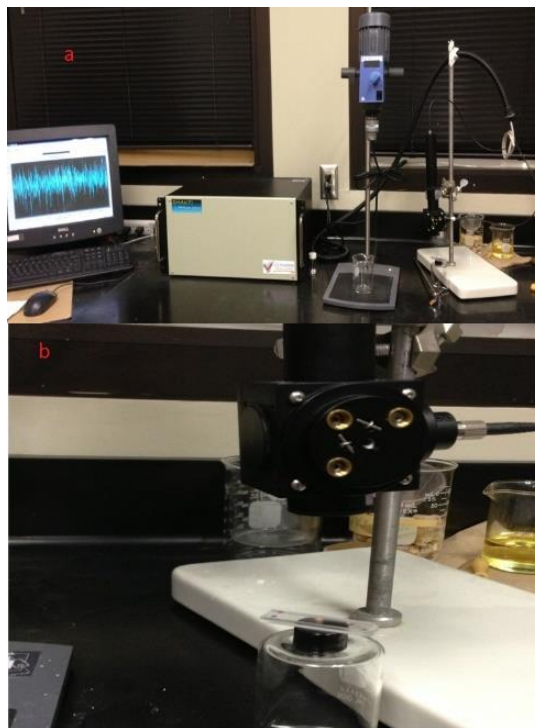


Figure 3.5 (a) Customer- made Macro-Raman spectrometer system. (b) Fresh Mammary gland tissue under the Macro-Raman microscope for testing

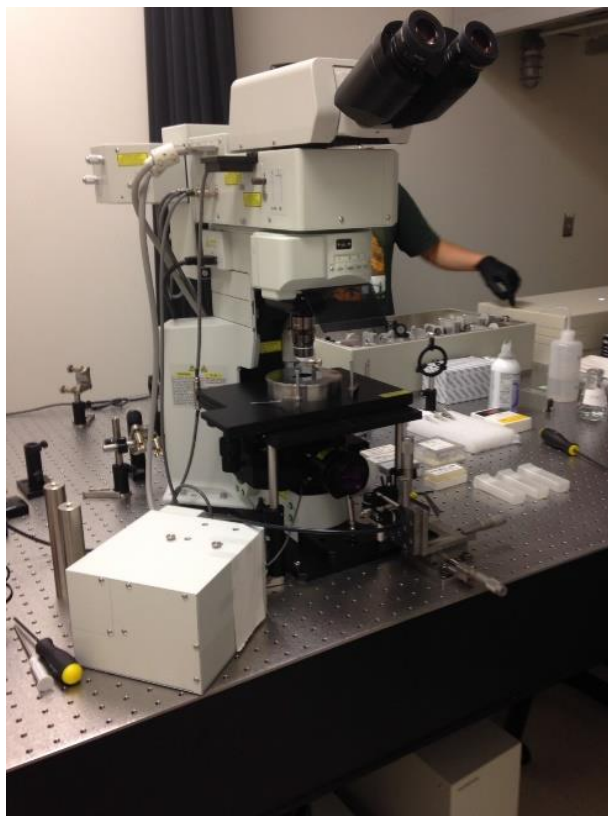


Figure 3.6 CRAS Instrument (most of the optic lens are not shown) used in this study

### 3.2.5 Test Conditions for the First Phase

In the first sets of experiments (SERS T1-T7, shown in Table 3-1), the Macro-Raman and Micro-Raman spectrometers were used to perform the tests on fresh, frozen and fixed tissues from mouse models. The laser wavelength of 785 nm was used to conduct the tests with Macro-Raman spectrometer and laser wavelength of 785 and 514 nm was used to perform Micro-Raman tests.

For tests with fresh and frozen tissues (SERS T2-T7, shown in Table 3-1), a small part of mammary gland tissue (around 3 mm in diameter) was placed on a glass slide under the Raman microscope lenses. The tests on fresh and frozen tissue were performed by mixing the tissue with one drop of silver or gold nanoparticles for enhancement. For the fixed tissue (SERS T1, shown in Table 3-1), one drop of silver or gold nanoparticles was dropped on the surface of tissues, dried in air for 10 minutes and then tested on the Raman microscope. From the first phase, we concluded that the optimum conditions to get the strongest SERS shifts is using fixed tissues on gold slides.

### **3.2.6 Test Conditions for the Second Phase**

The tests of SERS-T8, shown in Table 3.1 were conducted using SERS and CARS, using the fixed normal and tumor mammary gland tissues from mouse models. For the SERS tests (SERS-T8, shown in Table 3.1), the normal and tumor tissues were fixed on gold slides. In order to have a more accurate approximation of the difference between normal and cancer tissues, the spectra was acquired from 23 points of cancer tissue and 32 points of normal part.

CARS tests (RI-T1 &T2, CARS-IMG-T1 & T2, CARS-SPT-T1 & T2, shown in Table 3.1) were conducted as CARS imaging and CARS spectroscopy. In both sets, normal and tumor mammary gland tissues from mouse models were fixed on glass slides, and tests were conducted in the CH region ( $2800\text{--}3000\text{ cm}^{-1}$ ). The CARS images were taken in different depths of the fixed tissues and results were shown as a lipid concentration in

normal and tumor slides. 20 spectrum for both normal and tumor tissues were collected, and the method was tested using different mice. The pump and the broadband Stokes were set at 35 and 55 mw, respectively. From this phase, it was concluded that CARS spectroscopy has the highest potential to differentiate cancer tissues in different stages.

### **3.2.7 Test Conditions for the Third Phase**

The third phase of the tests (RU-UV-T1 & CARS-SPT-T3, shown in Table 3-1) was conducted using human breast tissues, fixed on glass slides. UV-Raman and CARS spectroscopy were used to perform tests in this stage. Three types of cancer tissues from different stages (Ductal carcinoma in situ (DCIS), invasive, and advanced) were selected for the sets of experiments. Figures 3.7 to 3.10 show human breast tissues fixed on glass slides in different stages.

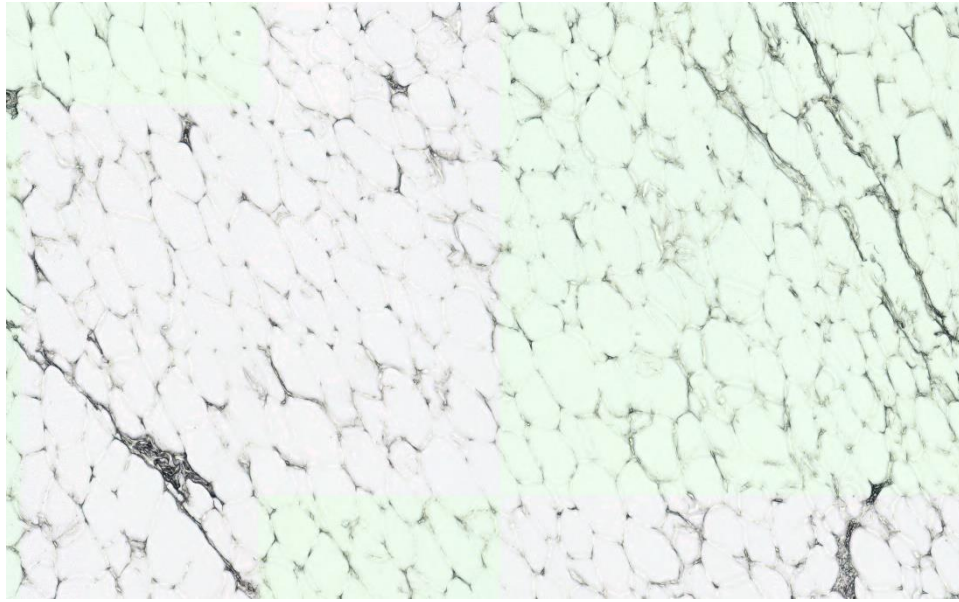


Figure 3.7 microscopic image of a benign breast tissue fixed on glass slide

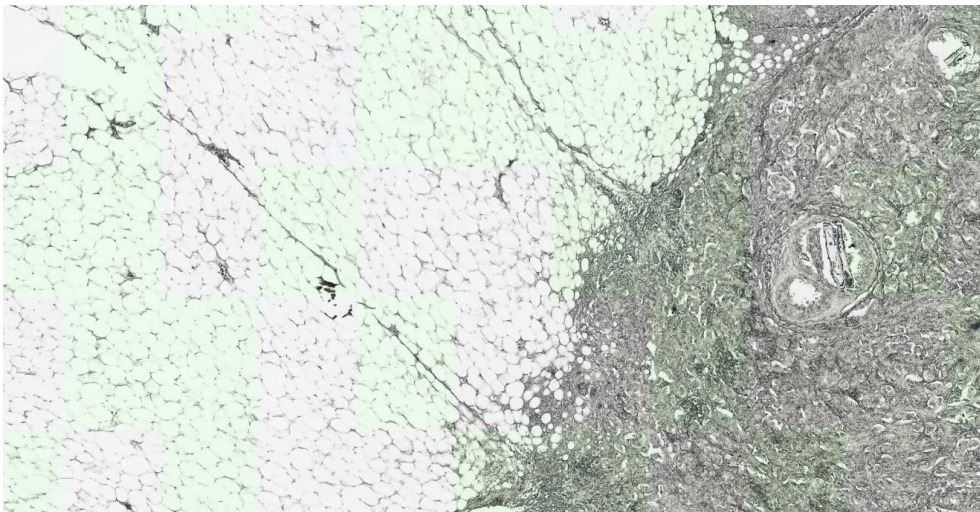


Figure 3.8 A microscopic image of a benign and stage-1 breast tumor tissue fixed on glass slide

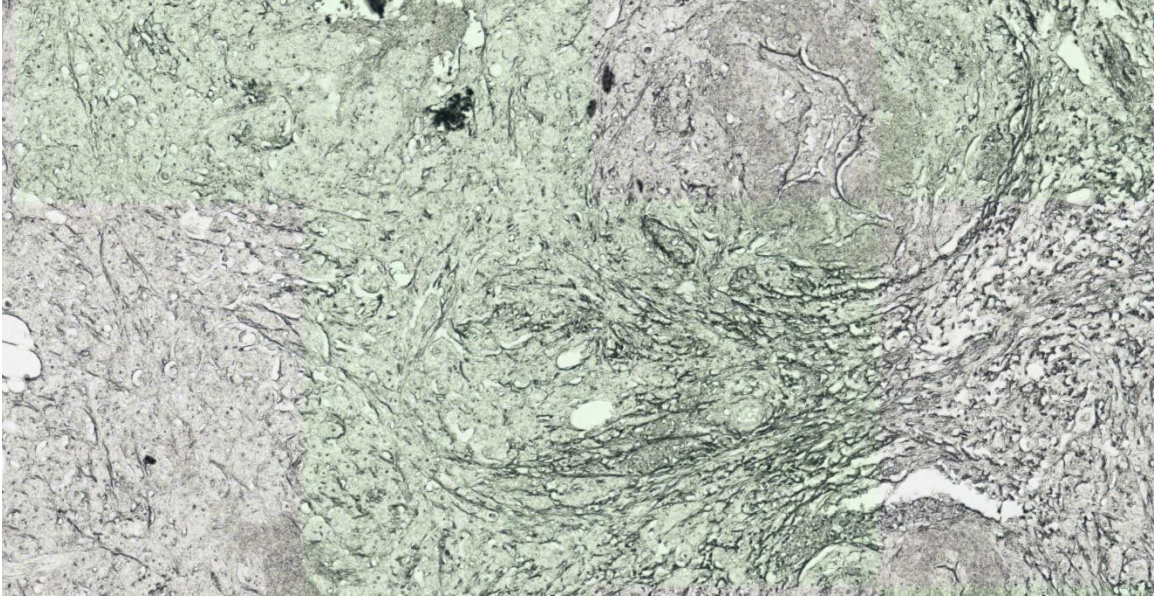


Figure 3.9 A microscopic image of a stage-2 breast tumor tissue fixed on glass slide



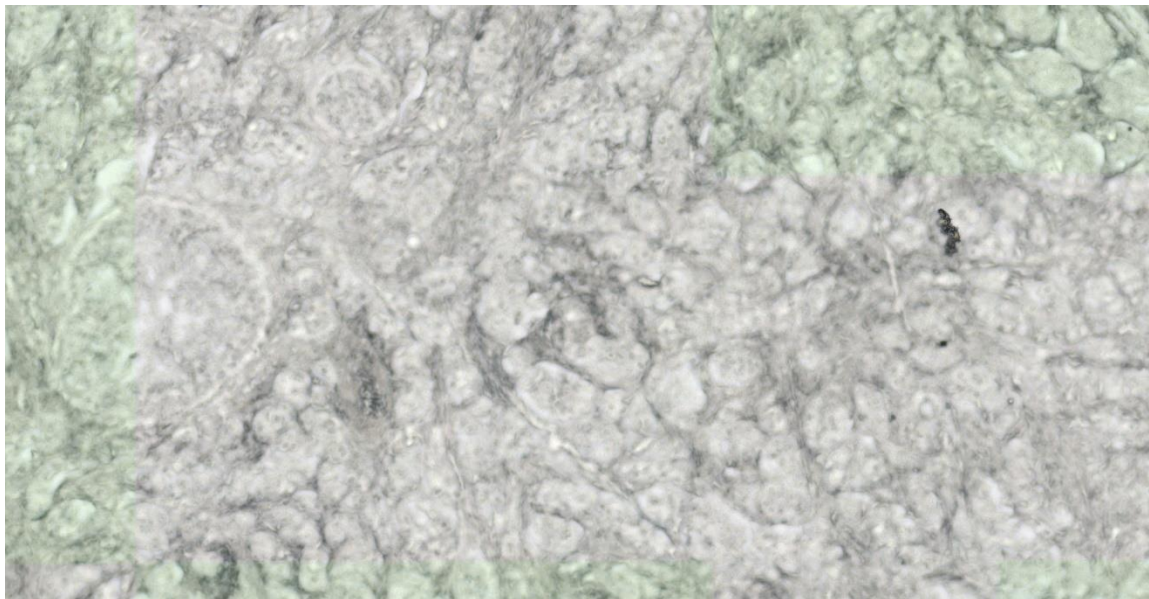


Figure 3.10 A microscopic image of a stage-3 breast tumor tissue fixed on glass slide

DCIS is a non-invasive cancer where abnormal cells are in the lining of the breast milk duct. Invasive cancer, happens once the tumor grows into surrounding, healthy tissues. Advanced cancer is when the tumor has started to spread out to other parts of the body. Figures 3.7 to 3.10 show the microscopic images of benign breast tissue and these three groups of cancer tissues. Each cancerous slide is composed of the benign and tumor tissues. Therefore, we tried to focus on the tumor part in all tests. The tests were conducted using UV-Raman and CARS. The total of 25 spectrum from each slide have been collected using UV-Raman. The wavelength of the UV-Raman used in tests was 244 nm, and the range of RS wavelength was from 400–1800  $\text{cm}^{-1}$ . The tests were repeated using CARS spectroscopy with the same conditions explained in Section 3.2.4.

### 3.2.8 Data Analysis

The first step in analyzing RS/SERS results is removing fluorescence background from SERS signals. The “Vancouver Raman Algorithm (VRA),” software (version 1.0.0, developed by BC cancer agency technology office) was used to remove fluorescence background. To do so, the raw data from the Raman system were imported into VRA, and the fluorescent background was removed before final analysis.

There are a couple of methods used to analyze the results of RS, SERS, and CARS in the second and third phases. Two band ratio, PCA, and PC-DFA are the methods which were used to analyze the results in this study.

Two band ratio method is one of the most common techniques used to analyze SERS data. This method has been mainly used to differentiate different types of bacteria according to SERS results of each category (Liu, Chao, Nou, & Chen, 2009). In this method, the intensity ratio between two Raman bands in Raman spectrum is calculated to describe the results. We used two-band ratio technique to analyze results of SERS and UV-Raman. Due to the lack of DNA peak, we used the RNA peak in  $806\text{ cm}^{-1}$  to describe the results.

Principal component analysis (PCA) is another statistical procedure to analyze big data, which is capable of detecting small spectral differences (Lin, et al., 2011). PCA converts a large number of observations (Raman Spectrum) from different points of samples into linear combinations of the few independent vectors called principal components (PC) (Guo, et al., 2009). PCA creates a new set of variables, defined as

principal components (PC) and scores (S) (Martin, Carter, de Oliveira Nunes, Arisawa, & Silveira Jr, 2004). The PCs represent the most important variation that occurs in all spectra; the first PCs describe most of the variation of the data, and the last one carry only noise. S values are also related to the weight of each PC to recreate the original data. The PCA method was used to analyze SERS results from the second part to differentiate normal and tumor mammary gland tissues in mouse models.

PCA techniques were used to analyze the SERS results. To create the model based on the PCA scores, the data sets composed of 55 Raman spectrums (23 points for normal and 32 points for cancer tissues) were used. The PC and S were extracted from the sets of data and among the 20 scores, the 2nd and 3rd scores were used as diagnostics. By using this type of analysis it was possible to differentiate the tissues into two groups: normal and cancer. The first PC vector is in the direction of data with the highest variance, and the second PC is in the direction of data with the second highest variance and so on. The PC1 and PC2 are usually enough to describe more than 90% of the data, and PC3, PC4, PC5, PC6, PC7 and PC8 don't contribute on the data analysis that much.

PC-DFA is another technique that has been used to differentiate normal and cancer mammary gland tissues from mouse model according to the results of CARS spectroscopy. PC-DFA utilizes the "mixOmics" package in R software. The PCA method was applied first to reduce the dimensionality of the CARS spectrum to 20 principal component scores (PCs), which accounted for greater than 80% of the variance. DFA was then performed on these PCs, which served as input-independent variables for the analysis

### 3.3 Results and Discussion

#### 3.3.1 Raman Spectrum from Mouse Models Using Macro-Raman (SERS T1-T3)

The results of the first phase of the tests are shown in Figures 3.11 to 3.13. As it is demonstrated, the Raman Signals are strong when the tissue with a small thickness (5 micron) is fixed for the test. Fresh and frozen tissues that have a larger diameter (3 mm) don't show very strong Raman signals.

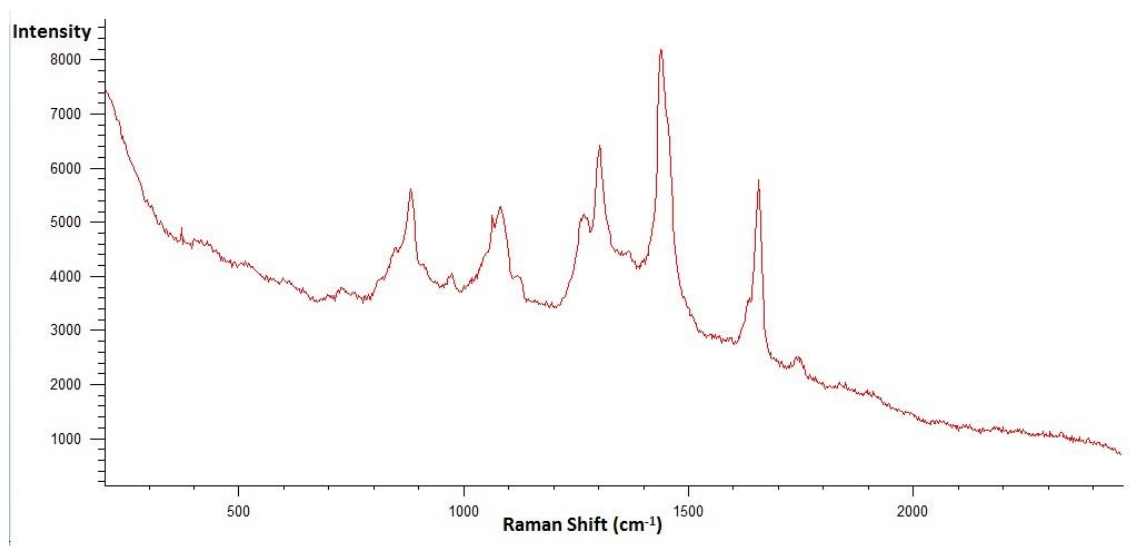


Figure 3.11 Raman spectrum of fixed normal mammary gland tissue using Macro- Raman (SERS-T1)

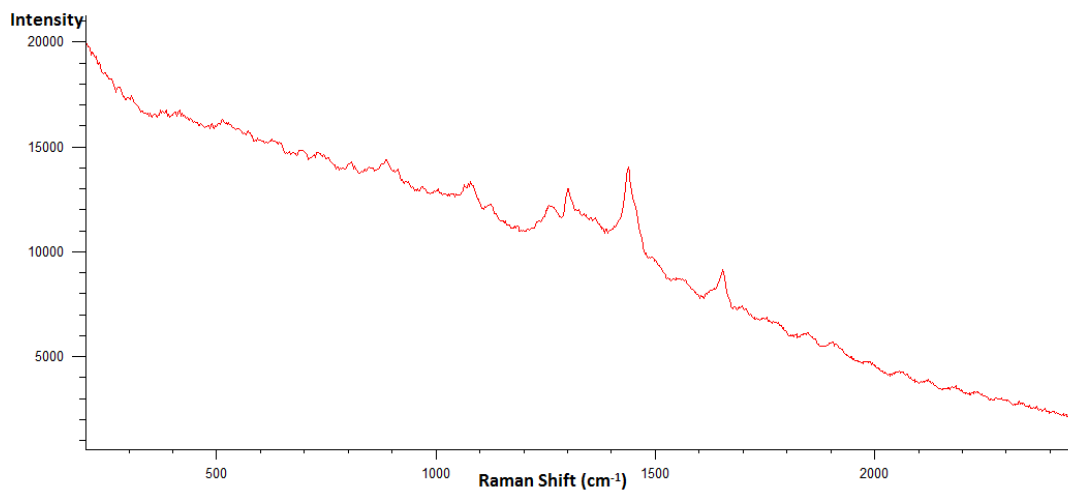


Figure 3.12 Raman spectrum of fresh normal mammary gland tissues using Macro-Raman (SERS-T2)

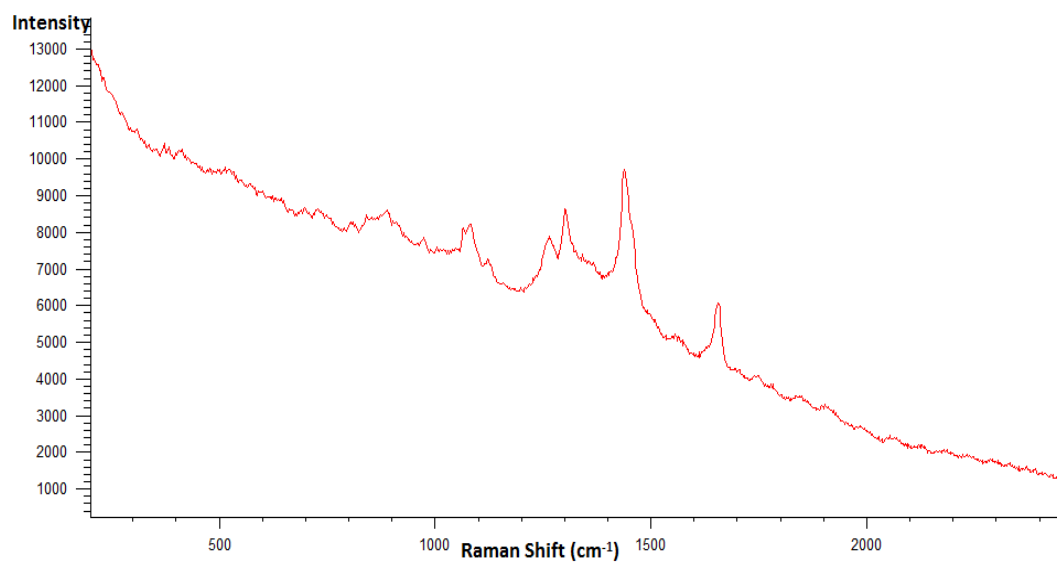


Figure 3.13 Raman spectrum of frozen normal mammary gland tissues using Macro-Raman (SERS-T3)

### 3.3.2 Raman Spectrum from Mouse Models Using Micro-Raman (SERS T4-T7)

The testing on fresh normal tissues using Micro-Raman spectrometer under the laser wavelength of 785 nm shows a stronger Raman spectrum, compared to the results from Macro-Raman. As shown in Figures 3.14 and 3.15, both types of nanoparticles (silver and gold) enhance Raman signals, but silver nanoparticles show higher enhancement which is consistent with the literature.

