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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 detruded 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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TABLE OF CONTENT

TABLE OF FIGURES

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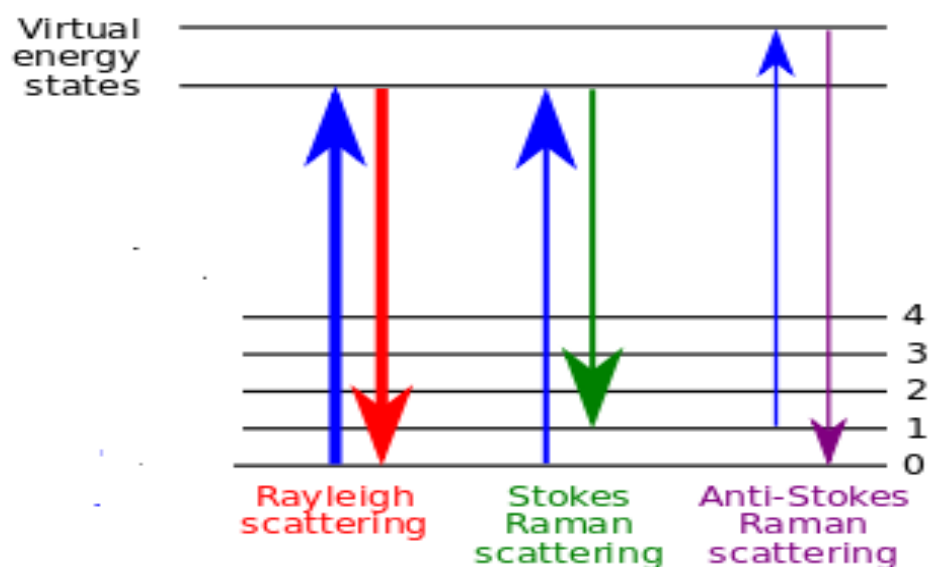
Figure 2.1 Energy- level diagram showing the states involved in Raman scattering process

In Raman effect, the laser beam is considered as an oscillating electromagnetic wave with electrical vector E that is determined by molecular polarizability α. 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 υ_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 υ_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 $\upsilon0$ and returns back to the same vibrational state and emits light with the same frequency υ_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 υ_0. Part of the photon's energy is transferred to the Raman-active mode with frequency υ_m and the frequency of scattered light is reduced to $\upsilon_0 - \upsilon_m$. 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 υ_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 $\upsilon_0 + \upsilon_m$. 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 $\upsilon_0 \pm \upsilon_m$ (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):

$$\frac{Nn}{Nm} = \frac{gn}{gm} \exp\left[\frac{-(En - Em)}{kT}\right] \qquad (2.1)$$

where:

Nn: Number of molecules in excited level n;

Nm: Number of molecules in ground level m;

g: Degeneracy of levels m and n;

$En - Em$: Difference in energy between levels of m and n;

k: Botltzman constant (1.3807×10^{-23} 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 eliminate 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).
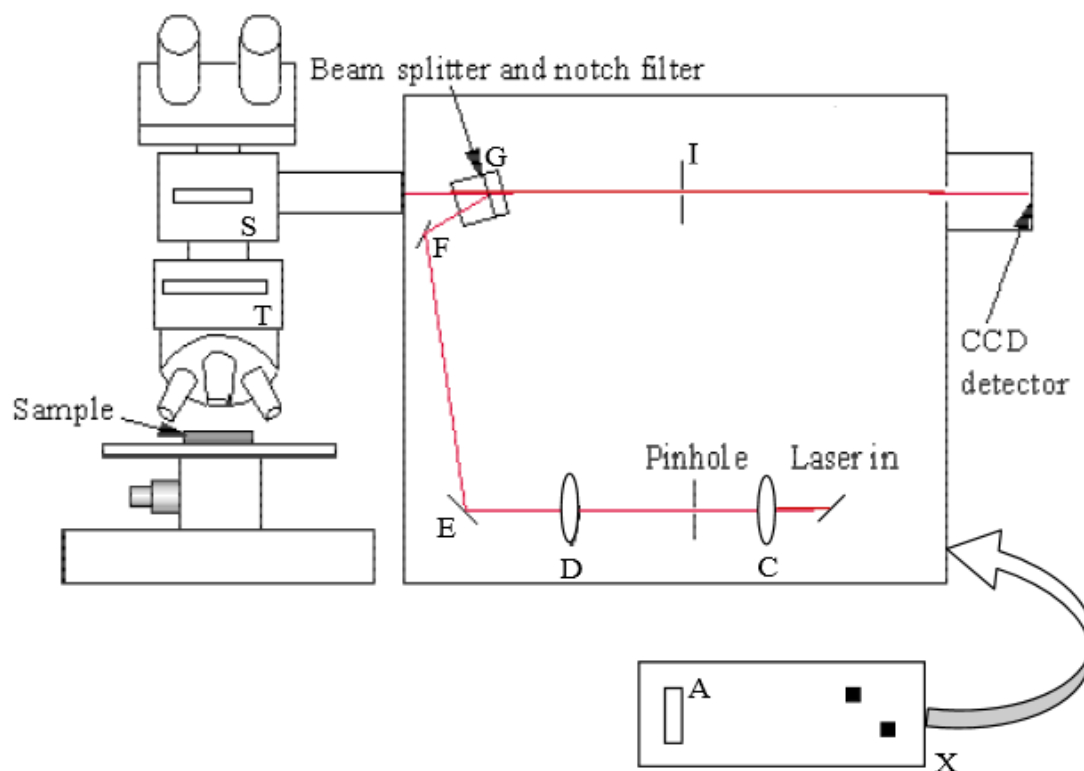
Figure 2.2 Internal Schematic of Renishaw Raman (Likodimos, Kontos, & Falaras, 2013)