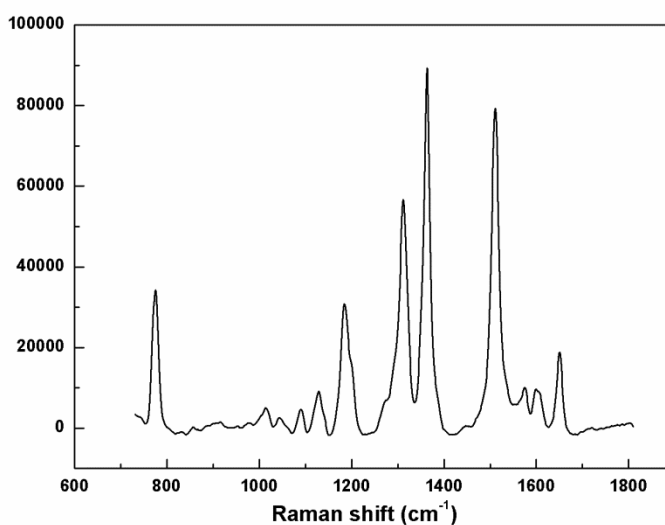


Figure 3.14 Raman spectrum from fresh normal mouse mammary gland tissues using gold nanoparticles (SERS-T4)

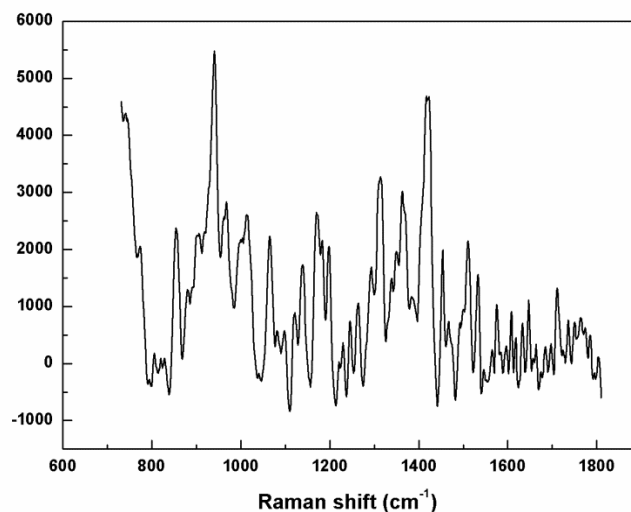


Figure 3.15 Raman spectrum from fresh normal mouse mammary gland tissues using silver nanoparticles (SERS-T5)

Figures 3.16 and 3.17 show the results of testing on the fresh sample from mouse mammary gland and lung using silver nanoparticles at the laser wavelength of 514 nm. As it is demonstrated, the Raman spectrum of Lung tissues show a high enhancement in the wavelength of 514 nm, compared to mammary gland tissues.

Although the Raman spectrum looks strong in these sets of experiments (i.e., SERS T1 to T6), the results are not easily repeatable. Because of the rough shape of the fresh tissue, the results may change by changing the focus point. Also, results highly depend on the position of the nanoparticles in the sample, and formation of hot spots. Therefore, the tests for the next steps were conducted using fixed tissues on gold slides.

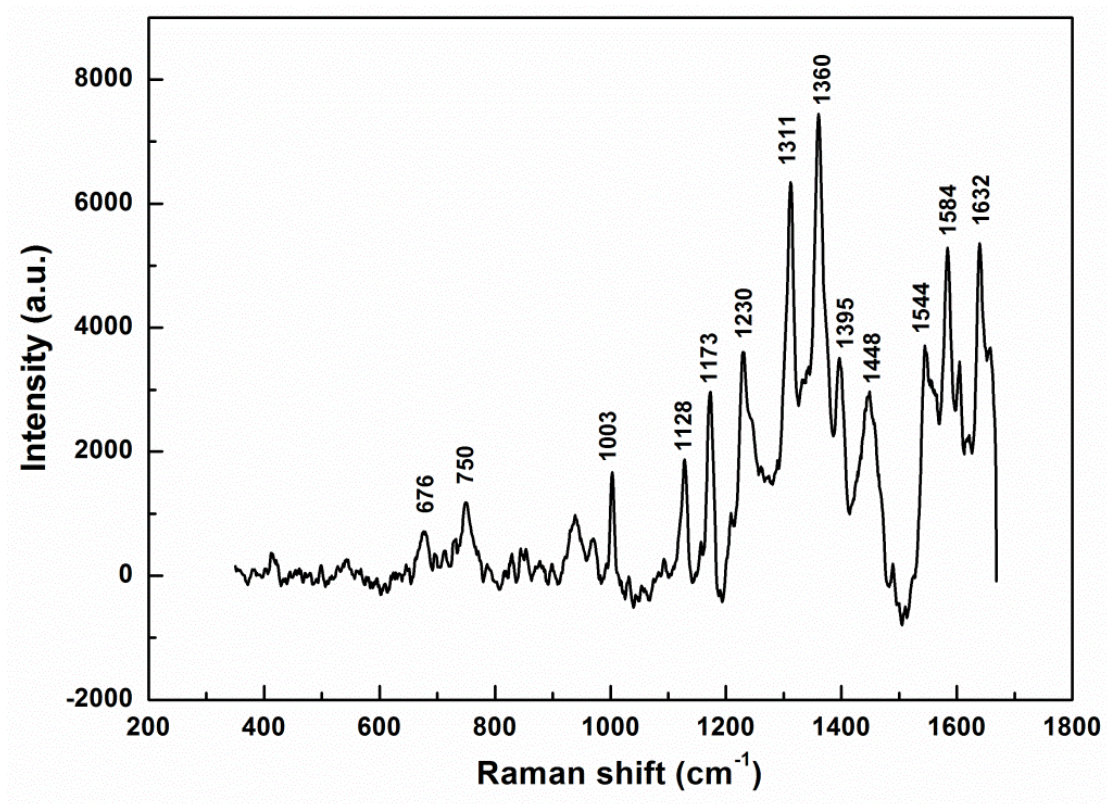


Figure 3.16 Raman spectrum from mouse lung using silver nanoparticles (SERS-T6)



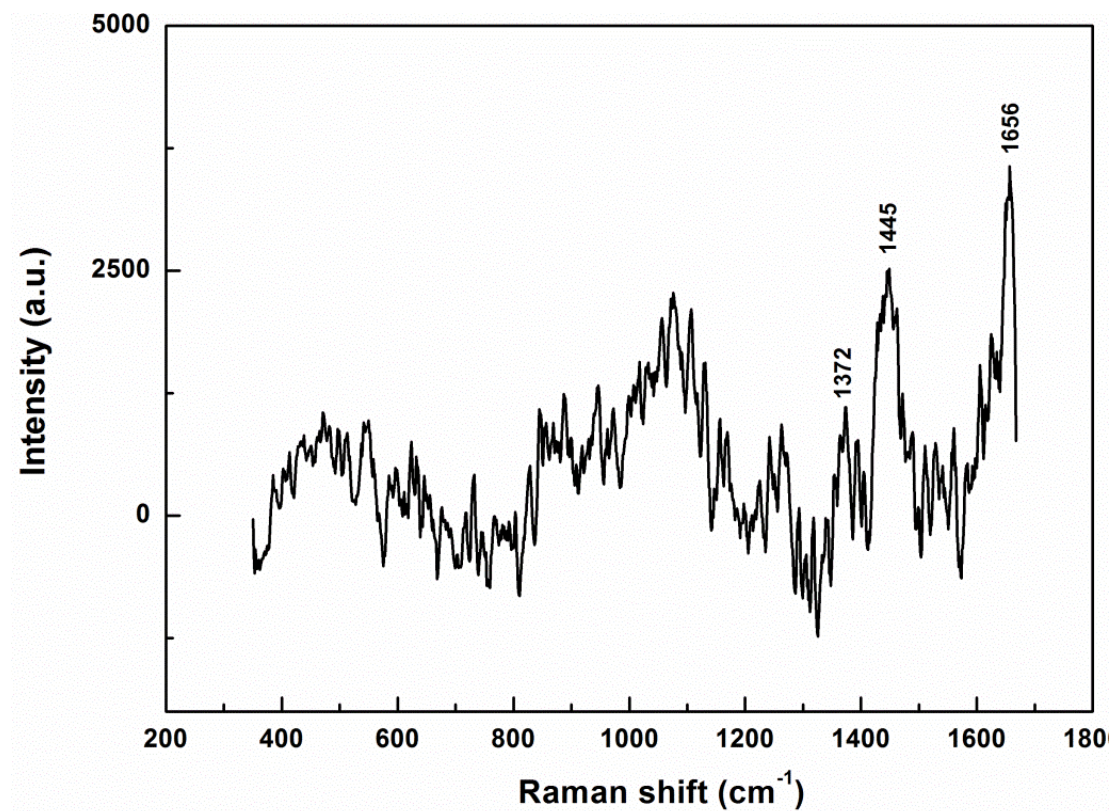


Figure 3.17 Raman spectrum from mouse mammary gland using silver nanoparticles (SERS-T7)

### 3.3.3 Raman Spectrum of Fixed Tissues on Gold Slides (SERS-T8)

The testing of mammary gland tissues fixed on gold slides was performed using a Micro-Raman spectrometer at the laser wavelength of 785 nm. Figure 3.18 shows the mammary gland tissues under the micro-Raman microscope. The cancer tissues have large numbers of irregularly shaped cells, which makes them easily distinguishable from normal tissues

under the Raman microscope. The averaged Raman results from normal and tumor tissues after applying VRA and removing the background are shown in Figures 3.19 and 3.20.

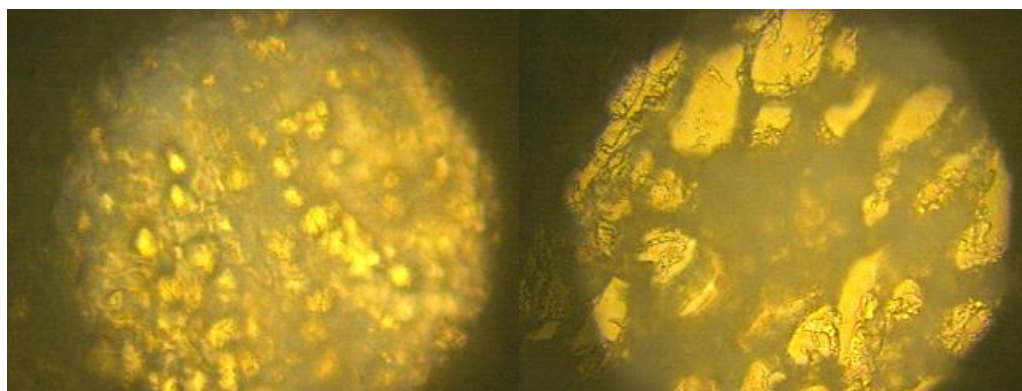


Figure 3.18 cancer (left) and normal (right) mammary gland tissue under Raman microscope

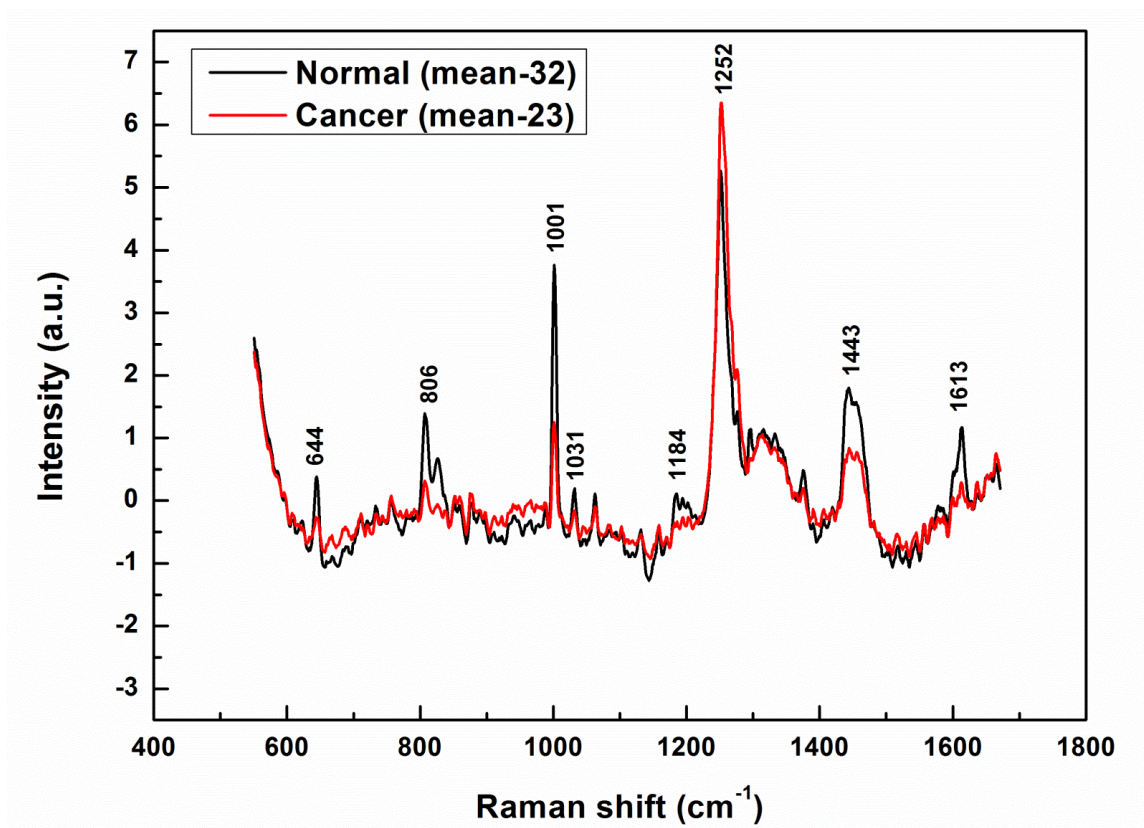


Figure 3.19 Averaged Raman spectrum from normal (black) and cancer (red) of mammary gland tissues (SERS-T8)

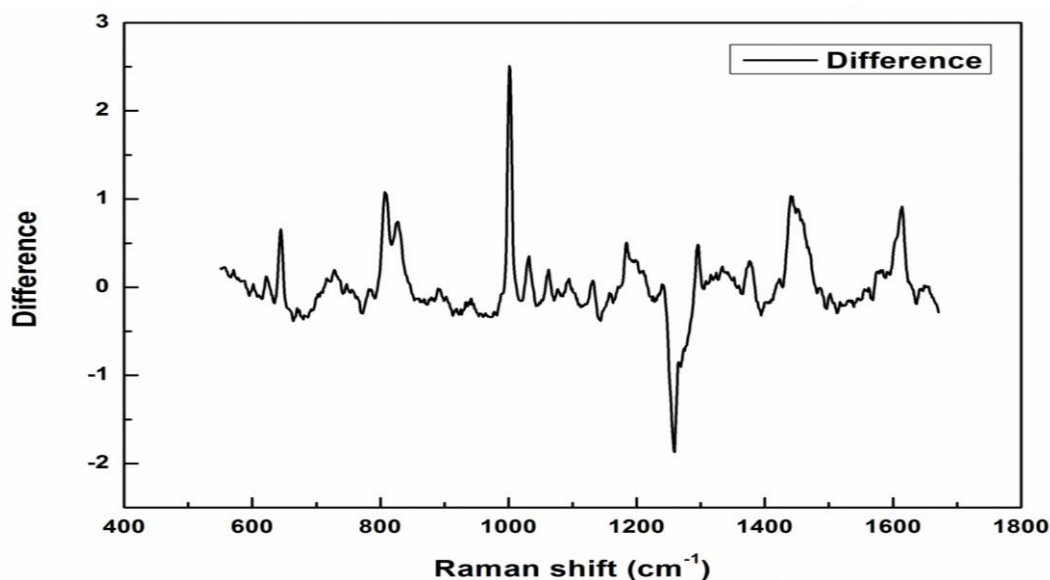


Figure 3.20 Difference graph between Raman spectra of normal and cancer tissues

Figure 3.19 shows that most of the Raman peaks from both tissues look similar, which reflects that the composition of both types of tissues are almost the same. According to Figure 3-20, Raman shifts were observed in the wavelengths of  $644\text{ cm}^{-1}$ ,  $806\text{ cm}^{-1}$ ,  $1001\text{ cm}^{-1}$ ,  $1184\text{ cm}^{-1}$ ,  $1252\text{ cm}^{-1}$ ,  $1443\text{ cm}^{-1}$ , and  $1613\text{ cm}^{-1}$ . The clear difference are in the peaks of  $644\text{ cm}^{-1}$ ,  $806\text{ cm}^{-1}$ ,  $1001\text{ cm}^{-1}$ ,  $1252\text{ cm}^{-1}$ , and  $1443\text{ cm}^{-1}$  that relate to the protein, RNA, phenylalanine, lipids, and collagen (phospholipids) (Aydin, Altaş, Kahraman, Bayrak, & Çulha, 2009). The difference in the peak of  $806\text{ cm}^{-1}$  evidences that once the tumor synthesizes, the RNA changes. It was also expected to see a change in DNA composition in the wavelength of  $722\text{--}725\text{ cm}^{-1}$  due to the changes in DNA of cancer tissues that was already confirmed in previous studies (Aydin, Altaş, Kahraman, Bayrak, & Çulha, 2009), but the DNA peaks were not visible in these sets of tests conducted in this study.

Figure 3.21 shows that the intensity ratio ( $I_{806}/I_{1251}$ ) has a significant difference for normal vs cancer tissues. The mean ratio of  $I_{806}/I_{1251}$  (Mean  $\pm$  Standard Division (SD)) is  $0.45 \pm 0.37$  for normal and  $3.11 \pm 0.87$  for cancer tissues, which are significantly different. A student t-test was also performed on the Raman spectrum which estimated the P- value of  $\sim 0.0001$  that indicates cancer and tumor tissues are extremely different. The results also demonstrate the capability of using lipid band to differentiate normal and cancer tissues, which was used in CARS imaging/spectroscopy later.

The PCA method was another technique to analyze Raman data. Figure 3-22 shows the bi-plot of PC1 versus PC2. PC1 and PC2 scores were plotted to separate the data into the two types of tissues according to the information found in each PC. As shown, some of the points in normal and cancer tissues overlap with each other, but around half of the points are completely separated. As the PCA method considers all the data acquired from Raman spectrum, the results look reasonable because the Raman spectrum of both tissues have a lot of similarities. In this case, the two band ratio model is more appropriate to differentiate two groups of tissues as it uses the two main peaks that are apparently different in normal and cancer tissues.

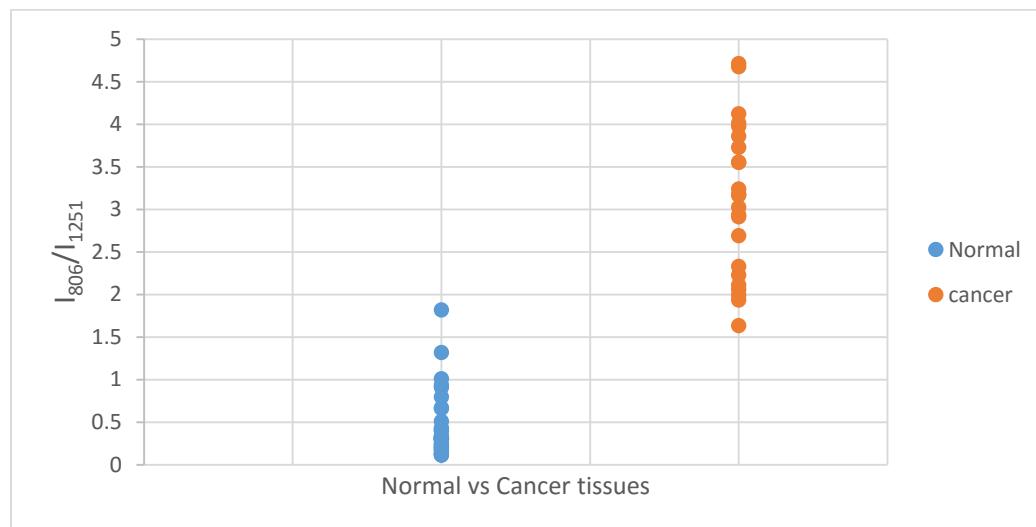


Figure 3.21 Difference between Raman spectrum of normal and cancer mammary gland tissues fixed on gold slides using two band ratio method (SERS-T8)

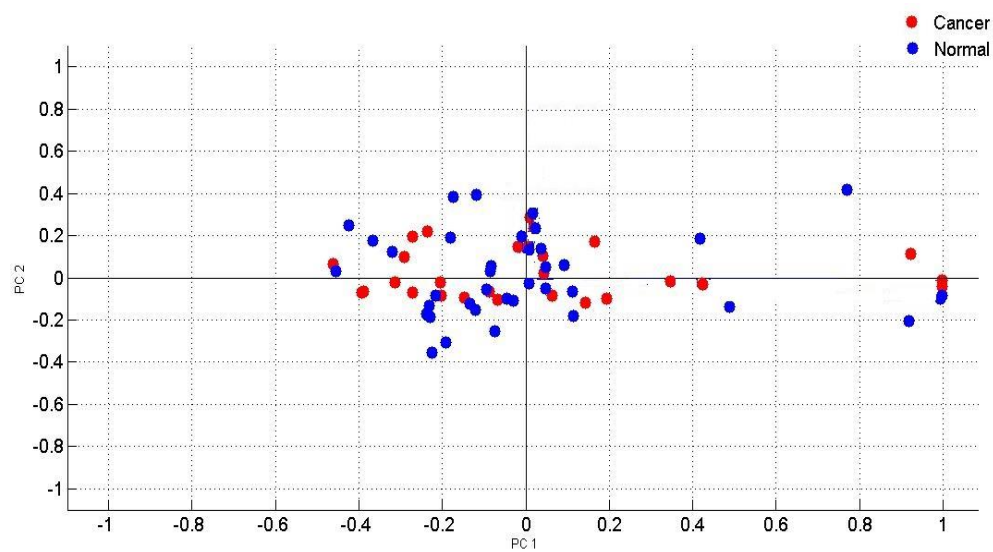


Figure 3.22 PCA results from Raman spectrum of normal and cancer tissues (SERS-T8)

### 3.3.5 Raman Imaging of Fresh Tissues (RI-T1-2)

The results of the Raman mapping from mammary gland and liver tissues are shown in Figures 3.23 and 3.24. The Raman microscope was focused on a  $50 \times 50 \mu\text{m}^2$  tissue area. Results were taken at the wavelength of  $1000 \text{ cm}^{-1}$  (phenylalanine) and  $1600 \text{ cm}^{-1}$  (lipids) that showed the strongest peaks on Raman spectrum. Red spots indicate the higher density of the composition (Phenylalanine or Lipids) and the black points show a lower density. Unfortunately, the images are not clear enough to provide additional useful information. In order to have a more visible Raman mapping, we need to focus on a larger area and get more points, but it would take a long time. Fortunately, CARS is able to provide results with a higher resolution in a shorter time (see below).

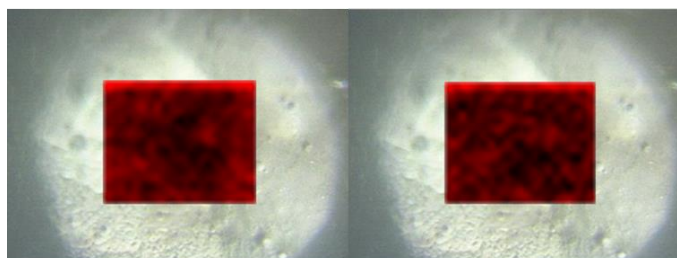


Figure 3.23 Raman imaging from mammary gland tissue at  $1600 \text{ cm}^{-1}$  (right) and  $1000 \text{ cm}^{-1}$  (left) (RI-T1)

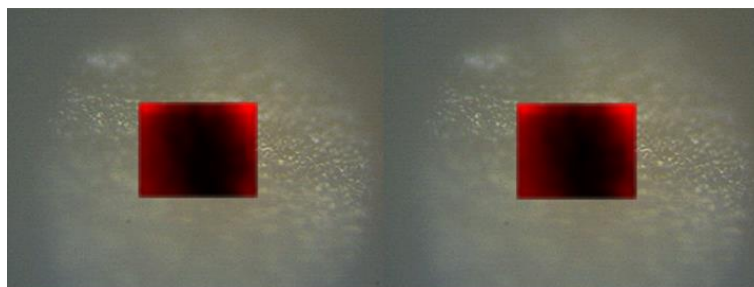


Figure 3.24 Raman imaging from liver tissue at  $1600 \text{ cm}^{-1}$  (right) and  $1000 \text{ cm}^{-1}$  (left) (RI-T2)

### 3.3.6 CARS Imaging of Fixed Tissues (CARS-IMG-T1-2)

The results of CARS imaging on normal and tumor mouse mammary gland tissues are shown in Figures 3.25 and 3.26, which indicate that the fibrous lipid in vessels of normal and tumor tissues can be identified by CARS microscopy. Tumor tissues need more blood vessels to deliver nutrients and thus, have more growth of vessels. Therefore, it might be possible to detect the cancer tissues and differentiate them from normal cells by quantifying the density of lipid fiber via CARS images. The results of CARS imaging show that there is a possibility of using the combination of Raman spectroscopy and CARS imaging as CARS spectroscopy with focusing on lipid concentration for cancer detection which will be shown in the next section.

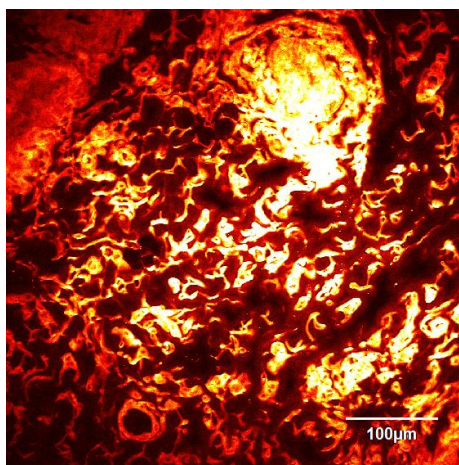


Figure 3.25 CARS imaging of fixed normal mammary gland tissue using lipid filter (CARS-IMG-T1)



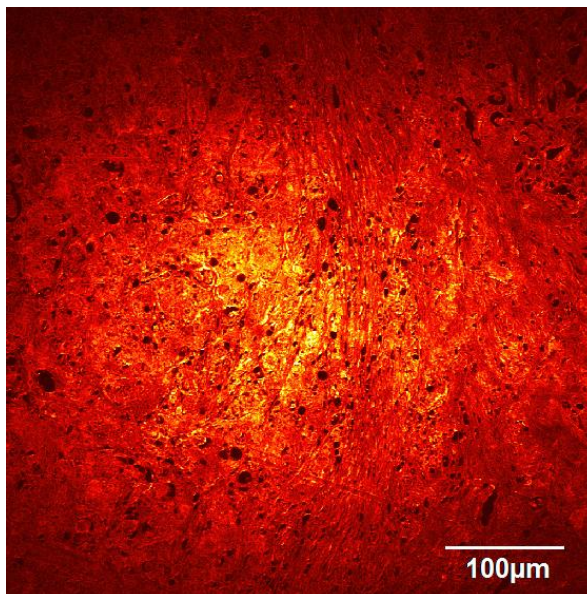


Figure 3.26 CARS imaging of fixed tumor mammary gland tissue using lipid filter (CARS-IMG-T2)

### **3.3.7 CARS Spectroscopy of Normal and Tumor Fixed Tissues from Mouse Model (CARS-SPT-T1 & T2)**

Figures 3.27 and 3.28 show the averaged CARS spectra in lipid band ( $2500 \sim 3100 \text{ cm}^{-1}$ ) from normal and tumor tissues fixed on glass slides. The PC-DFA technique was used to analyze data from CARS spectroscopy. As it is shown in Figure 3.28, that the two types of tissues can be differentiated using CARS spectra in lipid band ( $2500 \sim 3100 \text{ cm}^{-1}$ ).

As it is demonstrated, CARS spectroscopy is a fast technique capable to differentiate between normal and cancer tissues. Although there are some limitations about using CARS (e.g., the range of spectra is not as broad as Raman), it is a very fast technique

and an appropriate one for any diseases that are related to changes in the lipid content of the tissue. The accuracy of the data was confirmed using the cross validation method that is explained in Appendix A.

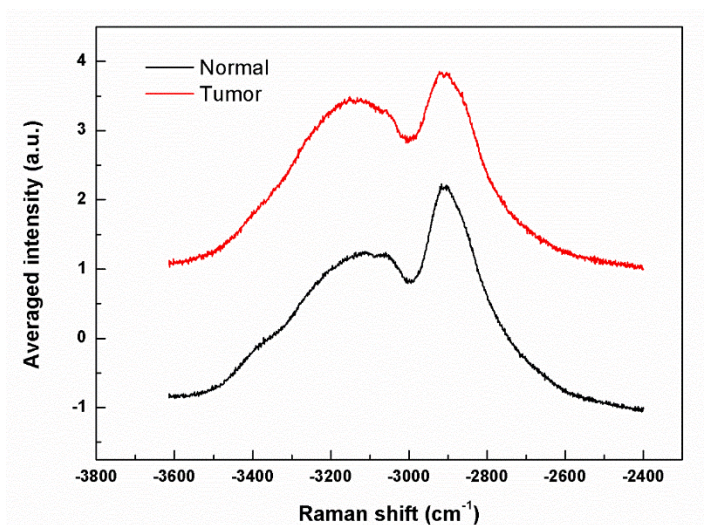


Figure 3.27 Normalized and averaged spectrum for tumor and normal mammary gland tissues fixed on glass slide (CARS-SPT-T1)

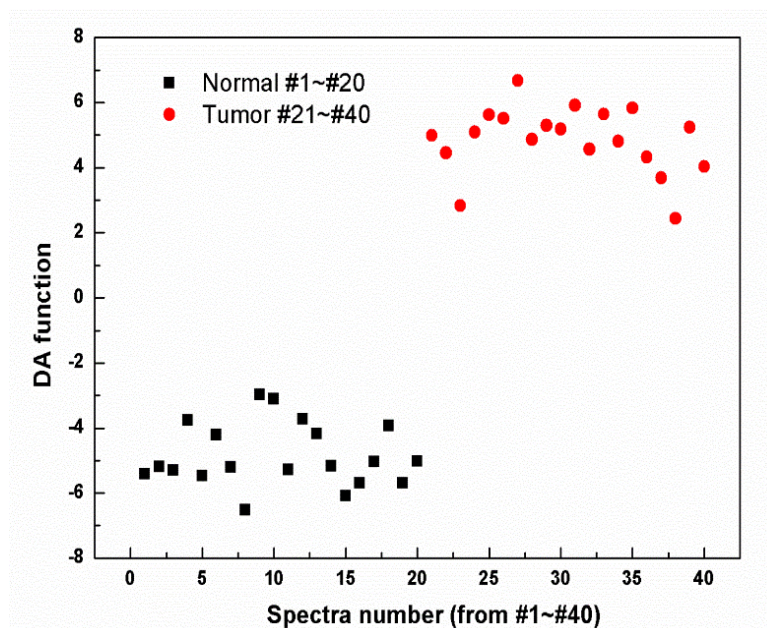


Figure 3.28 PC-DFA analysis of fixed normal and tumor mammary gland tissue using CARS

Figure 3.29 shows the averaged CARS spectra from the total of 40 spectra for normal and tumor tissues. Figure 3.30 shows that total 160 spectra are completely separated into two groups of cancer and normal as expected. The results show that CARS spectroscopy is able to differentiate normal and cancer tissues. Therefore, applying CARS spectroscopy and statistics is a feasible technique to distinguish different stages of cancer. The results of this section will be used in Section 3.3.6 to differentiate cancer in different stages using human tissues as models.

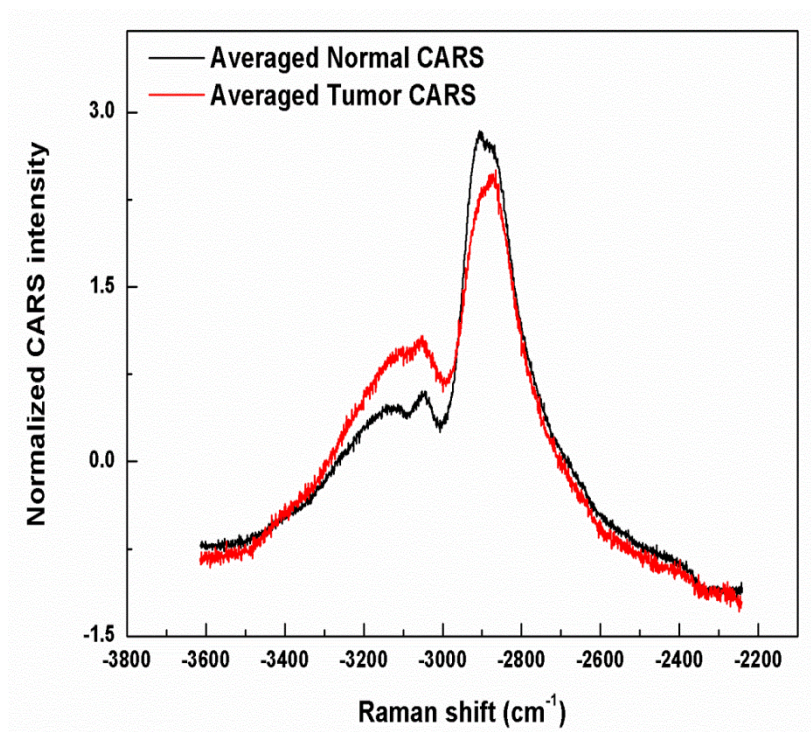


Figure 3.29 Averaged CARS spectrum of normal and tumor tissues (CARS-SPT-T2)

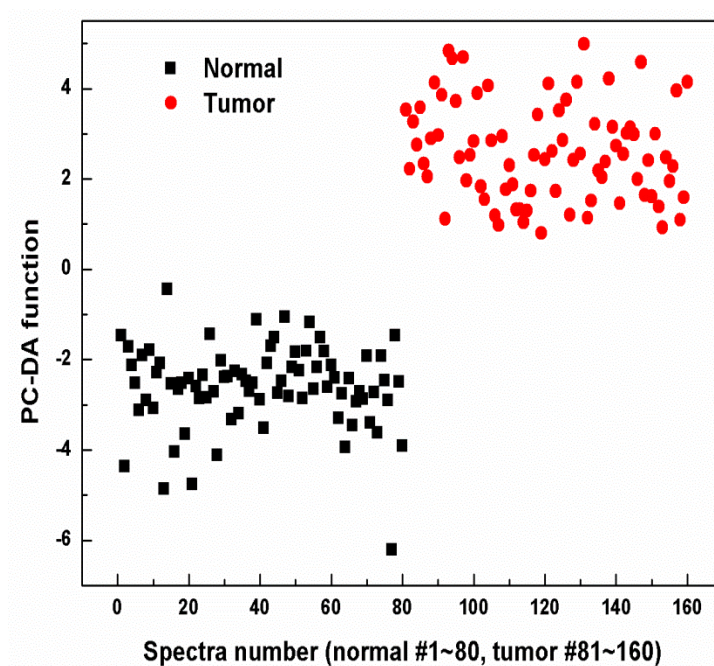


Figure 3.30 PC-DFA analysis of fixed normal and tumor mammary gland tissue from four different mice, using CARS

### 3.3.8 UV-Raman Spectroscopy of Human-Fixed Tissues (RS-UV-T1)

Figure 3.31 shows the averaged raw Raman spectra from the four different types of human tissues, including benign and stages 1–3, after background deduction. The general peaks of the spectra for all of the types are located in the same wavelengths because of the same nature of all tissues. The two-band ratio technique was chosen as the best statistical method to distinguish the minor differences between these four groups of the tissues. According to Figure 3.32, the RNA of benign and tumor tissues at the first stage are very similar. The difference between stage 1 and stage 2 is noticeable, but stages 2 and 3 are almost on the

same range. Once cancer develops from stage 1 to stage 2 and start becoming invasive (in stage 3), a big change occurs in the composition of RNA, which makes the RS diagnosis easier. Figure 3.33 shows the results of the two-band ratio model using RNA over lipid band. As it is shown, there is a clear change once the tumor develops from benign to stage 1. This is a very good indicator of the cancer development in the very early stage.

Comparison between Figure 3.32 and Figure 3.33 indicates that choosing the different two bands would result in very different ratios, which then can be used to differentiate tissues at different stages. Therefore, if a computer program is developed such that the computer can automatically analyze the Raman spectra and find the maximum differences between the two-band ratios of different tissues at different stages, it may be possible to use Raman-based technology for early cancer detection in the future. An excel program was developed to analyze the maximum difference between benign and stage-1 cancer tissues. The program was designed to calculate the  $I_n/I_{n+1}$ . The maximum difference was observed in the ratio of  $I_{470}/I_{1588}$  which corresponds to the polysaccharides over C–C stretching (protein) and guanine (Aydin, Altaş, Kahraman, Bayrak, & Çulha, 2009). As shown in Figure 3.34, the mean ratio of  $I_{470}/I_{1588}$  (Mean  $\pm$  Standard Division (SD)) is  $0.58 \pm 0.37$  for normal and  $2.11 \pm 0.23$  for stage 1 of cancer tissues, which are significantly different. A student t-test was also performed on the raw data which estimated the P- value of  $\sim 0.001$  that indicates benign and stage 1 of cancer tissues are extremely different. This analysis shows that the changes in the ratio of  $I_{470}/I_{1588}$  (i.e., polysaccharides to C-C

stretching of protein and guanine) of the tissues could be an indicator to diagnose cancer in early stages.

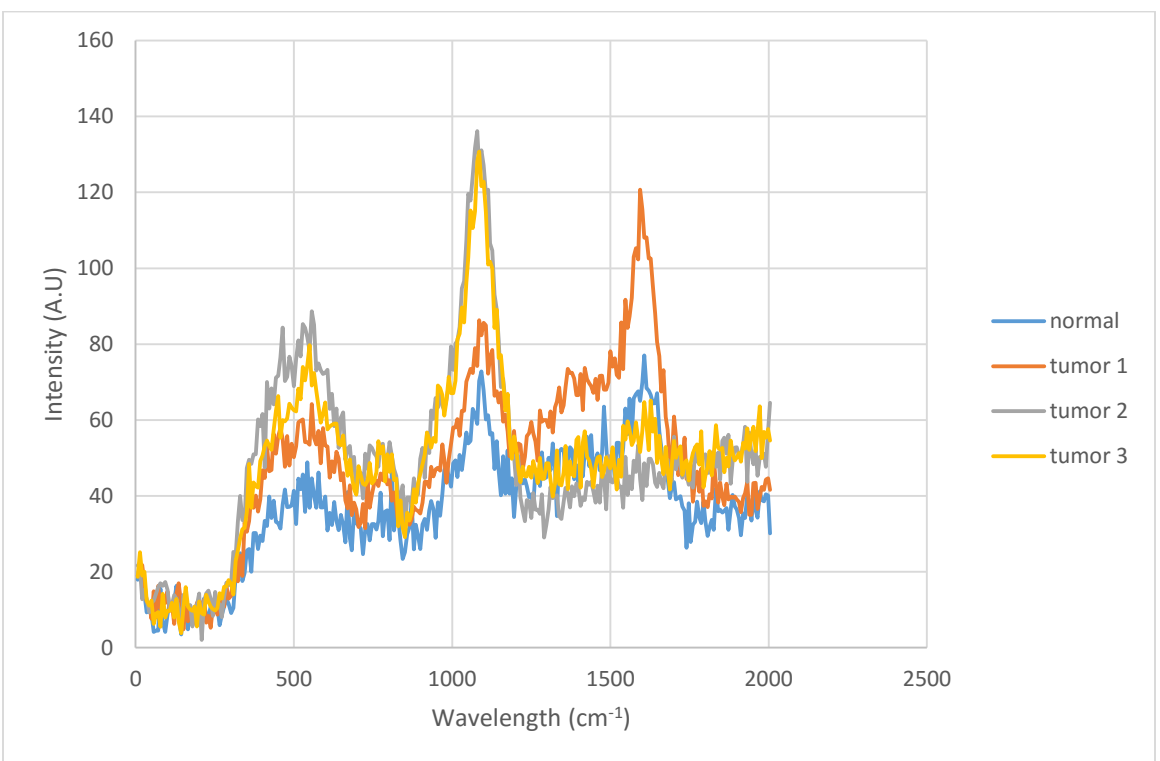


Figure 3.31 The averaged Raman spectrum of ten different Raman spectra from human tissues in each of the different stages using UV-Raman (RS-UV-T1)

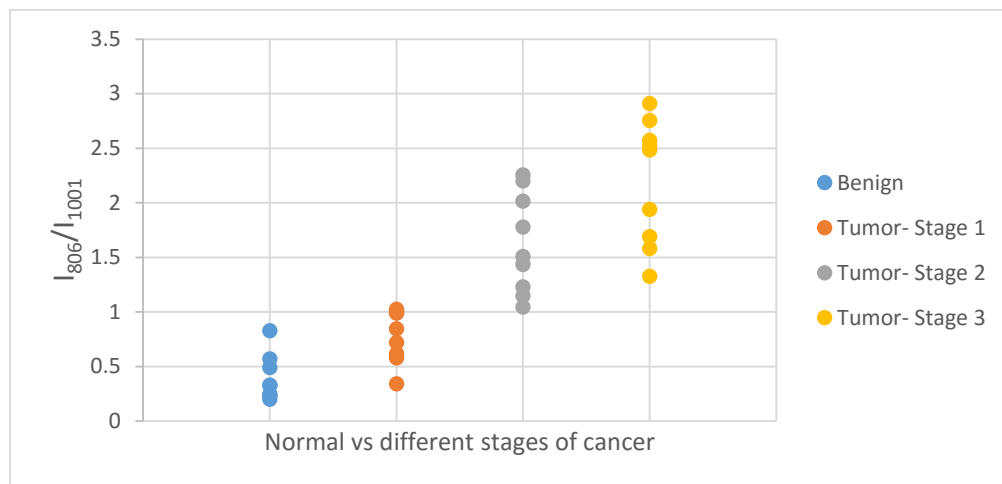


Figure 3.32 Results of using the two-band ratio ( $I_{806}/I_{1001}$ ) method to differentiate human tissues at different stages. Data of the two ratios is from Figure 3-31

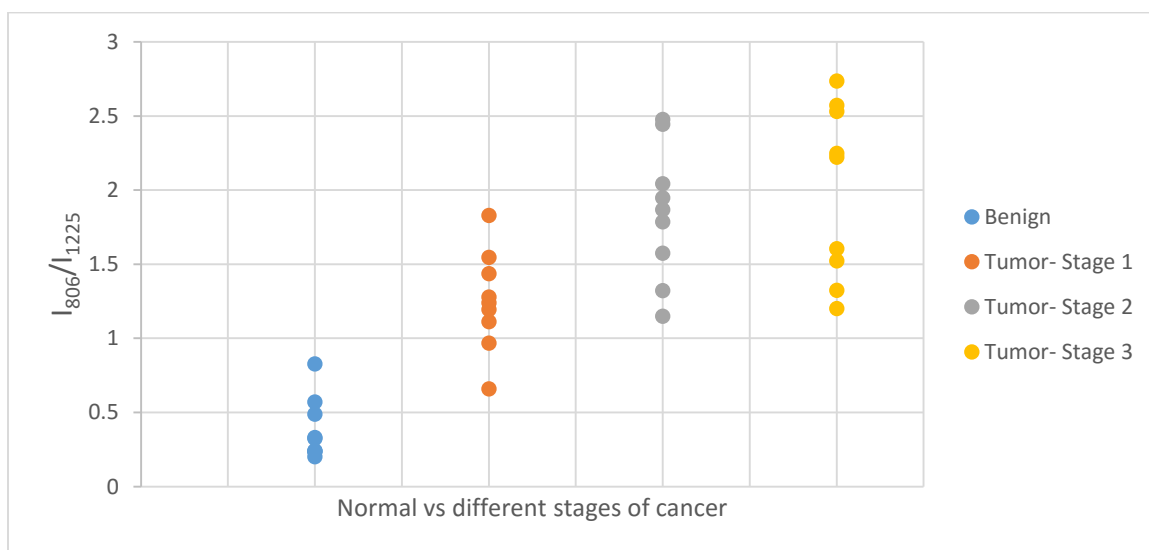


Figure 3.33 Results of using the two-band ratio ( $I_{806}/I_{1225}$ ) method to differentiate human tissues at different stages.



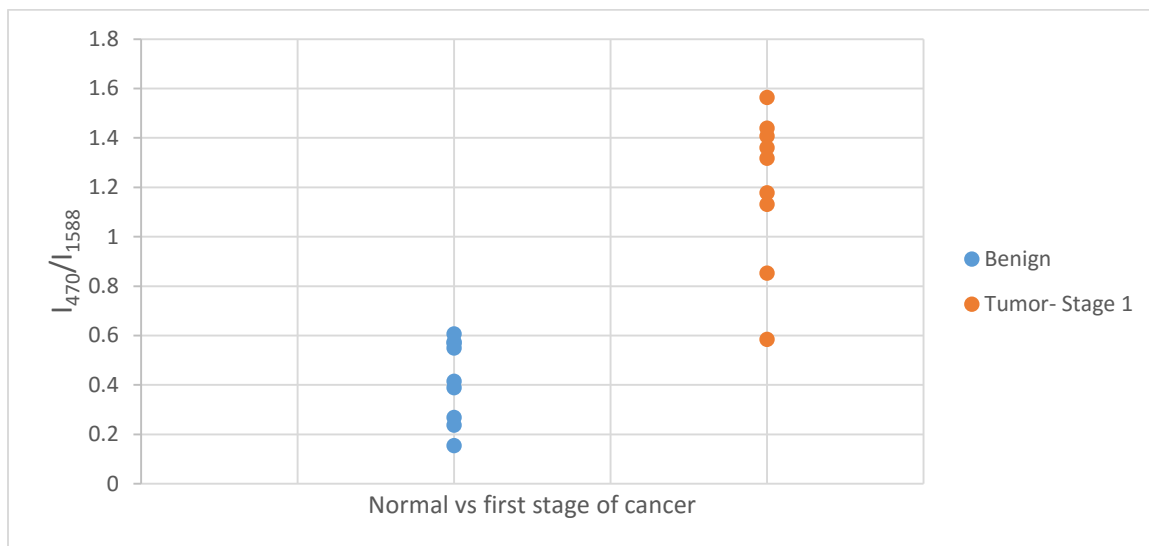


Figure 3.34 Results of using the two-band ratio ( $I_{470}/I_{1588}$ ) method to differentiate benign and tumor in stage 1

### 3.3.9 CARS Spectroscopy of Human-Fixed Tissues (CARS-SPT-T3)

In order to evaluate the feasibility of using CARS techniques as a fast method to distinguish different stages of cancer, the test (CARS-SPT-T3) was conducted using the CARS system. Like Section 3.3.6, three types of cancer tissues from different stages were selected for the sets of experiments. A total of 10 spectra from each slide have been collected using CARS. The range of CARS wavelength was in the lipid range, from 2200–3400  $\text{cm}^{-1}$ . Figure 3.34 shows the averaged CARS spectrum from benign and tumor tissues in different stages. PCA technique has been used to differentiate benign and three different stages of cancer. Figures 3.35 to 3.37 show that it is hard to distinguish benign and tumor tissues at the first stage, that was already confirmed in UV-Raman tests. The difference between stages 1 and

2 is very clear, while stages 2 and 3 have a lot of similarities that makes them difficult to distinguish.

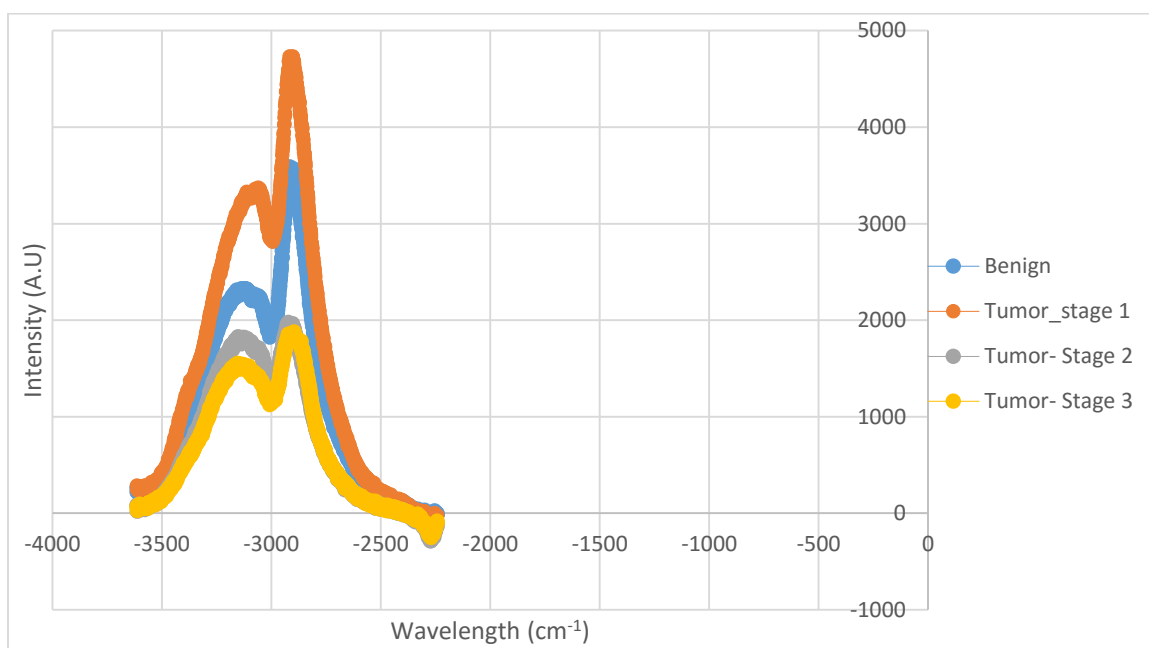


Figure 3.35 Averaged CARS spectrum from benign and tumor tissues in different stages (CARS-SPT-T3)

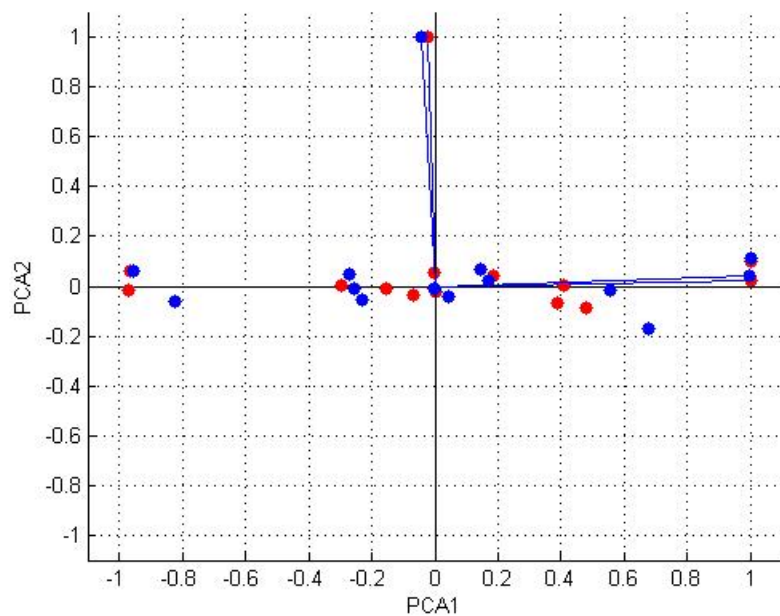


Figure 3.36 PCA results from benign and stage 1 of cancer. Blue dots represent benign and red dots show the tumor tissues

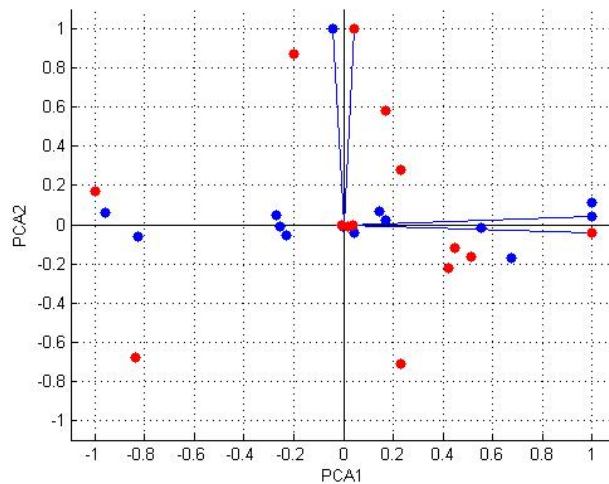


Figure 3.37 PCA results from stage 1 and stage 2 of cancer. Blue dots represent stage 1 and red dots show the stage 2

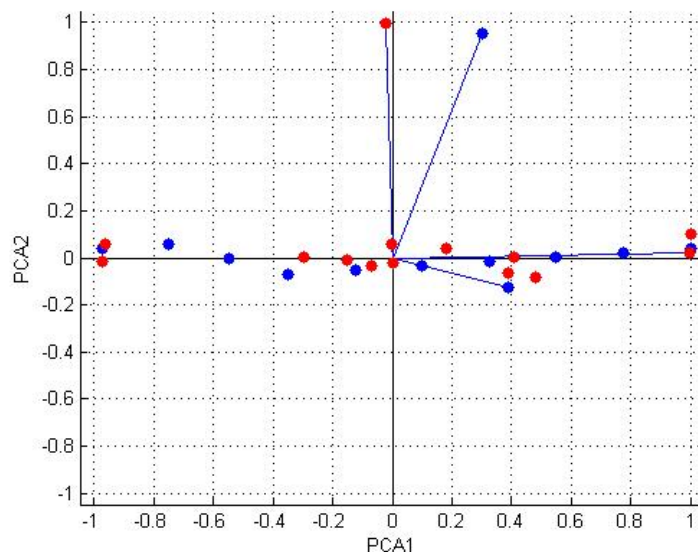


Figure 3.38 PCA results from stage 2 and stage 3 of cancer. Blue dots represent stage 1 and red dots show the stage 2

### 3.3.10 Summary

As it is demonstrated in this chapter, the RS, SERS and CARS are able to differentiate normal and cancer tissues by comparing the spectrum/imaging of each type. The tissues with small thickness (around 5 micron) and smooth surface are more appropriate for all Raman-based techniques. CARS also shows a strong capability to image the tissues and to demonstrate the concentration distribution of lipids and thus, can be used to distinguish normal and cancer cells. CARS spectroscopy is also capable to provide information about the tissues in specific wavelength ranges (e.g. lipids) and thus, is appropriate for clinical use for collect information in a short time. Using RNA and lipid bands in two- band ratio

model showed to be a very successful technique to diagnose cancer in very early stages (DCIS).

## CHAPTER 4 CONCLUSIONS AND RECOMMENDATIONS

### 4.1 Conclusions

The following conclusions can be drawn based on the tests conducted in this study:

- 1) The enhancement effect in SERS highly depends on the hot spots in the sample. A piece of a tissue is a solid sample, and it is very hard to make the tissue in a close contact with nanoparticles to make hot spots. SERS works well with fixed tissues, but once the sample under test is fresh tissue with a rough surface, it is hard to get the Raman spectrum from the tissue. Generally, Raman signals are very sensitive to the experimental conditions, and mainly the smoothness of the surface.
- 2) The CARS method works well to characterize lipids and to get spectrum in the lipid range within a short time.
- 3) RS and SERS are able to distinguish normal and cancer tissues using statistical techniques such as PCA, PC-DFA, and the two-band ratio methods. Also applying two-band ratio method in all spectra of benign and stage 1 of cancer showed that there is a significant difference in the ratio of polysaccharides over protein once tumor starts developing. This is a promising method that can be used to study cancer development in early stages.

### 4.2 Recommendations

Due to the weak Raman spectrum from fresh tissues and randomness of results, Raman-based techniques may not be appropriate to test fresh tissues directly. However, making

some changes in Raman or CARS spectrometers or developing Raman probes may solve this issue. In the future, we can develop a CARS probe that is able to record CARS spectrum results in fresh tissues within a short time. It will help to prepare a database that contains CARS spectrum from different stages of cancer, using different filters (lipid, protein, DNA). Lipid filter has already shown promising results on cancer detection. This database will be helpful to detect cancer in different stages, and also for early cancer diagnosis.

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## APPENDIX A: DATA ANALYSIS

### A.1 PCA method

A spectrum with  $n$  peaks can be plotted in an  $n$ -dimensional space. The PCA transforms the original coordinate system. The new coordinates are called principal components. The origin of the new coordinate system is located in the center of the data points. The first PC points are in the direction of highest variance. The second PC points in the direction of second highest variance and so on. In this study, PCA was performed on Math Works MATLAB® Software, and the PCA was taken on 55 Raman spectrum in the range of 600–1700 $\text{cm}^{-1}$  over the main 8 wavelengths of 644, 806, 1001, 1031, 1184, 1443 and 1613  $\text{cm}^{-1}$ . Two principal components (PC1 and PC2) were used to explain 98% of total variance in the Raman spectrum of cancer, and 97% of data in Raman spectrum of Normal tissues. Table A-1 and A-2 show the PC results for raw cancer and normal Raman data.

Table A-1 **PC values for Raman data from cancer tissues**

<b>PC</b>	<b>Value (%)</b>
PC 1	91.90
PC 2	6.52
PC 3	0.79

PC 4	0.35
PC 5	0.16
PC 6	0.12
PC 7	0.09
PC 8	0.04

**Table A-2** PC values for Raman data from normal tissues

<b>PC</b>	<b>Value (%)</b>
PC 1	71.95
PC 2	25.41
PC 3	0.83
PC 4	0.78
PC 5	0.47
PC 6	0.31
PC 7	0.16
PC 8	0.06

## A.2 Cross Validation Method

The cross-validation estimate method is a “leave-one out” method. To apply this method, the first spectra (training data) was removed from the data set, and the “rule” was established using the remaining spectra. The first observation was put back into the data set and the second one was removed. This method was continued until the “rule” on the whole spectra is established. In order to further test the rule, external data (10 spectra for normal, 10 spectra for tumor) were collected as test data, to test the accuracy of the rule. Figure A-3 shows the results of classification based on two groups of data.

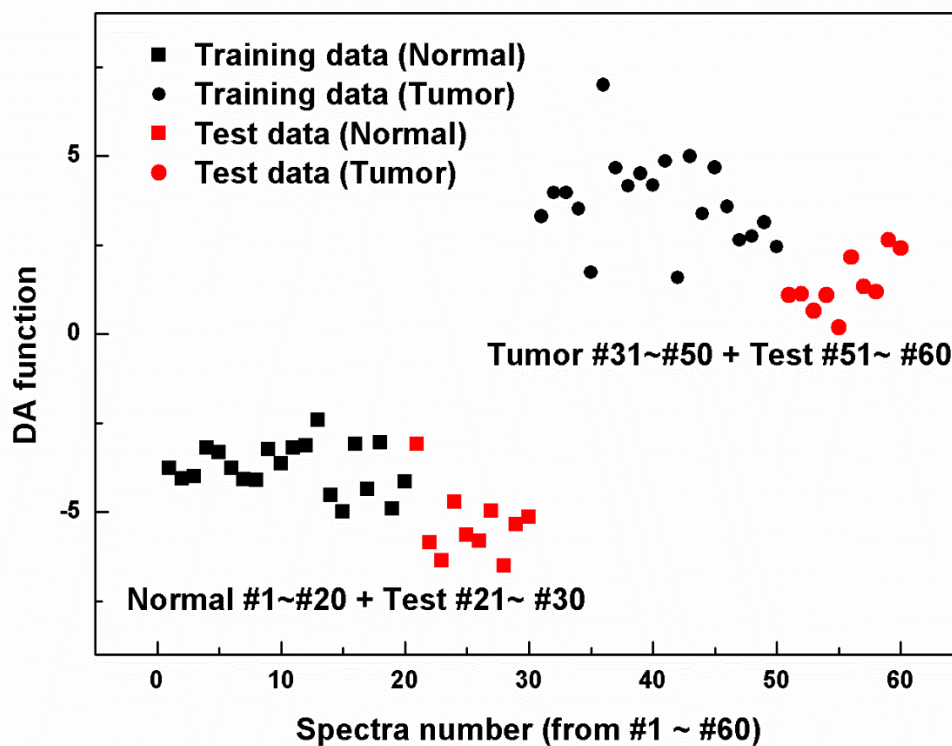


Figure A-1 PC-DFA plot for training and test data

## APPENDIX B: RAW RAMAN SPECTRUM DATA

### B.1 CARS Spectroscopy from human tissues

Here is the raw results of CARS spectroscopy from benign and stage 1-3 of breast cancer tissues from human models before any analysis. The Raman spectrums were taken from 10 different points on the tissues.

#### B.1.1- Benign Tissues

Wavelength (nm)	Intensity # 1	Intensity # 2	Intensity # 3	Intensity # 4	Intensity # 5	Intensity # 6	Intensity # 7	Intensity # 8	Intensity # 9	Intensity # 10
-3614.2	614	124	299	203	247	262	193	193	226	283
-3613.04	591	69	248	150	204	225	137	137	229	226
-3611.87	579	90	235	158	207	201	144	144	222	221
-3610.71	621	104	269	181	237	241	169	169	231	261
-3609.55	617	75	254	144	193	237	131	131	204	225
-3608.39	580	78	275	144	220	234	142	142	225	255
-3607.23	605	129	255	184	240	236	165	165	220	271
-3606.07	595	94	244	163	235	235	148	148	206	215
-3604.9	594	75	266	166	225	222	157	157	253	247
-3603.74	618	89	262	170	201	242	185	185	238	229
-3602.58	614	84	234	145	199	211	136	136	204	234
-3601.42	634	105	260	169	243	282	156	156	213	274
-3600.26	579	108	277	152	212	244	120	120	255	261
-3599.1	590	75	225	159	169	238	120	120	209	234
-3597.94	635	86	253	183	221	248	177	177	236	260
-3596.78	633	99	277	202	246	284	174	174	262	274
-3595.62	588	70	229	166	207	251	135	135	231	249
-3594.47	574	73	248	176	222	209	144	144	210	232
-3593.31	612	113	264	171	246	267	167	167	240	272
-3592.15	587	98	274	171	196	232	151	151	237	237
-3590.99	620	82	260	148	216	230	148	148	208	269
-3589.83	633	119	283	175	235	263	182	182	247	282

-3588.67	600	107	235	143	233	226	130	130	233	256
-3587.52	605	91	210	164	221	249	154	154	209	242
-3586.36	592	88	248	156	239	218	162	162	212	226
-3585.2	607	109	285	198	272	254	177	177	238	277
-3584.04	605	101	298	167	228	272	188	188	255	252
-3582.89	588	100	293	200	229	288	166	166	242	264
-3581.73	615	114	266	171	253	253	170	170	238	247
-3580.58	632	125	276	183	258	254	178	178	252	277
-3579.42	597	120	272	169	218	272	171	171	254	271
-3578.26	599	100	254	154	243	254	168	168	238	262
-3577.11	628	115	291	191	295	296	187	187	241	269
-3575.95	558	98	268	152	200	253	161	161	213	261
-3574.8	595	97	245	166	252	276	155	155	252	260
-3573.64	566	90	267	130	233	232	186	186	223	255
-3572.49	619	118	322	217	279	305	184	184	259	263
-3571.33	593	131	305	192	276	286	172	172	266	294
-3570.18	583	110	251	153	259	266	160	160	223	276
-3569.03	591	91	286	196	249	252	177	177	247	246
-3567.87	577	90	234	171	234	278	171	171	220	285
-3566.72	602	101	251	169	227	305	183	183	250	274
-3565.56	564	96	281	178	266	247	148	148	226	271
-3564.41	613	91	268	203	266	285	175	175	254	282
-3563.26	601	77	280	170	259	288	171	171	241	313
-3562.11	585	113	267	179	270	254	163	163	242	262
-3560.95	605	104	257	190	284	269	162	162	229	270
-3559.8	613	85	289	187	251	309	203	203	263	292
-3558.65	589	105	277	166	272	291	189	189	284	296
-3557.5	598	146	290	157	263	296	194	194	260	295
-3556.35	591	99	312	223	264	304	204	204	256	306
-3555.2	590	89	302	171	261	300	189	189	257	308
-3554.04	605	143	321	203	281	310	174	174	270	305
-3552.89	610	137	286	208	278	304	204	204	256	295
-3551.74	595	123	293	194	272	296	215	215	246	281
-3550.59	606	103	274	192	264	336	181	181	255	309
-3549.44	556	144	303	179	261	254	165	165	267	279
-3548.29	626	142	314	241	290	331	216	216	289	333
-3547.14	558	95	297	156	266	305	202	202	277	301
-3545.99	570	151	297	184	285	334	198	198	288	321

-3544.84	561	112	262	218	299	288	168	168	242	275
-3543.7	579	104	281	183	295	318	202	202	284	310
-3542.55	600	119	287	198	290	329	178	178	287	320
-3541.4	580	180	324	207	302	332	221	221	306	334
-3540.25	605	110	307	187	291	328	216	216	283	341
-3539.1	566	127	344	169	296	356	188	188	287	342
-3537.95	589	113	251	190	273	316	157	157	275	280
-3536.81	575	100	312	188	310	329	227	227	265	315
-3535.66	597	130	299	233	338	330	228	228	332	348
-3534.51	574	146	336	228	329	345	225	225	291	341
-3533.37	552	127	313	172	296	337	230	230	276	319
-3532.22	609	118	345	207	308	362	213	213	296	363
-3531.07	631	139	343	198	312	377	259	259	321	337
-3529.93	562	117	301	211	306	345	186	186	277	339
-3528.78	589	144	304	181	310	342	208	208	297	338
-3527.63	614	153	324	233	359	391	225	225	289	408
-3526.49	581	126	319	226	326	360	231	231	299	356
-3525.34	571	139	294	214	299	386	211	211	297	352
-3524.2	584	133	307	218	356	403	243	243	286	379
-3523.05	585	142	375	192	344	391	254	254	348	378
-3521.91	585	135	308	205	295	361	214	214	340	382
-3520.77	616	172	353	206	348	380	232	232	304	397
-3519.62	585	142	350	233	354	384	238	238	308	403
-3518.48	601	134	321	210	359	375	256	256	306	375
-3517.33	564	134	328	224	338	390	249	249	297	407
-3516.19	594	131	332	181	377	368	262	262	316	372
-3515.05	600	186	385	221	383	424	301	301	338	405
-3513.9	596	181	341	222	361	441	249	249	367	419
-3512.76	601	135	366	212	413	397	273	273	343	402
-3511.62	634	160	365	268	393	417	262	262	333	427
-3510.48	604	118	357	209	370	437	268	268	354	384
-3509.33	590	141	354	236	369	436	276	276	323	426
-3508.19	586	153	327	221	382	454	260	260	367	419
-3507.05	604	165	385	262	371	436	291	291	343	428
-3505.91	601	147	340	220	361	421	267	267	320	417
-3504.77	601	170	400	248	384	465	319	319	376	418
-3503.63	653	183	392	278	437	487	320	320	411	484
-3502.49	599	140	382	229	408	468	275	275	371	459

-3501.35	648	188	386	272	424	498	333	333	380	463
-3500.2	575	162	356	212	407	445	280	280	347	450
-3499.06	599	164	384	229	397	488	310	310	373	469
-3497.93	620	170	394	227	381	502	299	299	354	478
-3496.79	573	172	379	236	407	508	322	322	422	468
-3495.65	626	227	456	266	467	563	351	351	426	527
-3494.51	602	158	386	225	389	492	306	306	361	443
-3493.37	633	191	390	274	467	567	308	308	450	545
-3492.23	592	176	409	239	453	500	332	332	412	540
-3491.09	596	186	415	282	433	555	337	337	425	528
-3489.95	608	193	404	238	439	566	327	327	434	535
-3488.81	566	158	415	221	438	518	360	360	408	507
-3487.68	612	180	393	287	459	533	367	367	426	534
-3486.54	618	159	410	225	459	579	341	341	413	556
-3485.4	601	186	424	250	448	563	367	367	439	557
-3484.26	572	163	409	242	474	540	302	302	415	518
-3483.13	616	193	421	260	463	607	362	362	445	575
-3481.99	620	179	436	269	491	627	363	363	455	576
-3480.85	600	204	435	308	467	572	399	399	440	559
-3479.72	635	185	395	276	459	611	369	369	458	578
-3478.58	604	164	473	232	474	607	388	388	464	592
-3477.45	595	187	434	266	497	620	394	394	457	590
-3476.31	580	183	418	269	493	593	404	404	469	593
-3475.18	594	193	439	300	520	624	387	387	477	625
-3474.04	604	188	451	265	532	652	394	394	490	626
-3472.91	616	179	455	298	529	659	428	428	511	634
-3471.77	656	201	476	321	551	722	414	414	530	659
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-3466.1	623	213	472	297	578	726	469	469	540	699
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-3428.82	696	326	727	394	844	1114	733	733	885	1182
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-3418.69	689	321	738	438	920	1196	801	801	916	1280
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-3406.34	722	362	827	457	985	1326	935	935	1065	1459
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-3368.33	759	492	1030	603	1220	1644	1165	1165	1340	1906
-3367.22	744	481	1026	584	1236	1626	1141	1141	1332	1863
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-3362.76	737	444	1033	522	1235	1591	1166	1166	1354	1914
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-3358.31	784	491	1042	586	1258	1633	1193	1193	1343	1927
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-3352.75	772	507	1078	586	1315	1647	1225	1225	1418	1954
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-3350.53	783	483	1059	585	1254	1728	1193	1193	1434	1972
-3349.42	772	495	1035	581	1288	1659	1227	1227	1413	1951
-3348.31	802	512	1139	625	1255	1722	1249	1249	1413	2017
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-3197.13	1008	902	2214	1126	2526	3097	2252	2252	2588	3681
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-3182.02	1007	974	2327	1156	2571	3175	2301	2301	2629	3742
-3180.94	1012	981	2310	1151	2592	3190	2344	2344	2626	3783
-3179.87	978	980	2326	1166	2558	3231	2284	2284	2657	3768
-3178.79	987	972	2325	1157	2534	3144	2298	2298	2638	3729
-3177.71	1068	994	2377	1204	2602	3232	2269	2269	2698	3797
-3176.63	1007	1015	2349	1167	2578	3151	2277	2277	2658	3777
-3175.56	1038	948	2322	1176	2553	3224	2267	2267	2673	3800
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-3154.07	991	1019	2435	1204	2611	3346	2322	2322	2683	3778
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-3143.35	963	1032	2393	1243	2661	3436	2323	2323	2627	3831
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-3139.07	1024	1022	2468	1243	2686	3546	2368	2368	2690	3815
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-3134.79	969	1018	2440	1201	2667	3473	2318	2318	2593	3858
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-3128.39	1029	1065	2454	1282	2687	3550	2354	2354	2595	3843
-3127.32	950	1047	2484	1249	2680	3536	2318	2318	2609	3829
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-3124.12	974	1069	2455	1270	2686	3582	2336	2336	2592	3812
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-3090.09	874	999	2327	1238	2538	3634	2235	2235	2382	3610
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-3080.55	888	1079	2308	1247	2546	3642	2241	2241	2386	3540
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-3033.13	798	979	2058	1162	2186	3556	2127	2127	2076	3420
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-3028.94	825	932	1983	1154	2225	3503	2076	2076	2048	3374
-3027.89	792	949	1953	1136	2256	3484	2096	2096	2017	3302
-3026.84	783	974	2010	1154	2213	3480	2063	2063	2071	3307
-3025.79	771	1004	1949	1135	2189	3562	2088	2088	2045	3323
-3024.75	763	947	1938	1129	2174	3526	2068	2068	2001	3287
-3023.7	786	974	1975	1153	2199	3443	2065	2065	1935	3245
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-3021.6	747	939	1939	1091	2119	3486	2037	2037	1977	3162
-3020.56	813	937	2015	1109	2186	3446	2072	2072	1976	3215
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-3017.42	745	910	1885	1072	2073	3444	1931	1931	1885	3098
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-3003.83	700	858	1767	1020	1916	3276	1960	1960	1915	3018
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-2999.66	701	887	1774	1030	1912	3298	1959	1959	1982	3060
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-2992.37	745	872	1800	1022	1878	3237	2039	2039	2099	3100
-2991.33	725	891	1746	1057	1942	3198	2047	2047	2106	3163
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-2956.04	1006	953	2048	1113	2325	3399	2922	2922	3865	4595
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-2946.74	1131	1010	2276	1181	2587	3659	3353	3353	4545	5219
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-2942.61	1221	1009	2332	1228	2680	3702	3402	3402	4815	5599
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-2935.39	1323	1065	2543	1318	2913	3859	3749	3749	5406	6144
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-2933.33	1291	1053	2515	1293	2919	3949	3762	3762	5446	6210
-2932.3	1325	1092	2575	1313	2989	3936	3819	3819	5530	6303
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-2930.24	1356	1057	2590	1258	3013	3912	3853	3853	5601	6400
-2929.21	1373	1095	2691	1312	3082	4010	3966	3966	5607	6393
-2928.18	1385	1079	2602	1317	3037	3909	3896	3896	5662	6422
-2927.15	1414	1081	2659	1398	3122	3964	3948	3948	5659	6532
-2926.12	1408	1093	2636	1360	3105	4021	3922	3922	5790	6558

-2925.09	1385	1067	2676	1359	3193	4017	4006	4006	5797	6641
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-2917.9	1448	1140	2733	1359	3227	4197	4072	4072	6027	6918
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-2913.79	1434	1095	2746	1393	3335	4174	4059	4059	6047	6933
-2912.76	1472	1079	2707	1398	3321	4243	4043	4043	6074	6957
-2911.74	1445	1109	2690	1348	3314	4168	4023	4023	5994	6943
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-2909.69	1510	1126	2686	1392	3315	4187	4018	4018	5989	6966
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-2881.04	1407	1034	2655	1285	3176	3948	3688	3688	5568	6347
-2880.02	1385	1035	2700	1299	3153	3966	3681	3681	5523	6320
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-2849.51	1135	908	2264	1111	2715	3485	3014	3014	4533	5242
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-2847.48	1144	898	2279	1143	2713	3519	2966	2966	4460	5161
-2846.47	1155	881	2215	1098	2683	3460	2950	2950	4389	5084
-2845.45	1120	871	2222	1085	2662	3473	2980	2980	4289	5091
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-2837.35	1111	863	2138	1091	2565	3300	2774	2774	3958	4709
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-2831.29	1022	826	2018	981	2392	3201	2531	2531	3661	4323
-2830.27	1037	798	1968	979	2337	3106	2525	2525	3698	4343
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-2823.2	966	783	1889	972	2254	2977	2358	2358	3313	4027
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-2821.19	929	736	1867	948	2154	2954	2369	2369	3185	3899
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-2814.13	896	750	1739	911	2031	2883	2142	2142	2976	3654
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-2809.09	839	686	1737	880	1969	2697	2062	2062	2848	3502
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-2803.06	853	674	1647	831	1900	2647	1933	1933	2669	3317
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-2801.05	801	656	1626	817	1879	2561	1915	1915	2607	3265
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-2789	766	607	1529	755	1665	2341	1730	1730	2310	2843
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-2785.99	742	595	1456	731	1655	2380	1638	1638	2211	2836
-2784.99	709	611	1431	760	1642	2344	1663	1663	2201	2810
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-2780.98	739	589	1441	714	1570	2183	1584	1584	2153	2720
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-2772.98	714	580	1375	721	1493	2122	1473	1473	1994	2523
-2771.98	670	505	1297	668	1445	2134	1428	1428	1946	2445

-2770.98	671	531	1288	640	1425	2091	1456	1456	1902	2416
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-2768.98	684	528	1321	661	1448	2033	1471	1471	1915	2472
-2767.98	680	530	1304	656	1401	2044	1462	1462	1931	2391
-2766.98	635	513	1262	626	1394	1971	1404	1404	1885	2346
-2765.99	684	532	1247	643	1436	2024	1385	1385	1844	2295
-2764.99	667	512	1245	637	1383	1972	1369	1369	1839	2307
-2763.99	662	530	1272	657	1378	1981	1347	1347	1827	2329
-2762.99	653	523	1254	669	1354	1952	1388	1388	1842	2296
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-2760	647	487	1242	598	1363	1911	1303	1303	1743	2278
-2759	652	498	1229	638	1343	1978	1310	1310	1755	2241
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-2757.01	660	481	1218	589	1325	1904	1299	1299	1734	2215
-2756.01	667	531	1243	616	1394	1918	1296	1296	1770	2164
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-2750.03	616	465	1182	594	1294	1839	1201	1201	1688	2059
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-2748.04	601	464	1126	605	1248	1799	1177	1177	1592	2008
-2747.05	615	469	1123	586	1228	1766	1231	1231	1601	2006
-2746.05	589	434	1083	534	1189	1752	1164	1164	1571	1964
-2745.06	619	453	1141	563	1188	1781	1193	1193	1549	1950
-2744.07	599	458	1086	536	1195	1721	1137	1137	1515	1924
-2743.07	593	477	1098	562	1168	1725	1154	1154	1546	1946
-2742.08	538	455	1100	530	1210	1694	1106	1106	1479	1946
-2741.08	614	484	1111	588	1164	1779	1166	1166	1537	1921
-2740.09	549	417	1059	522	1196	1728	1107	1107	1462	1891
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-2737.11	605	432	1045	549	1133	1655	1102	1102	1443	1887
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-2735.12	568	406	1047	542	1121	1573	1016	1016	1382	1827
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-2733.14	564	431	1056	533	1120	1627	1082	1082	1418	1797
-2732.15	558	436	1046	551	1063	1612	1032	1032	1374	1741
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-2730.16	573	418	1030	510	1101	1588	1044	1044	1341	1722
-2729.17	583	437	1021	527	1074	1548	999	999	1372	1700
-2728.18	557	378	948	514	1085	1504	1007	1007	1346	1716
-2727.19	518	368	978	478	1063	1529	982	982	1276	1717
-2726.2	561	436	977	498	1033	1544	1000	1000	1355	1693
-2725.21	571	404	964	530	1036	1500	945	945	1339	1673
-2724.22	548	428	980	474	1017	1498	991	991	1297	1645
-2723.23	533	416	975	507	1040	1552	969	969	1301	1636
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-2721.24	544	420	949	495	1051	1466	980	980	1268	1629
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-2719.26	544	384	938	497	1012	1466	962	962	1279	1584
-2718.28	522	391	926	471	977	1402	922	922	1230	1604
-2717.29	531	367	911	491	986	1432	915	915	1187	1553
-2716.3	539	381	922	460	1006	1398	926	926	1206	1549
-2715.31	481	324	882	436	916	1378	878	878	1186	1518
-2714.32	534	392	904	458	972	1426	928	928	1206	1535
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-2711.35	516	345	888	484	941	1383	836	836	1165	1459
-2710.37	516	344	888	421	934	1331	862	862	1131	1495
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-2708.39	509	353	888	440	904	1311	902	902	1179	1486
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-2702.47	524	381	874	496	920	1340	845	845	1139	1435
-2701.48	505	334	828	403	864	1288	824	824	1058	1366
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-2699.51	506	343	848	412	855	1253	794	794	1053	1349
-2698.52	493	336	842	447	886	1327	816	816	1079	1384
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-2692.61	499	321	790	391	832	1171	741	741	1021	1276
-2691.63	503	324	827	449	849	1210	751	751	1000	1289
-2690.64	500	311	767	409	822	1196	696	696	947	1277
-2689.66	504	344	777	407	820	1198	722	722	994	1267
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-2686.71	480	288	749	396	816	1130	724	724	980	1239
-2685.72	510	290	762	401	807	1155	720	720	935	1236
-2684.74	484	321	736	403	810	1137	688	688	937	1220
-2683.76	469	274	730	351	779	1127	695	695	924	1181
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-2681.79	519	301	750	433	804	1176	687	687	954	1173
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-2673.94	471	294	730	396	758	1092	646	646	908	1146
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-2670.01	455	242	689	358	714	1032	609	609	838	1093
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-2662.17	455	270	684	360	679	954	604	604	827	1030
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-2660.22	465	279	674	349	677	978	616	616	828	1018
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-2653.37	457	277	669	345	648	968	558	558	774	992
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-2650.44	475	270	634	342	664	913	531	531	683	903
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-2647.51	446	233	595	330	614	881	524	524	685	882
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-2643.61	474	264	615	305	621	852	537	537	702	894
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-2641.66	442	248	569	351	597	822	500	500	655	818
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-2631.91	369	201	527	254	519	748	433	433	595	780
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-2628.99	379	186	515	215	487	711	415	415	571	757
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-2624.13	385	224	532	295	457	695	440	440	552	715
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-2621.22	390	194	480	248	462	689	389	389	530	683
-2620.25	380	173	472	229	488	623	379	379	512	672
-2619.28	397	191	493	282	474	669	431	431	537	675
-2618.31	390	191	466	242	468	649	381	381	556	647
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-2608.61	367	180	448	234	435	644	360	360	501	642
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-2606.67	335	161	403	201	400	557	311	311	434	580
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-2603.76	381	173	432	210	403	588	308	308	464	569
-2602.8	362	123	401	185	429	547	324	324	395	610
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-2600.86	361	169	404	230	376	587	275	275	433	549
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-2565.18	303	103	343	171	299	411	223	223	303	409
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-2550.79	338	111	328	168	279	407	246	246	287	379
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-2547.92	345	123	287	186	274	403	239	239	278	385
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-2538.35	293	107	260	117	238	311	178	178	248	329
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-2535.48	289	94	291	149	234	343	172	172	273	335
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-2528.8	303	100	240	131	232	324	169	169	247	313
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-2518.31	294	94	222	109	226	277	153	153	220	277
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-2511.65	300	118	265	136	230	289	169	169	258	291
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-2502.15	285	82	212	131	202	276	141	141	203	242
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-2449.27	269	59	142	109	99	179	98	98	163	178
-2448.33	245	56	147	97	136	166	101	101	153	168
-2447.39	277	77	165	93	129	186	111	111	136	174
-2446.45	267	48	157	113	118	153	88	88	144	171
-2445.51	245	59	145	72	127	178	74	74	123	151
-2444.57	265	73	170	108	139	140	77	77	151	170
-2443.63	256	69	182	93	132	148	93	93	118	161
-2442.7	248	67	119	69	104	156	76	76	127	165
-2441.76	249	65	183	92	143	172	96	96	160	159
-2440.82	262	62	152	106	135	172	121	121	132	171

-2439.88	257	52	141	96	112	153	66	66	122	162
-2438.94	245	70	195	95	110	162	84	84	108	149
-2438.01	243	79	149	91	133	140	96	96	152	133
-2437.07	240	27	152	90	107	158	90	90	91	152
-2436.13	207	51	139	106	99	159	63	63	104	141
-2435.2	226	42	136	106	98	146	51	51	125	102
-2434.26	214	51	141	81	102	164	87	87	115	154
-2433.32	255	88	152	109	108	183	94	94	127	174
-2432.39	217	60	125	75	104	143	93	93	141	131
-2431.45	245	59	138	92	117	148	96	96	120	142
-2430.51	252	73	128	94	107	158	92	92	98	133
-2429.58	240	48	131	95	117	149	87	87	124	128
-2428.64	251	55	125	87	101	147	84	84	137	139
-2427.71	229	67	130	96	97	125	82	82	122	136
-2426.77	236	39	94	99	99	132	80	80	107	124
-2425.84	208	35	120	56	93	135	66	66	100	96
-2424.9	247	85	143	89	116	150	78	78	118	158
-2423.97	217	21	112	91	99	105	58	58	88	114
-2423.03	232	57	147	81	110	144	72	72	118	137
-2422.1	229	56	142	102	114	163	97	97	103	115
-2421.16	193	33	89	51	100	103	55	55	76	110
-2420.23	269	50	159	98	130	165	91	91	137	185
-2419.29	233	64	150	95	104	157	85	85	151	131
-2418.36	240	53	143	105	127	127	61	61	139	144
-2417.43	240	29	102	47	84	104	76	76	99	91
-2416.49	240	55	148	101	128	141	83	83	124	147
-2415.56	205	64	137	95	101	145	75	75	111	129
-2414.63	210	85	143	62	117	125	91	91	114	143
-2413.69	256	67	159	109	122	156	96	96	126	146
-2412.76	194	42	117	62	67	115	68	68	103	98
-2411.83	241	47	104	54	102	128	90	90	72	99
-2410.9	227	42	138	85	105	157	86	86	118	121
-2409.96	255	62	152	86	131	138	84	84	139	139
-2409.03	198	21	130	67	95	109	74	74	81	103
-2408.1	201	50	124	70	104	115	88	88	103	124
-2407.17	212	32	147	75	129	133	51	51	106	131
-2406.24	222	53	128	63	90	121	35	35	107	112
-2405.3	198	24	89	56	93	107	52	52	77	102

-2404.37	186	32	104	49	95	66	46	46	68	85
-2403.44	254	64	144	120	100	142	63	63	117	141
-2402.51	195	31	100	74	58	117	68	68	89	107
-2401.58	184	50	87	91	78	85	47	47	83	117
-2400.65	234	64	139	100	116	138	81	81	127	147
-2399.72	175	61	123	73	82	131	78	78	93	94
-2398.79	178	26	79	55	70	85	9.0001	9.0001	89	102
-2397.86	185	15	129	60	105	96	58	58	72	86
-2396.93	204	70	131	96	93	122	80	80	94	125
-2396	168	24	89	37	64	112	44	44	77	82
-2395.07	190	46	86	66	93	104	65	65	80	106
-2394.14	225	63	120	103	106	114	102	102	107	106
-2393.21	174	33	107	56	62	101	72	72	84	99
-2392.28	201	56	109	83	124	122	110	110	119	97
-2391.35	163	34	117	61	101	92	45	45	83	82
-2390.42	177	34	101	71	68	117	44	44	63	99
-2389.5	164	20	110	48	69	94	44	44	62	72
-2388.57	163	54	111	77	71	94	74	74	112	84
-2387.64	176	38	101	59	69	103	25	25	72	58
-2386.71	167	26	74	65	75	110	67	67	60	114
-2385.78	150	11	48	51	53	100	46	46	80	69
-2384.86	152	51	116	54	95	74	57	57	92	101
-2383.93	182	26	85	81	107	102	66	66	71	87
-2383	183	54	108	59	88	101	58	58	100	81
-2382.07	151	24	95	46	84	94	51	51	102	64
-2381.15	130	29	67	46	42	69	23	23	35	69
-2380.22	178	40	96	72	84	82	73	73	84	95
-2379.29	143	17	89	53	57	84	39	39	52	60
-2378.37	116	22	61	30	33	55	27	27	36	62
-2377.44	166	20	59	45	57	56	14	14	34	70
-2376.51	149	14	82	35	70	72	42	42	73	64
-2375.59	139	24	71	82	70	81	30	30	84	73
-2374.66	114	9.0001	88	58	70	88	36	36	34	64
-2373.74	144	9.0001	97	63	67	89	58	58	77	94
-2372.81	146	37	91	75	70	102	65	65	96	103
-2371.89	125	35	69	35	49	52	52	52	51	51
-2370.96	149	25	82	62	69	78	44	44	36	86
-2370.04	120	37	77	32	60	75	20	20	50	59

-2369.11	138	58	84	62	65	63	40	40	57	81
-2368.19	103	20	62	23	59	50	32	32	26	65
-2367.26	127	32	88	71	63	90	69	69	65	87
-2366.34	104	35	78	63	80	85	35	35	64	78
-2365.41	72	-9.9999	43	21	48	48	24	24	24	58
-2364.49	135	10	90	61	67	45	27	27	16	47
-2363.57	111	18	75	21	59	40	32	32	59	62
-2362.64	64	17	38	22	5.9999	52	13	13	37	45
-2361.72	90	14	61	20	48	41	39	39	32	53
-2360.79	91	-10	26	23	42	53	6.9998	6.9998	34	34
-2359.87	73	17	42	22	16	49	12	12	28	40
-2358.95	71	14	37	40	24	36	12	12	21	57
-2358.03	109	16	35	37	56	59	33	33	55	59
-2357.1	55	22	78	32	47	31	30	30	54	69
-2356.18	94	50	35	56	11	65	22	22	45	47
-2355.26	75	18	67	33	64	38	18	18	30	39
-2354.34	75	5.9999	58	37	35	52	27	27	40	70
-2353.41	57	42	71	42	16	88	32	32	57	38
-2352.49	7.9999	-	-3.0001	19	22	8.9999	-0.0001	-0.0001	-16	17
-2351.57	45	12	42	23	65	37	19	19	14	37
-2350.65	57	25	60	30	19	40	15	15	44	39
-2349.73	35	-10	-5	-4	0	13	-29	-29	-16	13
-2348.81	37	-3	19	-9	25	-6	3	3	3	-4
-2347.89	63	29	65	76	59	66	40	40	39	47
-2346.97	-24	-7.9999	-6.9999	-37	-18	10	-33	-33	-33	-20
-2346.05	8.9999	0.99988	3.9999	0.99988	-0.0001	16	2.9999	2.9999	-16	30
-2345.12	26	45	36	44	32	34	19	19	15	67
-2344.2	-4.9998	-	13	-4.9998	-3.9998	11	-3.9998	-3.9998	16	12
-2343.28	34	-5.0001	15	13	13	5.9999	26	26	-2.0001	26
-2342.36	18	-5.9999	8.0001	-3.9999	30	7.0001	17	17	5.0001	-6.9999
-2341.44	47	-5.9998	28	31	39	45	-2.9998	-2.9998	17	27
-2340.53	-11	-11	17	-13	-7.9999	15	-4.9999	-4.9999	-14	7.0001
-2339.61	9.9999	1.9999	-12	8.9999	-12	9.9999	-9.0001	-9.0001	-4.0001	13
-2338.69	23	20	64	4.9999	30	0.99988	19	19	3.9999	22
-2337.77	17	5.9998	29	15	-2.0002	25	11	11	-0.0002	5.9998
-2336.85	8	-17	2	2	-20	17	-22	-22	-46	8
-2335.93	-13	14	18	-8.0001	14	-22	-22	-22	-26	-28

-2335.01	36	27	25	52	12	30	13	13	28	10
-2334.09	-6.0002	-12	7.9998	-16	19	-0.0002	-17	-17	-12	6.9998
-2333.17	-11	-20	3.9999	-19	-3.0001	6.9999	-17	-17	4.9999	-11
-2332.26	39	31	50	9.9998	34	15	22	22	-3.0002	21
-2331.34	-23	0.99976	-3.0002	-13	-28	21	1.9998	1.9998	-23	1.9998
-2330.42	6.0002	1.0002	10	-2.9998	19	15	-11	-11	-27	8.0002
-2329.5	-34	-13	20	-21	2.9998	-24	-21	-21	-43	-11
-2328.59	54	36	47	46	51	70	20	20	22	50
-2327.67	-40	-26	-9.9998	-33	-6.9998	-14	-26	-26	-32	-20
-2326.75	-22	-28	-2.9998	9.0002	-20	21	-35	-35	-5.9998	-27
-2325.83	19	4.9999	22	3.9999	23	19	-8.0001	-8.0001	-21	14
-2324.92	0.00024	-19	21	-6.9998	0.00024	25	-6.9998	-6.9998	3.0002	11
-2324	0.99988	-20	1.9999	-3.0001	11	6.9999	-13	-13	-22	-0.0001
-2323.09	-2	8	20	1	9	-14	-7	-7	24	-17
-2322.17	13	-	-	-	-	-	-	-	-	-
-2322.17	13	0.99988	-1.9999	11	9.0001	15	-35	-35	-6.9999	-6.9999
-2321.25	7.0002	19	11	13	7.0002	-2.9998	-9.9998	-9.9998	7.0002	-0.9998
-2320.34	-3.9998	3.0002	9.0002	5.0002	-16	28	-24	-24	-1.9998	-3.9998
-2319.42	-16	8.0001	9.0001	-12	10	-0.9999	-29	-29	-0.9999	-8.9999
-2318.51	10	3	30	48	28	31	21	21	4	30
-2317.59	8.9998	-13	-12	-9.0002	13	16	-7.0002	-7.0002	4.9998	2.9998
-2316.68	1.9999	0.99988	-10	-20	-0.0001	-18	-39	-39	-7.0001	-22
-2315.76	11	0.00012	23	22	20	28	14	14	2.0001	13
-2314.85	1.9998	-29	7.9998	-8.0002	1.9998	-13	-14	-14	-2.0002	-12
-2313.93	-6.0001	-32	8.9999	-0.0001	-11	-8.0001	-18	-18	-17	0.99988
-2313.02	-16	20	9.0002	12	21	8.0002	15	15	9.0002	0.00024
-2312.1	0.99988	12	0.99988	5.9999	13	-12	-20	-20	-19	-17
-2311.19	-18	-30	-5	-2	-26	8	-22	-22	-20	-4
-2310.27	-28	-9.9998	20	-11	19	-1.9998	-20	-20	-24	-25
-2309.36	0.99976	-25	-8.0002	-10	-12	-24	-14	-14	-14	-13
-2308.45	10	7.0001	29	12	34	25	4.0001	4.0001	16	-2.9999
-2307.53	3	26	41	23	20	34	9	9	-3	6
-2306.62	-38	6	1	-12	32	-3	-4	-4	-26	-7
-2305.71	28	8.9998	18	8.9998	24	22	-5.0002	-5.0002	9.9998	24
-2304.79	-16	-1.9999	13	13	17	9.0001	2.0001	2.0001	7.0001	8.0001
-2303.88	-6.9999	-12	-13	-12	-23	-15	-8.9999	-8.9999	-5.9999	-8.9999
-2302.97	-2.0001	-18	-19	-8.0001	11	-12	-2.0001	-2.0001	-42	-0.0001
-2302.05	9.0002	-1.9998	18	15	20	14	-14	-14	13	-14

-2301.14	-1.0002	-29	-6.0002	-17	-12	-12	-40	-40	-22	-14
-2300.23	2.0002	9.0002	17	-1.9998	32	16	-12	-12	-33	15
-2299.32	35	27	64	31	35	41	26	26	17	1.0002
-2298.41	-14	-4	30	0	3	23	10	10	-2	3
-2297.49	-11	-9.9999	14	-27	9.0001	-1.9999	-32	-32	-23	-16
-2296.58	-19	-11	-1.0001	-7.0001	5.9999	-8.0001	-22	-22	-54	-18
-2295.67	-	15	42	15	35	3.9999	-17	-17	13	-5.0001
-2294.76	1.9998	-20	-14	-29	-18	14	-24	-24	-13	-24
-2293.85	-2.0001	3.9999	12	3.9999	13	-2.0001	-21	-21	-31	-7.0001
-2292.94	7.0002	-18	2.0002	12	27	4.0002	-36	-36	-8.9998	-2.9998
-2292.03	-	7.0002	8.0002	5.0002	-1.9998	13	-17	-17	3.0002	-3.9998
-2291.12	-21	-9.0002	-15	-7.0002	-28	-29	-46	-46	-45	-13
-2290.21	-1	-19	-14	-17	31	-10	-20	-20	-32	-10
-2289.3	1.9999	6.9999	47	15	27	31	-9.0001	-9.0001	-5.0001	17
-2288.39	27	-22	3.9998	-16	-11	14	-8.0002	-8.0002	-20	-24
-2287.48	-24	-19	-7.0002	-15	31	-7.0002	-15	-15	-43	-24
-2286.57	20	2	20	19	26	27	-8	-8	-27	12
-2285.66	-29	-14	-0.9999	0.00012	-33	-14	-39	-39	-47	-51
-2284.75	0.00012	-20	-9.9999	-23	-15	5.0001	-44	-44	-39	-26
-2283.84	13	17	8.0002	2.0002	-7.9998	19	-12	-12	-44	-14
-2282.93	-	25	28	31	19	-0.9998	-35	-35	-41	-23
-2282.02	8	-13	-27	0	-1	3	-14	-14	-37	-25
-2281.11	-8.0001	5.9999	37	6.9999	23	-20	-37	-37	-36	-35
-2280.2	0	-11	23	13	5	-2	-26	-26	-36	-32
-2279.29	-34	-12	-1.0001	-26	-20	9.9999	-38	-38	-31	-41
-2278.39	-7	7	-15	-17	2	10	-56	-56	-66	-33
-2277.48	-14	8.9998	-24	24	2.9998	-5.0002	-46	-46	-31	-24
-2276.57	-15	-19	-12	-8.0002	-16	-36	-85	-85	-79	-36
-2275.66	18	-14	9.9999	14	-28	32	-44	-44	-40	-31
-2274.76	-17	-5.9998	-15	7.0002	6.0002	-29	-67	-67	-24	-59
-2273.85	19	6.0002	13	-8.9998	8.0002	16	-34	-34	-34	-13
-2272.94	-38	-25	12	-2.9998	-24	15	-40	-40	-66	-38
-2272.03	3.9999	-37	-38	1.9999	-33	-9.0001	-61	-61	-61	-52
-2271.13	-45	-46	-9.0002	-42	-14	-36	-65	-65	-74	-60
-2270.22	31	39	36	49	15	10	-28	-28	-36	-1.9998
-2269.31	-9.0001	-29	-7.0001	5.9999	-14	-15	-55	-55	-62	-58

-2268.41	-12	11	0.99976	4.9998	13	-13	-51	-51	-31	-49
-2267.5	22	22	29	19	33	23	-25	-25	-46	-41
-2266.59	-32	-25	3	-18	5	-3	-55	-55	-51	-55
-2265.69	-26	-13	2.9998	-15	-15	4.9998	-66	-66	-72	-45
-2264.78	3.0001	23	41	-9.9999	27	-18	-29	-29	-44	-25
-2263.88	-19	-38	-33	2	-19	3	-39	-39	-66	-53
-2262.97	-4.9998	-34	9.0002	-28	9.0002	-3.9998	-40	-40	-47	-23
-2262.07	13	28	35	43	26	-12	-48	-48	-55	-51
-2261.16	16	-16	-22	6.0001	11	-27	-69	-69	-65	-62
-2260.26	-3.0002	8.9998	37	-14	20	-8.0002	-47	-47	-57	-17
-2259.35	-5.9999	-16	-17	-5.9999	-22	23	-46	-46	-37	-62
-2258.45	-17	5.9998	-9.0002	-23	15	-19	-40	-40	-50	-41
-2257.54	-5.9999	-4.9999	-14	-22	-19	8.0001	-56	-56	-82	-74
-2256.64	2.0002	-38	12	-22	8.0002	-36	-76	-76	-58	-63
-2255.73	-6.9999	6.0001	26	32	-13	27	-27	-27	-30	-24
-2254.83	21	-5.9999	11	5.0001	-12	-17	-38	-38	-59	-17
-2253.93	31	9.9998	48	41	58	47	7.9998	7.9998	-2.0002	2.9998
-2253.02	-7.9998	-29	5.0002	-17	-2.9998	17	-45	-45	-40	-36
-2252.12	-12	0.99976	-16	-40	1.9998	-13	-50	-50	-55	-7.0002
-2251.22	25	1.0001	27	35	29	23	-8.9999	-8.9999	-39	0.00012
-2250.31	9.0001	-13	0.00012	0.00012	13	-3.9999	-62	-62	-9.9999	-37
-2249.41	-14	-9	-12	-36	0	-17	-49	-49	-40	-37
-2248.51	2.9998	44	33	20	27	7.9998	-18	-18	-47	-23
-2247.6	-4	6	20	-11	12	-5	-36	-36	-32	-21
-2246.7	10	12	26	17	2.0002	23	-16	-16	-13	-2.9998
-2245.8	-1.0001	-26	-11	-14	-13	-14	-49	-49	-43	-40
-2244.9	-16	-34	-17	-4	-6	-15	-19	-19	-47	-35
-2244	-6.9998	-30	0.00024	19	-11	-25	-41	-41	-22	-5.9998
-2243.09	-10	0	-27	18	13	21	7	7	-29	-23
-2242.19	-12	-11	15	-20	33	-26	-3.0002	-3.0002	-41	-35

### B.1.2. Cancer- stage 1

Wavelength (nm)	Intensity # 1	Intensity # 2	Intensity # 3	Intensity # 4	Intensity # 5	Intensity # 6	Intensity # 7	Intensity # 8	Intensity # 9	Intensity # 10
-3614.2	208	260	240	146	151	393	338	573	265	296
-3613.04	159	216	203	130	100	330	278	524	229	246



-3611.87	147	232	191	110	126	357	284	545	249	245
-3610.71	172	243	203	128	143	375	300	566	296	257
-3609.55	166	231	228	125	124	344	305	540	254	273
-3608.39	170	212	174	132	92	348	285	561	220	256
-3607.23	158	190	207	109	92	348	280	538	253	244
-3606.07	206	265	219	116	130	370	296	531	251	277
-3604.9	149	240	202	142	115	358	319	545	260	272
-3603.74	155	222	203	110	116	301	314	541	218	301
-3602.58	177	248	177	139	130	349	306	549	225	282
-3601.42	190	233	233	146	125	391	301	535	274	285
-3600.26	161	221	173	131	112	334	303	561	251	283
-3599.1	174	221	191	101	108	355	253	530	230	231
-3597.94	228	256	214	119	129	387	336	547	279	289
-3596.78	184	256	212	117	139	367	304	572	256	316
-3595.62	174	243	208	108	117	331	271	518	225	252
-3594.47	174	231	182	125	99	351	286	533	224	249
-3593.31	205	252	237	146	149	359	317	533	261	308
-3592.15	173	271	223	104	117	332	321	518	247	277
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-3567.87	171	250	224	126	109	355	296	553	246	278
-3566.72	219	243	238	105	131	389	325	527	238	329
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-3564.41	198	254	236	111	167	376	305	531	231	305
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-3559.8	206	296	240	141	178	351	304	569	246	347
-3558.65	229	306	226	116	167	382	322	550	238	339
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-3268.93	2194	2816	2834	440	2413	3010	1733	1863	529	3735
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-2810.1	2130	3016	4110	481	3030	3902	2113	2093	756	4923
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-2808.09	2117	2998	4118	520	2997	3867	2088	2021	758	4871
-2807.08	2071	2941	3978	461	2942	3831	2017	2044	755	4774
-2806.08	2040	2925	3986	501	2959	3708	2030	2017	738	4830
-2805.07	2091	2902	3975	502	2969	3781	2048	2078	774	4779
-2804.06	1993	2943	3958	469	2937	3697	1992	2035	739	4700
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-2802.05	2016	2923	3876	449	2849	3650	1927	1993	733	4665
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-2800.04	1953	2815	3826	447	2837	3586	1892	1968	726	4576
-2799.04	1954	2827	3716	472	2781	3557	1882	1971	704	4554
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-2797.03	1900	2684	3593	427	2744	3403	1825	1874	697	4423
-2796.02	1926	2711	3627	452	2715	3452	1819	1941	691	4423
-2795.02	1882	2698	3612	442	2654	3379	1878	1916	687	4375
-2794.02	1859	2740	3558	435	2651	3367	1790	1900	693	4337
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-2790	1824	2676	3546	426	2567	3301	1775	1831	613	4164
-2789	1787	2556	3414	428	2531	3232	1732	1867	635	4196
-2788	1823	2525	3355	406	2521	3163	1726	1797	615	4115
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-2781.99	1706	2444	3202	412	2466	3046	1624	1743	605	3869
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-2765.99	1456	2106	2845	361	2091	2590	1407	1536	512	3361
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-2761	1437	2022	2664	325	2012	2543	1347	1458	496	3265
-2760	1452	1988	2651	322	1956	2506	1310	1454	502	3245
-2759	1424	2028	2708	326	2005	2456	1331	1447	504	3174
-2758	1407	2016	2715	341	1975	2473	1353	1437	509	3161
-2757.01	1392	1971	2628	312	1921	2464	1279	1453	519	3139
-2756.01	1467	1994	2650	353	1938	2469	1321	1488	515	3147
-2755.01	1399	1985	2633	365	1925	2438	1373	1441	505	3148
-2754.02	1375	1926	2608	286	1892	2401	1267	1400	466	3056

-2753.02	1351	1896	2561	309	1873	2392	1282	1425	503	3075
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-2751.03	1305	1892	2457	331	1815	2337	1243	1345	466	2960
-2750.03	1344	1878	2505	325	1888	2360	1241	1395	505	2955
-2749.04	1358	1843	2449	326	1790	2294	1229	1383	497	2925
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-2744.07	1231	1767	2373	308	1745	2219	1165	1292	431	2847
-2743.07	1248	1780	2355	278	1729	2134	1145	1297	450	2788
-2742.08	1219	1728	2291	308	1686	2186	1138	1290	457	2740
-2741.08	1258	1782	2324	347	1740	2158	1176	1294	473	2773
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-2738.1	1187	1688	2245	262	1654	2063	1121	1257	441	2649
-2737.11	1181	1664	2252	307	1664	2113	1093	1245	420	2722
-2736.12	1182	1688	2215	286	1614	2054	1083	1278	439	2635
-2735.12	1128	1684	2177	274	1593	2014	1094	1243	466	2612
-2734.13	1148	1671	2178	260	1618	2006	1029	1210	409	2574
-2733.14	1186	1629	2160	310	1585	2038	1061	1282	454	2594
-2732.15	1142	1604	2091	289	1568	1961	1043	1192	448	2578
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-2730.16	1143	1610	2104	289	1550	1988	1050	1161	459	2515
-2729.17	1110	1620	2082	254	1502	1948	1059	1231	389	2454
-2728.18	1109	1575	2058	270	1509	1918	1023	1160	416	2423
-2727.19	1109	1531	2017	257	1501	1914	1010	1131	420	2425
-2726.2	1117	1552	2028	283	1519	1929	1018	1170	448	2469
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-2724.22	1088	1546	1955	224	1434	1834	1020	1151	407	2388
-2723.23	1066	1513	1990	264	1489	1842	983	1108	421	2401
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-2721.24	1080	1481	1908	290	1465	1838	964	1114	401	2311
-2720.25	1051	1510	1949	282	1432	1784	965	1130	416	2321
-2719.26	1056	1510	1923	296	1426	1788	974	1152	400	2316
-2718.28	1009	1463	1891	286	1391	1782	946	1089	378	2261
-2717.29	1098	1435	1884	256	1382	1722	969	1093	366	2271
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-2715.31	980	1411	1830	220	1322	1692	905	1038	344	2182
-2714.32	1005	1388	1835	229	1336	1706	926	1074	364	2193
-2713.33	1010	1401	1827	238	1356	1702	928	1080	401	2230
-2712.34	1008	1390	1804	258	1351	1660	903	1063	353	2151
-2711.35	981	1361	1742	251	1282	1641	873	1068	366	2104
-2710.37	949	1382	1760	239	1285	1664	886	1069	380	2085
-2709.38	988	1341	1829	277	1274	1643	914	1080	414	2144
-2708.39	974	1356	1753	244	1268	1632	904	1066	354	2086
-2707.4	945	1326	1714	195	1271	1653	871	1023	350	2049
-2706.41	972	1283	1736	215	1269	1576	888	1053	390	2069
-2705.43	956	1326	1723	254	1243	1605	866	1050	377	2066
-2704.44	895	1293	1693	238	1264	1559	860	1003	354	2002
-2703.45	958	1245	1642	217	1212	1585	855	972	398	2054
-2702.47	937	1300	1704	260	1255	1549	868	1043	369	2008
-2701.48	896	1293	1634	221	1182	1545	847	984	368	1988
-2700.49	889	1256	1662	253	1203	1610	855	986	357	1993
-2699.51	872	1283	1603	202	1194	1487	798	973	364	1960
-2698.52	899	1252	1654	242	1199	1527	840	1000	374	1976
-2697.54	870	1215	1582	223	1158	1463	819	981	332	1874
-2696.55	882	1233	1595	242	1213	1462	767	991	366	1897
-2695.57	863	1198	1564	226	1140	1456	808	959	322	1885
-2694.58	818	1198	1542	230	1109	1451	757	948	343	1847
-2693.6	841	1185	1546	193	1124	1492	763	911	317	1842
-2692.61	847	1182	1526	208	1110	1434	832	915	336	1839
-2691.63	853	1158	1528	241	1119	1411	755	942	330	1873
-2690.64	852	1201	1517	190	1126	1368	738	948	329	1828
-2689.66	839	1143	1504	191	1086	1423	785	967	336	1762
-2688.67	880	1168	1537	237	1067	1385	762	958	315	1763
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-2686.71	795	1139	1475	194	1051	1394	755	919	303	1733
-2685.72	829	1133	1476	206	1066	1329	758	883	318	1768
-2684.74	806	1161	1422	185	1039	1315	723	895	316	1767
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-2682.77	811	1094	1423	215	1020	1356	746	951	323	1682
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-2680.81	762	1088	1383	200	969	1308	727	864	314	1662
-2679.83	813	1109	1403	224	1011	1300	732	913	319	1648
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-2674.92	764	1039	1312	208	957	1213	704	852	306	1621
-2673.94	757	1041	1301	205	957	1248	703	861	327	1600
-2672.95	744	1029	1342	165	962	1217	668	845	280	1582
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-2653.37	666	898	1128	205	816	1058	641	769	322	1316
-2652.39	567	831	1036	125	747	943	544	732	192	1252
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-2649.46	600	888	1034	165	783	1030	585	727	269	1286
-2648.49	581	820	1037	145	746	1004	545	714	259	1258
-2647.51	607	805	1044	166	740	987	583	725	296	1264
-2646.53	568	795	1038	157	751	944	554	719	249	1247
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-2644.58	593	797	1017	142	751	994	575	699	267	1226
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-2640.68	535	772	983	159	692	929	515	693	263	1193
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-2638.73	549	711	911	133	695	886	515	654	250	1154
-2637.76	534	718	904	118	659	913	513	662	249	1118
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-2635.81	521	756	870	129	660	866	509	640	244	1075
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-2628.02	467	663	852	136	609	814	487	590	235	1032
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-2618.31	428	609	700	103	543	731	429	561	214	932
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-2611.51	387	556	692	122	493	659	395	546	204	829
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-2603.76	413	501	658	117	496	624	381	529	189	808
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-2596.99	376	487	594	124	426	602	366	482	150	694
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-2593.13	316	487	571	82	403	592	319	477	163	703
-2592.16	352	489	588	95	435	580	333	488	190	719
-2591.19	332	505	522	96	404	578	337	489	194	689
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-2589.26	357	469	563	90	409	528	313	466	177	679
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-2587.33	330	463	548	93	405	505	347	465	158	661
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-2567.11	282	411	459	107	313	457	303	436	179	582
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-2302.97	-44	-29	1.9999	-32	-1.0001	-54	-17	-25	-22	-12
-2302.05	-12	0.99976	-11	2.0002	-0.9998	-5.9998	22	-35	-28	10
-2301.14	-22	-19	-39	-28	-38	-17	-35	-9.0002	-63	-16
-2300.23	13	-5.9998	7.0002	33	-12	30	8.0002	-9.9998	-2.9998	8.0002
-2299.32	42	29	31	24	17	11	36	12	-4.9998	16
-2298.41	-20	17	-25	9	-30	-8	15	7	1	-28
-2297.49	-7.9999	-21	-29	-12	-7.9999	-8.9999	-9.9999	-24	-48	-11
-2296.58	0.00012	-16	-7.0001	-19	-10	-38	-11	-8.0001	-10	-5.0001
-2295.67	-18	3.9999	-11	-16	-13	-1.0001	23	-9.0001	-33	-17
-2294.76	-27	-39	-22	-21	-38	-13	-18	-32	-59	-29
-2293.85	8.9999	8.9999	-10	-10	-10	1.9999	-6.0001	-14	-2.0001	-9.0001
-2292.94	5.0002	-24	-7.9998	-24	-20	-12	-19	-27	-28	-30
-2292.03	-2.9998	9.0002	-11	23	-14	-19	-14	-35	-22	0.00024
-2291.12	-43	-32	-27	-33	-27	-20	-23	-69	-60	-35
-2290.21	-6	2	-7	-30	-36	-25	-38	-5	-24	-16
-2289.3	-10	-8.0001	-10	-8.0001	2.9999	11	25	-5.0001	-24	-13
-2288.39	-5.0002	-4.0002	-15	-39	-23	-30	-4.0002	-31	-58	-10
-2287.48	-4.0002	-17	-20	-29	-40	-27	-43	-38	-34	-41
-2286.57	-14	-34	2	-2	-2	0	6	-3	-35	-20
-2285.66	-37	-11	-43	-23	-54	-63	-39	-56	-65	-52

-2284.75	-48	-26	-51	-32	-53	-22	-44	-24	-60	-65
-2283.84	-20	-15	-26	-9.9998	-12	-22	-32	-28	-34	-22
-2282.93	-25	7.0002	-26	-23	0.00024	-23	-33	-27	-37	-42
-2282.02	-31	-23	-31	-38	-35	-4	-38	-29	-68	-48
-2281.11	-31	-13	-33	-14	-19	-20	-29	-37	-30	-14
-2280.2	-18	-18	-31	-35	-27	-2	-21	-30	-35	-24
-2279.29	-32	1.9999	-48	-12	-57	-59	-33	-44	-52	-57
-2278.39	-21	-31	-49	-9	-50	-42	-56	-36	-68	-49
-2277.48	-30	-27	-36	-46	-34	-45	-36	-71	-53	-8.0002
-2276.57	-55	-41	-75	-29	-55	-68	-64	-63	-66	-64
-2275.66	-34	-8.0001	-33	-13	-60	-23	-16	-36	-43	-47
-2274.76	-32	-15	-45	-51	-75	-26	-58	-64	-76	-60
-2273.85	-5.9998	-19	-20	-24	-44	-35	-30	-46	-42	-18
-2272.94	-35	-32	-62	-20	-60	-49	-42	-74	-80	-47
-2272.03	-23	-40	-59	-40	-38	-10	-67	-52	-71	-34
-2271.13	-58	-88	-55	-38	-71	-87	-60	-79	-42	-66
-2270.22	-11	0.99976	8.0002	-20	10	-14	5.0002	-20	-34	-25
-2269.31	-68	-27	-57	-35	-37	-41	-33	-59	-48	-34
-2268.41	-17	-24	-37	-20	-39	-33	-36	-59	-50	-36
-2267.5	0.00024	-1.9998	-35	-29	-11	-44	-28	-28	-43	-17
-2266.59	-68	-48	-47	-32	-39	-33	-35	-49	-73	-54
-2265.69	-35	-55	-23	-36	-57	-42	-51	-41	-66	-33
-2264.78	-12	-21	-28	-2.9999	-25	-48	-24	-21	-20	-13
-2263.88	-45	-27	-58	-36	-49	-45	-19	-77	-71	-47
-2262.97	-37	-46	-38	-34	-53	-17	-32	-39	-60	-20
-2262.07	-14	-22	-29	-17	-33	-34	-16	-23	-62	-31
-2261.16	-38	-33	-51	-61	-22	-48	-65	-52	-69	-32
-2260.26	-35	-20	-12	-15	-46	-17	-39	-32	-27	-31
-2259.35	-33	-38	-40	-48	-64	-46	-45	-35	-76	-55
-2258.45	-16	-32	-34	-42	-49	-44	-61	-74	-58	-34
-2257.54	-56	-43	-62	-53	-45	-57	-50	-64	-79	-57
-2256.64	-68	-38	-76	-30	-52	-68	-52	-59	-68	-50
-2255.73	-34	2.0001	-31	-22	-33	-38	-33	-36	-64	-39
-2254.83	0.00012	3.0001	-40	-55	-53	-41	-52	-53	-58	-26
-2253.93	-6.0002	30	11	-7.0002	-0.0002	1.9998	7.9998	-23	-22	6.9998
-2253.02	-29	-39	-51	-44	-62	-26	-29	-31	-87	-9.9998
-2252.12	-21	-20	-42	-24	-49	-34	-51	-57	-43	-34

-2251.22	-15	-19	-9.9999	-21	-5.9999	-12	-5.9999	-20	-7.9999	-20
-2250.31	-38	-20	-40	-20	-35	-5.9999	-25	-34	-33	-24
-2249.41	-35	-7	-53	-38	-61	-42	-26	-57	-60	-45
-2248.51	-22	-9.0002	-9.0002	-18	-5.0002	-20	2.9998	-30	-36	23
-2247.6	-32	-9	-14	-13	-11	-28	-13	-30	-39	-28
-2246.7	7.0002	-2.9998	-13	-35	-26	-11	6.0002	-3.9998	-42	-20
-2245.8	-21	-16	-30	-9.0001	-48	-36	-36	-55	-55	-23
-2244.9	-25	-19	-28	-34	-25	-17	-34	-12	-36	-24
-2244	-39	-21	-33	-0.9998	-45	-32	-8.9998	-22	-36	-19
-2243.09	-5	13	-21	-25	-9	-28	-26	-21	-54	-28
-2242.19	-27	-18	-16	-26	-33	-25	-43	-52	-37	-6.0002

### B.1.3. Cancer – Stage 2

Wavelength (nm)	Intensity # 1	Intensity # 2	Intensity # 3	Intensity # 4	Intensity # 5	Intensity # 6	Intensity # 7	Intensity # 8	Intensity # 9	Intensity # 10
-3614.2	48	35	51	87	53	346	43	55	49	69
-3613.04	65	35	79	90	74	64	27	54	58	98
-3611.87	42	6.9998	-8.0002	50	37	6.9998	15	21	22	29
-3610.71	65	64	55	78	68	83	24	61	54	82
-3609.55	46	14	61	60	66	85	31	70	47	76
-3608.39	86	40	34	90	70	51	69	44	52	78
-3607.23	71	38	21	85	58	71	26	48	48	52
-3606.07	40	34	17	72	70	46	33	29	50	58
-3604.9	62	29	31	80	77	44	41	45	72	83
-3603.74	43	28	23	57	39	39	13	18	17	28
-3602.58	102	56	61	109	89	90	63	93	92	107
-3601.42	49	22	22	73	82	59	22	45	43	66
-3600.26	58	48	67	73	71	50	34	47	51	76

-3599.1	70	50	62	106	77	80	47	63	63	79
-3597.94	54	52	32	86	82	49	38	51	42	82
-3596.78	52	34	40	84	49	44	49	50	32	75
-3595.62	77	73	64	77	72	61	34	47	49	74
-3594.47	81	32	44	92	67	46	60	56	65	81
-3593.31	39	32	40	87	62	43	29	43	44	55
-3592.15	73	39	77	96	66	34	75	33	56	79
-3590.99	54	16	25	92	48	53	37	53	50	56
-3589.83	97	49	38	93	82	59	63	65	61	93
-3588.67	47	25	67	87	80	36	58	49	54	78
-3587.52	64	34	48	98	80	65	22	54	45	77
-3586.36	70	63	51	129	69	80	64	87	81	81
-3585.2	42	48	42	93	57	51	52	40	42	82
-3584.04	79	59	56	92	101	78	48	50	47	97
-3582.89	65	18	67	93	51	80	59	58	25	72
-3581.73	89	39	45	77	86	74	52	40	68	101
-3580.58	58	13	18	97	72	34	44	61	76	53
-3579.42	41	33	28	72	51	51	-7.0001	7.9999	15	73
-3578.26	80	56	57	117	75	81	73	71	68	70
-3577.11	50	32	43	102	89	65	56	84	44	51
-3575.95	67	64	64	85	94	46	29	61	60	87
-3574.8	94	49	52	117	65	70	43	43	42	82
-3573.64	82	59	54	100	103	52	56	68	66	99
-3572.49	66	69	52	112	95	76	42	78	88	103
-3571.33	69	50	49	101	75	67	52	49	54	98
-3570.18	89	50	67	155	90	100	98	92	85	91
-3569.03	58	51	22	97	89	59	60	69	67	80
-3567.87	68	46	75	102	98	61	65	69	40	91
-3566.72	86	78	83	110	66	77	41	69	69	109
-3565.56	64	48	38	100	72	81	55	56	41	72
-3564.41	58	52	17	88	64	58	35	52	55	78
-3563.26	71	67	85	131	112	104	71	98	82	131
-3562.11	83	40	48	126	86	77	70	50	67	83
-3560.95	100	85	82	117	100	94	57	88	94	97
-3559.8	110	85	89	154	131	101	94	86	77	131
-3558.65	124	99	86	158	101	94	85	84	82	119
-3557.5	124	72	61	120	99	50	76	89	77	101
-3556.35	63	61	55	109	71	75	53	75	50	72

-3555.2	106	81	64	116	116	75	76	85	91	132
-3554.04	100	83	74	148	89	81	64	77	77	100
-3552.89	101	108	91	124	118	113	58	110	88	109
-3551.74	85	71	54	87	110	79	86	84	87	100
-3550.59	136	105	89	131	94	124	65	69	108	144
-3549.44	121	94	85	151	118	107	111	109	97	107
-3548.29	92	69	69	136	87	81	71	79	72	131
-3547.14	129	89	90	143	131	76	77	86	116	119
-3545.99	87	76	63	137	79	61	71	75	72	96
-3544.84	74	66	54	108	99	88	67	83	102	96
-3543.7	89	99	80	139	136	78	62	99	100	111
-3542.55	120	136	122	162	117	104	121	127	84	149
-3541.4	123	93	89	150	125	97	105	76	92	113
-3540.25	125	90	63	171	144	125	107	125	101	116
-3539.1	107	109	96	165	143	125	110	101	118	149
-3537.95	120	122	108	171	105	100	94	98	93	137
-3536.81	125	106	93	151	167	137	108	118	121	157
-3535.66	124	123	93	184	164	124	135	138	129	158
-3534.51	135	104	104	157	126	115	122	106	95	133
-3533.37	130	113	92	166	141	117	104	133	116	154
-3532.22	126	103	40	159	134	113	106	104	93	140
-3531.07	100	70	84	144	124	99	89	97	71	139
-3529.93	124	135	107	155	139	116	87	112	124	145
-3528.78	140	138	92	174	152	150	88	133	109	128
-3527.63	108	120	110	192	162	146	135	152	136	154
-3526.49	108	93	82	143	136	117	106	116	96	153
-3525.34	122	91	70	132	115	75	81	67	73	113
-3524.2	127	144	95	175	164	130	119	152	123	164
-3523.05	136	129	111	176	180	117	119	133	132	173
-3521.91	127	135	102	155	144	106	106	122	117	159
-3520.77	139	125	104	172	151	128	150	145	129	151
-3519.62	139	128	114	198	158	152	132	174	150	183
-3518.48	145	127	117	186	164	109	126	122	132	173
-3517.33	109	100	87	177	134	113	110	112	93	133
-3516.19	169	157	85	210	182	160	163	166	171	187
-3515.05	138	112	94	157	129	121	124	126	125	148
-3513.9	148	146	127	188	185	123	108	150	132	168
-3512.76	181	144	128	209	199	178	135	175	161	175

-3511.62	131	145	110	206	162	141	140	130	157	197
-3510.48	166	153	130	205	208	162	156	138	168	201
-3509.33	171	133	115	196	155	157	154	154	132	167
-3508.19	148	131	95	180	172	147	123	151	136	175
-3507.05	152	114	115	191	178	131	155	151	118	172
-3505.91	162	166	117	217	171	195	127	155	129	202
-3504.77	152	186	118	239	193	163	165	169	169	209
-3503.63	91	104	91	193	165	127	127	131	124	169
-3502.49	141	136	112	182	193	133	144	137	144	182
-3501.35	176	149	125	208	172	171	143	173	157	198
-3500.2	182	169	103	236	207	157	176	162	158	214
-3499.06	154	146	116	216	192	161	137	172	154	193
-3497.93	155	130	121	197	194	150	162	165	140	217
-3496.79	201	145	131	257	203	167	176	186	189	208
-3495.65	175	195	135	231	237	185	191	218	195	201
-3494.51	151	160	105	214	186	157	155	146	158	217
-3493.37	205	188	148	256	180	172	176	181	162	237
-3492.23	209	196	162	249	240	184	222	201	159	228
-3491.09	180	195	127	248	214	187	206	216	196	241
-3489.95	216	214	146	280	224	176	200	211	195	254
-3488.81	180	153	124	270	203	184	180	166	184	225
-3487.68	202	209	189	281	249	204	207	224	231	254
-3486.54	182	161	152	244	210	190	189	179	172	240
-3485.4	206	188	182	284	236	204	219	199	211	232
-3484.26	221	206	174	280	271	224	221	261	243	259
-3483.13	173	147	127	238	213	180	186	176	174	240
-3481.99	217	208	135	281	238	203	201	243	184	260
-3480.85	193	180	145	272	211	188	221	195	202	247
-3479.72	224	245	171	302	249	204	203	231	229	259
-3478.58	206	220	153	275	260	241	258	219	204	288
-3477.45	201	191	164	276	231	204	203	183	238	231
-3476.31	251	247	154	320	251	229	241	249	246	253
-3475.18	201	213	153	314	236	237	235	218	196	255
-3474.04	274	245	177	331	298	255	238	269	258	296
-3472.91	235	219	162	273	252	211	220	231	187	263
-3471.77	216	242	174	300	287	255	243	248	246	266
-3470.64	227	216	169	302	297	222	252	245	251	278
-3469.5	257	257	209	346	277	243	280	230	242	304

-3468.37	253	279	199	382	324	293	281	286	283	302
-3467.23	225	218	162	322	300	247	258	267	257	277
-3466.1	266	245	217	342	320	272	250	260	260	295
-3464.97	249	241	207	335	296	265	291	260	259	315
-3463.83	264	303	231	353	329	282	288	275	298	333
-3462.7	248	259	171	359	315	248	269	250	253	297
-3461.57	233	292	228	355	296	277	289	256	270	315
-3460.44	302	291	197	354	300	294	303	246	287	324
-3459.3	258	251	197	356	315	287	267	259	274	320
-3458.17	292	310	237	401	317	326	344	314	286	372
-3457.04	317	314	262	406	331	299	330	310	290	353
-3455.91	305	306	193	413	370	306	323	329	291	381
-3454.78	272	312	215	390	327	332	302	286	293	338
-3453.65	308	321	256	432	359	324	337	318	319	371
-3452.51	327	311	238	429	373	347	327	341	320	393
-3451.38	297	326	221	411	354	297	323	296	301	363
-3450.25	288	305	238	417	368	322	327	327	300	388
-3449.12	328	311	230	391	329	308	319	299	284	367
-3447.99	350	326	232	431	374	342	356	346	314	380
-3446.86	331	358	262	438	385	332	372	339	339	416
-3445.73	337	350	253	448	361	321	326	339	340	393
-3444.6	316	311	212	453	343	329	331	331	291	363
-3443.48	326	342	258	432	414	347	344	339	360	379
-3442.35	361	349	258	462	438	342	383	363	342	392
-3441.22	383	389	286	479	375	344	374	352	351	423
-3440.09	365	328	272	491	428	364	419	358	362	402
-3438.96	322	358	256	436	417	315	372	343	348	402
-3437.83	350	363	287	452	414	367	360	350	361	405
-3436.71	349	356	237	465	417	371	410	367	377	445
-3435.58	340	373	267	478	438	350	404	409	399	431
-3434.45	378	398	261	476	441	385	427	393	374	447
-3433.32	393	412	319	521	459	369	401	413	382	459
-3432.2	412	417	268	539	427	416	417	416	403	464
-3431.07	351	372	252	478	434	363	428	348	363	408
-3429.94	392	416	304	538	469	385	448	416	388	481
-3428.82	404	411	280	523	472	424	416	441	398	461
-3427.69	395	425	307	539	483	401	450	407	426	485
-3426.57	419	431	314	546	484	400	474	444	404	492



-3425.44	412	448	342	524	481	453	464	417	391	495
-3424.31	415	406	309	573	475	430	474	435	431	496
-3423.19	415	449	329	586	510	401	491	441	419	517
-3422.06	484	468	350	570	511	455	471	473	450	516
-3420.94	428	465	337	556	520	436	484	447	482	548
-3419.82	453	462	329	606	543	475	491	463	476	515
-3418.69	455	484	374	574	555	470	500	500	472	555
-3417.57	468	477	365	614	532	451	507	444	476	535
-3416.44	489	478	366	578	532	457	500	466	464	516
-3415.32	471	520	365	590	575	499	554	510	503	546
-3414.2	479	524	383	633	559	478	513	490	481	542
-3413.07	486	509	370	634	581	484	540	507	503	569
-3411.95	518	531	380	632	588	482	561	529	522	551
-3410.83	453	473	379	644	552	513	535	519	488	587
-3409.71	526	556	409	650	602	479	548	503	481	590
-3408.58	512	547	382	652	590	513	599	529	487	554
-3407.46	508	582	403	681	578	489	552	496	487	604
-3406.34	536	546	384	683	632	525	539	541	493	611
-3405.22	550	552	433	676	619	531	580	569	531	583
-3404.1	543	539	409	712	610	524	579	563	548	615
-3402.98	499	545	350	668	572	493	544	528	511	606
-3401.85	557	583	423	703	655	537	632	578	570	642
-3400.73	521	557	392	689	598	534	603	537	534	613
-3399.61	556	603	421	708	624	559	620	569	549	647
-3398.49	572	576	434	724	661	549	649	545	538	654
-3397.37	598	569	448	717	657	534	608	559	575	652
-3396.25	601	630	462	731	650	555	644	624	611	653
-3395.13	545	563	424	729	649	563	640	576	599	638
-3394.01	559	598	425	741	646	555	612	594	563	666
-3392.9	569	560	417	702	646	558	631	573	525	651
-3391.78	566	625	424	677	679	562	620	597	569	646
-3390.66	590	636	434	746	685	551	649	562	570	668
-3389.54	598	667	450	768	686	563	667	613	598	678
-3388.42	579	612	467	771	691	564	664	609	628	669
-3387.3	596	616	418	748	694	577	691	611	591	695
-3386.18	611	676	508	783	712	598	634	651	640	705
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-3377.25	663	699	524	843	760	639	737	666	635	720
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-3372.79	684	706	496	875	751	641	742	674	655	746
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-3368.33	667	744	509	863	772	688	747	720	680	789
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-3364.99	695	741	518	894	779	696	767	681	658	794
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-2856.61	1408	1536	1207	1887	1651	1205	1621	1578	1560	1838
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-2843.43	1276	1410	1056	1689	1501	1064	1469	1461	1451	1668
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-2827.24	1090	1196	907	1410	1285	935	1190	1208	1275	1394
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-2797.03	807	885	674	1063	966	721	907	877	932	1011
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-2794.02	752	838	639	1089	951	722	893	883	864	960
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-2786.99	774	789	622	1025	894	693	830	839	857	926
-2785.99	705	784	600	996	898	657	817	769	797	889
-2784.99	756	855	655	998	891	716	838	814	830	921
-2783.99	752	807	585	965	895	684	797	797	829	870
-2782.99	721	767	591	956	856	675	764	764	819	890
-2781.99	712	761	596	968	875	644	795	784	764	883
-2780.98	675	763	612	917	845	673	773	758	768	861



-2779.98	736	787	572	958	843	660	790	789	838	888
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-2773.98	659	714	553	876	767	612	745	745	741	810
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-2762.99	612	672	494	843	699	582	706	635	701	731
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-2759	598	648	478	783	665	570	659	646	658	693
-2758	597	636	505	796	721	563	642	638	658	732
-2757.01	610	678	506	809	688	565	675	671	654	727
-2756.01	582	625	476	773	668	546	635	612	635	705
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-2732.15	473	550	403	629	531	463	515	511	523	547
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-2730.16	480	537	397	637	592	490	502	517	500	557
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-2695.57	380	374	261	495	414	331	401	379	387	427
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-2692.61	307	331	242	438	406	310	317	347	366	385
-2691.63	342	374	285	475	432	321	362	380	389	402
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-2682.77	283	318	259	415	344	271	343	320	349	364
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-2679.83	313	350	275	430	358	309	337	335	375	392
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-2674.92	279	311	206	396	320	261	293	298	297	331
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-2672.95	306	297	228	390	334	275	279	282	331	356
-2671.97	310	300	234	404	343	291	318	315	321	338
-2670.99	282	306	223	385	316	273	271	312	344	351
-2670.01	275	282	223	367	321	262	285	290	313	348
-2669.03	263	316	252	398	334	258	314	305	300	344
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-2667.07	262	290	200	370	346	264	291	292	298	325
-2666.09	267	290	218	388	315	228	290	262	294	319
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-2636.78	214	209	148	311	236	193	200	212	237	237
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-2634.83	213	212	193	273	268	206	194	244	240	244
-2633.86	211	233	162	276	236	205	245	222	233	261
-2632.89	183	207	125	290	232	163	203	198	199	216
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-2629.97	188	183	155	266	237	159	201	212	225	247
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-2361.72	-9.0002	-12	-0.0002	-13	-46	-9.0002	-58	-34	-42	-40
-2360.79	37	-24	-19	-5.9999	-6.9999	0.00012	-25	-9.9999	-25	-9.9999
-2359.87	8.0001	-26	-11	-31	-13	-30	-34	-39	-36	-26
-2358.95	29	-11	8.9998	0.99976	-34	-13	-46	-29	-51	-18
-2358.03	-14	4.0001	-32	-19	-25	-34	-48	-12	-55	-13
-2357.1	1.0001	-13	-38	-40	-50	2.0001	-38	-21	-30	-15
-2356.18	-15	-8.0001	1.9999	-16	0.99988	-21	-31	-4.0001	-43	-24
-2355.26	9.0001	-20	0.00012	-37	-28	-21	-39	-30	-41	-28
-2354.34	-20	-35	-47	-7.0001	-27	-16	-45	-13	-65	-38
-2353.41	-18	-46	-19	-24	-37	-20	-51	-64	-49	-49
-2352.49	-53	-68	-39	-60	-64	-68	-69	-93	-89	-95
-2351.57	4.9999	-20	-26	-37	-12	3.9999	-37	-24	-47	-28
-2350.65	-2	-62	-70	-54	-51	-56	-74	-58	-87	-55
-2349.73	-47	-70	-27	-45	-20	-48	-68	-48	-101	-56
-2348.81	-15	-20	-21	-49	-67	-35	-52	-47	-78	-79
-2347.89	-27	-35	-20	-27	-37	-19	-47	-44	-74	-49
-2346.97	4.0002	-42	-40	-30	-26	-19	-46	-50	-53	-16
-2346.05	0.99976	-41	-2.0002	-38	-40	-40	-51	-54	-66	-44

-2345.12	-30	-15	-47	-53	-56	-52	-18	-50	-66	-56
-2344.2	5.0002	-35	-52	-30	-41	-51	-73	-59	-71	-33
-2343.28	-45	-44	-47	-54	-32	-62	-49	-49	-83	-60
-2342.36	-36	-46	-28	-69	-75	-48	-72	-68	-84	-57
-2341.44	-58	-81	-76	-83	-83	-102	-113	-89	-89	-96
-2340.53	-11	-31	-46	-54	-26	-31	-17	-34	-74	-28
-2339.61	-38	-46	-25	-45	-37	-36	-77	-76	-75	-47
-2338.69	-22	-16	-18	-51	-27	-57	-38	-52	-55	-38
-2337.77	4.0001	-55	-66	-25	-49	-40	-71	-68	-97	-44
-2336.85	-68	-42	-48	-51	-46	-52	-74	-62	-81	-26
-2335.93	-28	-80	-53	-69	-73	-35	-70	-81	-107	-57
-2335.01	-51	-81	-81	-70	-85	-66	-74	-70	-105	-76
-2334.09	-22	-40	-63	-64	-51	-67	-73	-63	-86	-67
-2333.17	-58	-88	-52	-58	-50	-82	-87	-87	-105	-55
-2332.26	22	0.00024	-5.9998	-15	-23	-2.9998	-7.9998	-22	-32	-24
-2331.34	-15	-73	-69	-46	-80	-55	-77	-62	-63	-41
-2330.42	-68	-63	-57	-58	-52	-79	-95	-62	-103	-52
-2329.5	-23	-50	-45	-49	-72	-43	-56	-44	-103	-61
-2328.59	-40	-54	-71	-51	-48	-51	-73	-71	-92	-86
-2327.67	-4.0002	-65	-49	-41	-48	-53	-76	-54	-104	-59
-2326.75	-75	-61	-50	-64	-49	-58	-56	-87	-116	-68
-2325.83	-14	-74	-52	-50	-65	-58	-90	-79	-74	-90
-2324.92	-25	-72	-71	-33	-63	-74	-65	-69	-96	-74
-2324	-71	-79	-77	-83	-80	-65	-98	-95	-132	-106
-2323.09	-46	-58	-42	-51	-69	-60	-82	-87	-82	-79
-2322.17	-58	-77	-87	-93	-87	-78	-85	-96	-136	-85
-2321.25	-29	-35	-54	-63	-98	-66	-72	-52	-111	-86
-2320.34	-32	-43	-47	-52	-57	-64	-67	-73	-66	-68
-2319.42	2.0002	-33	-16	-54	-42	-51	-54	-61	-76	-68
-2318.51	-23	-54	-68	-81	-60	-62	-74	-105	-91	-85
-2317.59	-46	-47	-50	-81	-64	-65	-90	-88	-103	-63
-2316.68	-27	-45	-76	-82	-81	-85	-114	-103	-135	-114
-2315.76	-33	-58	-69	-80	-64	-60	-66	-115	-133	-94
-2314.85	-25	-68	-37	-86	-78	-106	-117	-93	-127	-101
-2313.93	-54	-82	-79	-88	-102	-87	-117	-111	-144	-112
-2313.02	-69	-73	-44	-70	-112	-91	-89	-128	-132	-105
-2312.1	-31	-80	-69	-92	-66	-87	-138	-90	-124	-101
-2311.19	-52	-68	-68	-99	-84	-100	-82	-122	-163	-96

-2310.27	-88	-84	-66	-71	-98	-82	-124	-134	-153	-120
-2309.36	-70	-74	-89	-105	-111	-83	-128	-110	-152	-129
-2308.45	-19	-78	-81	-110	-94	-97	-118	-111	-154	-111
-2307.53	-106	-124	-120	-148	-114	-153	-159	-146	-190	-154
-2306.62	-40	-92	-95	-104	-121	-114	-140	-127	-158	-136
-2305.71	-31	-90	-109	-118	-129	-121	-131	-122	-171	-141
-2304.79	-57	-66	-98	-93	-103	-121	-108	-123	-160	-122
-2303.88	-61	-82	-82	-97	-109	-100	-143	-146	-169	-103
-2302.97	-67	-67	-73	-124	-106	-138	-109	-146	-170	-142
-2302.05	-59	-98	-96	-126	-149	-121	-140	-146	-205	-145
-2301.14	-76	-122	-102	-142	-153	-169	-135	-150	-209	-144
-2300.23	-68	-89	-95	-111	-151	-137	-131	-136	-182	-170
-2299.32	-90	-100	-101	-120	-129	-151	-154	-144	-199	-168
-2298.41	-32	-88	-89	-103	-116	-121	-143	-139	-156	-111
-2297.49	-85	-116	-115	-140	-156	-136	-175	-161	-207	-180
-2296.58	-93	-107	-109	-141	-164	-162	-187	-175	-216	-193
-2295.67	-51	-107	-110	-131	-166	-137	-159	-161	-225	-180
-2294.76	-103	-141	-137	-148	-154	-177	-198	-202	-244	-188
-2293.85	-94	-119	-109	-152	-151	-173	-175	-192	-241	-198
-2292.94	-75	-119	-127	-141	-157	-154	-162	-163	-236	-201
-2292.03	-70	-5	-122	-146	-179	-178	-192	-205	-213	-169
-2291.12	-133	-56	-153	-163	-217	-195	-206	-208	-288	-219
-2290.21	-91	-134	-120	-214	-199	-210	-233	-239	-270	-219
-2289.3	-87	-128	-157	-174	-192	-182	-206	-219	-258	-194
-2288.39	-99	-140	-139	-195	-176	-200	-224	-216	-270	-197
-2287.48	-87	-134	-113	-180	-196	-207	-197	-238	-269	-221
-2286.57	-122	-134	-171	-229	-229	-220	-222	-260	-315	-243
-2285.66	-81	-109	-134	-180	-189	-209	-228	-242	-282	-225
-2284.75	-131	-139	-145	-214	-217	-219	-253	-250	-309	-261
-2283.84	-133	-143	-163	-239	-242	-220	-260	-272	-318	-249
-2282.93	-119	-179	-218	-238	-250	-277	-279	-288	-335	-294
-2282.02	-104	-166	-180	-232	-223	-238	-252	-293	-347	-242
-2281.11	-142	-169	-173	-247	-258	-243	-283	-298	-360	-275
-2280.2	-97	-142	-204	-224	-209	-256	-246	-293	-361	-259
-2279.29	-110	-163	-180	-227	-235	-259	-299	-306	-356	-267
-2278.39	-112	-122	-168	-214	-217	-224	-253	-264	-317	-251
-2277.48	-87	-152	-188	-244	-270	-245	-275	-274	-356	-286
-2276.57	-138	-197	-242	-256	-286	-271	-305	-313	-395	-305

-2275.66	-103	-143	-179	-216	-227	-242	-260	-299	-351	-257
-2274.76	-164	-195	-193	-238	-294	-292	-302	-322	-418	-328
-2273.85	-157	-193	-217	-290	-285	-307	-299	-353	-423	-342
-2272.94	-122	-177	-236	-270	-263	-278	-296	-337	-388	-322
-2272.03	-110	-132	-189	-234	-236	-275	-297	-326	-376	-281
-2271.13	-145	-176	-213	-267	-300	-311	-316	-330	-425	-318
-2270.22	-126	-186	-259	-281	-296	-283	-295	-325	-425	-352
-2269.31	-109	-170	-215	-260	-240	-316	-295	-316	-390	-318
-2268.41	-134	-187	-177	-256	-281	-272	-313	-325	-432	-327
-2267.5	-110	-149	-199	-249	-267	-273	-282	-321	-381	-311
-2266.59	-97	-152	-185	-244	-245	-252	-273	-278	-369	-288
-2265.69	-94	-145	-176	-251	-222	-283	-294	-321	-386	-295
-2264.78	-115	-155	-180	-228	-272	-255	-287	-308	-371	-309
-2263.88	-99	-168	-195	-218	-257	-256	-278	-298	-365	-300
-2262.97	-107	-159	-173	-235	-238	-276	-314	-293	-401	-299
-2262.07	-130	-174	-156	-216	-226	-241	-238	-266	-383	-278
-2261.16	-120	-156	-179	-228	-261	-280	-265	-297	-361	-281
-2260.26	-103	-139	-173	-258	-244	-284	-308	-303	-395	-315
-2259.35	-55	-137	-148	-215	-187	-219	-246	-269	-345	-233
-2258.45	-114	-151	-184	-209	-243	-255	-266	-295	-346	-292
-2257.54	-123	-177	-195	-245	-247	-259	-255	-294	-406	-283
-2256.64	-30	-46	-121	-188	-189	-186	-199	-221	-296	-208
-2255.73	-109	-133	-170	-195	-200	-243	-233	-254	-340	-256
-2254.83	-53	-115	-125	-192	-199	-201	-206	-244	-306	-225
-2253.93	-76	-127	-131	-209	-183	-213	-229	-246	-315	-241
-2253.02	-108	-149	-145	-205	-178	-190	-203	-243	-314	-216
-2252.12	-69	-125	-124	-161	-198	-215	-220	-242	-286	-231
-2251.22	-74	-102	-133	-184	-170	-203	-174	-217	-277	-213
-2250.31	-74	-113	-151	-156	-184	-185	-226	-221	-264	-221
-2249.41	-53	-123	-104	-148	-173	-181	-184	-210	-274	-188
-2248.51	-35	-58	-97	-121	-154	-134	-140	-174	-237	-158
-2247.6	-29	-95	-118	-162	-130	-158	-186	-165	-237	-167
-2246.7	-47	-83	-106	-120	-151	-129	-151	-196	-241	-159
-2245.8	-58	-96	-73	-111	-111	-135	-156	-173	-220	-151
-2244.9	-37	-71	-79	-135	-117	-160	-166	-157	-212	-159
-2244	-5.9998	-53	-101	-125	-123	-124	-157	-160	-203	-127
-2243.09	-76	-81	-94	-120	-142	-151	-149	-161	-226	-145
-2242.19	-12	-50	-66	-109	-101	-98	-138	-118	-178	-128

### B.1.4. Cancer stage 3

Wavelength (nm)	Intensity # 1	Intensity # 2	Intensity # 3	Intensity # 4	Intensity # 5	Intensity # 6	Intensity # 7	Intensity # 8	Intensity # 9	Intensity # 10
-3614.2	59	75	117	62	28	94	45	72	66	75
-3613.04	76	62	84	63	24	57	26	90	64	85
-3611.87	49	36	43	34	-6.0002	27	-10	32	36	49
-3610.71	51	75	116	79	39	98	45	74	63	94
-3609.55	62	59	76	65	16	88	32	77	62	74
-3608.39	89	83	79	30	55	104	40	81	55	58
-3607.23	52	63	103	52	57	62	18	58	59	60
-3606.07	60	77	84	44	33	79	14	34	35	63
-3604.9	48	44	84	67	15	67	19	66	51	74
-3603.74	60	44	50	26	26	66	8.9998	34	29	67
-3602.58	71	96	131	129	79	112	50	103	81	107
-3601.42	45	45	74	48	9	72	22	60	61	71
-3600.26	84	79	82	52	43	73	36	62	59	56
-3599.1	61	73	102	68	32	83	30	93	89	72
-3597.94	62	98	118	62	38	81	47	66	47	73
-3596.78	64	47	89	55	40	45	35	53	40	67
-3595.62	57	54	95	65	38	68	7.0001	50	84	61
-3594.47	71	85	111	76	61	98	57	77	89	90
-3593.31	60	62	86	51	31	74	16	32	46	70
-3592.15	58	67	78	60	40	92	17	78	77	73
-3590.99	58	71	70	54	53	67	20	39	48	58
-3589.83	73	76	106	45	43	91	40	77	53	82
-3588.67	63	73	58	58	19	46	11	69	41	50
-3587.52	63	71	106	75	49	67	22	39	103	91
-3586.36	81	80	113	84	57	103	68	92	83	82
-3585.2	81	90	135	77	33	87	23	48	48	65
-3584.04	77	51	114	76	36	95	43	78	94	104
-3582.89	72	53	110	57	32	78	6.9999	37	66	74
-3581.73	74	86	108	80	73	64	30	65	75	81
-3580.58	65	53	80	72	30	63	26	60	72	69
-3579.42	72	63	89	50	30	59	-18	33	70	73
-3578.26	86	100	117	96	41	87	43	79	97	76



-3577.11	69	70	123	86	31	78	10	60	72	98
-3575.95	61	55	136	49	60	45	-0.9998	72	63	95
-3574.8	78	64	87	80	57	62	21	71	69	65
-3573.64	82	97	140	79	59	68	46	89	92	98
-3572.49	105	77	128	69	45	96	48	73	75	103
-3571.33	68	86	114	95	57	83	17	77	86	76
-3570.18	74	73	138	113	86	90	65	82	107	103
-3569.03	82	78	106	113	42	97	3.9999	39	59	84
-3567.87	74	81	145	91	68	78	33	93	103	94
-3566.72	99	88	117	99	54	97	49	88	93	98
-3565.56	59	87	113	75	45	88	7.9999	77	55	84
-3564.41	59	47	95	41	37	47	-1.9998	59	64	71
-3563.26	80	92	125	96	87	83	33	90	103	98
-3562.11	87	90	115	91	60	77	24	95	86	85
-3560.95	101	109	154	107	76	101	61	87	104	118
-3559.8	114	75	148	123	86	107	59	119	144	106
-3558.65	134	113	130	125	84	108	56	116	107	131
-3557.5	91	88	114	116	85	88	25	94	101	81
-3556.35	80	81	151	70	47	60	2.0001	77	81	116
-3555.2	117	107	121	75	111	111	42	103	95	107
-3554.04	91	100	114	98	79	69	34	74	89	93
-3552.89	137	108	164	139	72	109	40	93	130	134
-3551.74	81	75	280	81	75	87	8	76	90	96
-3550.59	108	111	156	117	105	106	36	87	101	122
-3549.44	146	123	150	131	86	135	46	119	101	123
-3548.29	105	94	137	88	84	79	42	62	111	94
-3547.14	84	114	142	94	86	92	61	110	113	116
-3545.99	102	104	124	117	86	62	14	81	75	90
-3544.84	129	102	171	124	73	76	12	89	111	124
-3543.7	97	100	129	116	96	106	54	101	96	120
-3542.55	132	128	190	139	108	95	50	93	156	128
-3541.4	101	103	151	124	84	111	41	111	105	123
-3540.25	113	133	185	143	100	106	58	115	130	126
-3539.1	129	140	154	123	118	110	57	101	127	119
-3537.95	143	115	145	144	110	102	19	93	112	136
-3536.81	139	147	206	172	116	115	67	109	150	142
-3535.66	147	135	185	167	97	108	91	129	128	161
-3534.51	142	152	182	143	120	102	21	92	128	133

-3533.37	135	90	194	151	86	120	55	108	128	151
-3532.22	147	133	178	149	97	93	19	108	106	153
-3531.07	104	110	160	101	78	46	22	90	101	76
-3529.93	155	141	159	136	111	98	40	120	122	119
-3528.78	135	181	203	171	116	91	57	107	127	155
-3527.63	164	156	182	161	89	101	48	128	152	125
-3526.49	161	102	161	135	113	87	29	101	131	132
-3525.34	118	107	186	116	90	75	41	59	135	125
-3524.2	166	155	177	181	112	113	56	113	148	144
-3523.05	153	147	203	179	147	105	44	104	129	157
-3521.91	133	138	201	138	115	92	19	111	143	139
-3520.77	148	169	188	189	128	83	47	129	146	156
-3519.62	174	142	209	172	123	100	43	136	143	134
-3518.48	176	146	194	160	127	112	46	124	168	171
-3517.33	119	107	183	164	115	99	19	99	148	120
-3516.19	200	185	230	211	122	156	81	155	172	169
-3515.05	166	139	169	137	128	90	45	118	147	138
-3513.9	149	163	224	169	136	103	27	123	183	133
-3512.76	167	197	222	196	157	132	95	138	176	166
-3511.62	185	145	222	166	115	99	40	135	149	142
-3510.48	189	174	249	194	166	127	51	136	197	178
-3509.33	182	170	193	199	145	130	69	135	186	138
-3508.19	162	159	220	199	139	84	51	132	159	165
-3507.05	174	152	200	168	159	94	22	123	170	129
-3505.91	185	168	245	177	165	116	37	124	176	186
-3504.77	204	185	248	222	165	141	89	139	166	180
-3503.63	171	148	207	186	110	67	5.0001	91	114	125
-3502.49	186	153	236	193	145	100	17	114	174	174
-3501.35	183	146	202	178	153	112	54	147	178	166
-3500.2	187	206	270	235	153	109	44	127	195	203
-3499.06	192	163	245	206	136	92	54	159	194	178
-3497.93	181	155	223	188	158	108	33	158	190	176
-3496.79	185	201	260	228	183	96	57	161	214	185
-3495.65	227	199	233	203	160	121	70	146	196	208
-3494.51	185	165	247	189	162	111	25	107	192	200
-3493.37	216	211	255	215	164	124	59	178	206	187
-3492.23	224	230	281	241	193	149	59	152	212	232
-3491.09	233	183	277	218	186	150	54	183	199	205

-3489.95	232	218	256	256	216	126	63	182	244	237
-3488.81	225	218	258	227	182	88	54	163	198	175
-3487.68	251	210	303	252	205	132	73	168	224	219
-3486.54	183	199	279	244	189	110	47	150	225	209
-3485.4	234	229	274	268	163	158	80	197	231	222
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-3453.65	386	330	401	384	336	175	63	260	389	343
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-3450.25	376	311	405	393	313	148	59	238	362	338
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-3191.73	1852	1553	2110	1806	1659	614	422	1251	1816	1566
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-3109.2	2015	1666	2221	2030	1754	612	379	1238	1725	1524
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-2918.92	1922	1639	1773	1492	1775	880	723	1993	3582	2561
-2917.9	1878	1668	1838	1479	1777	881	748	1934	3513	2557
-2916.87	1875	1649	1869	1468	1774	878	721	2005	3544	2529
-2915.84	1915	1690	1846	1498	1791	870	773	2017	3523	2579



-2914.82	1900	1659	1852	1475	1782	853	741	2001	3547	2572
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-2912.76	1869	1633	1841	1467	1746	835	754	1995	3575	2547
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-2896.37	1890	1612	1971	1511	1791	836	775	2212	3627	2543
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-2881.04	1720	1510	1938	1407	1662	803	723	2174	3425	2458
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-2870.85	1647	1423	2124	1398	1588	709	690	2305	3353	2325
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-2670.99	305	285	445	295	296	137	103	315	490	408
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-2668.05	356	307	454	304	286	175	124	328	468	419
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-2665.11	359	301	418	345	299	207	108	331	499	405
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-2661.2	263	250	396	243	256	139	68	276	403	345
-2660.22	299	269	417	295	268	167	105	272	446	340
-2659.24	283	269	404	279	271	134	100	273	404	341
-2658.26	272	264	392	276	247	140	87	255	405	354
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-2655.33	314	271	390	283	290	190	117	289	460	383
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-2652.39	287	254	377	263	246	141	63	274	376	352
-2651.42	301	271	412	309	270	161	120	293	434	362
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-2638.73	266	223	329	238	240	129	61	215	342	276
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-2634.83	225	215	325	221	209	148	92	231	389	264
-2633.86	238	236	339	238	212	131	92	240	323	323
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-2404.37	24	49	49	50	20	27	15	48	31	57
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-2398.79	-1.0002	-30	19	-6.0002	-12	-10	-53	-12	7.9998	-20
-2397.86	28	35	45	44	0.99976	35	5.9998	37	43	46
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-2391.35	11	19	1.9998	19	-9.0002	21	6.9998	22	-16	22
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-2387.64	-16	19	15	-2	-16	11	-6	15	6	8
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-2384.86	-31	0.00012	-11	19	-18	20	9.0001	21	-2.9999	9.0001
-2383.93	7.0002	20	13	18	-11	4.0002	-26	7.0002	23	36
-2383	-1.0002	-17	4.9998	-11	-12	35	-34	-10	-19	-0.0002
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-2379.29	13	-1.0002	9.9998	17	14	30	3.9998	23	9.9998	19
-2378.37	4.0001	19	-2.9999	10	-5.9999	3.0001	-12	-13	21	26
-2377.44	1.0002	-1.9998	-20	-29	-13	2.0002	-42	-8.9998	-37	-6.9998
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-2372.81	-22	-33	-12	6	-29	1	-19	-30	-14	-13
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-2355.26	-23	-40	-23	-2.9999	-19	-3.9999	-27	-18	-16	-3.9999
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