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 absorbed 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).
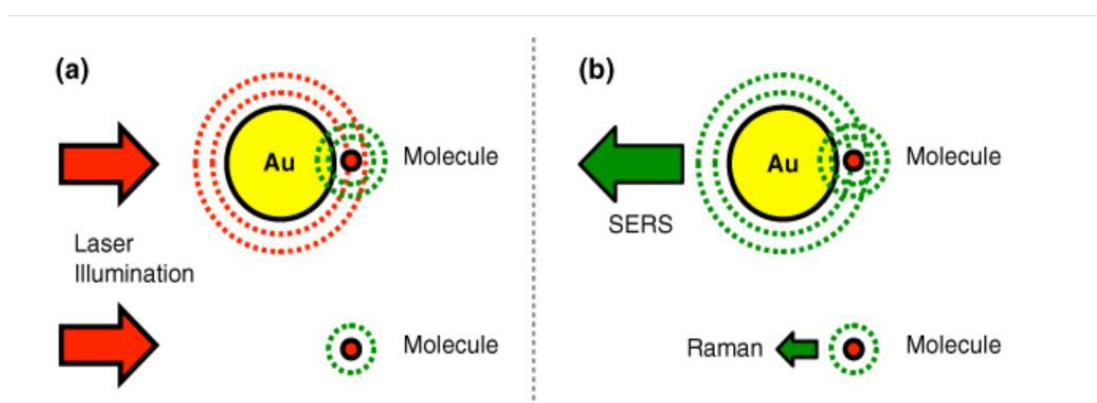
Figure 2.3 Basic SERS electromagnetic enhancement. A gold nanoparticle will enhance both (a) the incident field and (b) the scattered field, significantly enhancing the Raman signal from a proximate molecule. Since the intensity is enhanced twice, i.e. the nanoparticle acts as both a receiver and a transmitter, 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:

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

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)

θ: The angle relative to the direction of electric field (degree)

g: Related to the dielectric constant: $g = \left(\frac{\varepsilon_1(\vartheta_L) - \varepsilon_0}{\varepsilon_1(\vartheta_L) + 2\varepsilon_0}\right)$

where ε_0 & ε_1 dielectric constants of the medium are surrounded metal sphere (F/m), and ϑ_L is the frequency of incident light beam (Hz). Once the value of g is maximum, the value of ε_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., $AgNO_3$) 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 μm). These nanopipettes are largely suitable for probing a cell nucleus and cytoplasm. Carbon nanotube-based nanoprobes 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 [8]. 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., cethyltrimethylamonium 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 introduce 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 ω_p and a Stokes beam at frequency of ω_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.
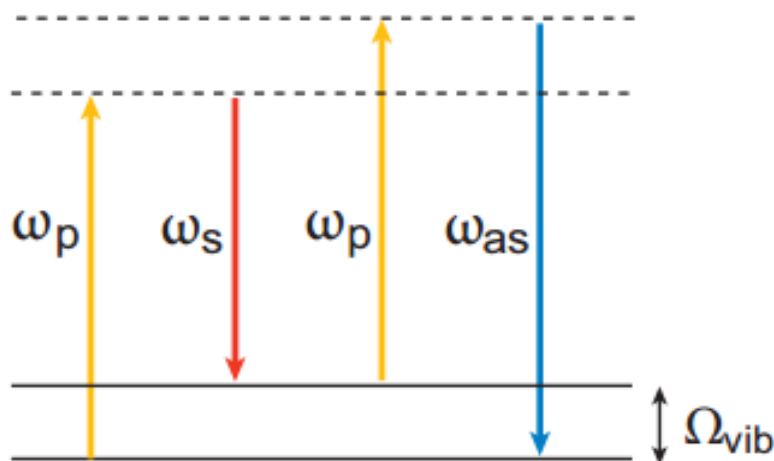
Figure 2.4 Energy diagram of CARS. When the difference between the pump and Stokes frequencies ($\omega_p - \omega_s$) matches the molecular vibrational frequency, Ω_{vib}, the anti-Stokes signal is generated at a frequency $\omega_{as} = 2\omega_p - \omega_s$ (Evans & Xie, 2008)

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 CH2 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 μm 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).
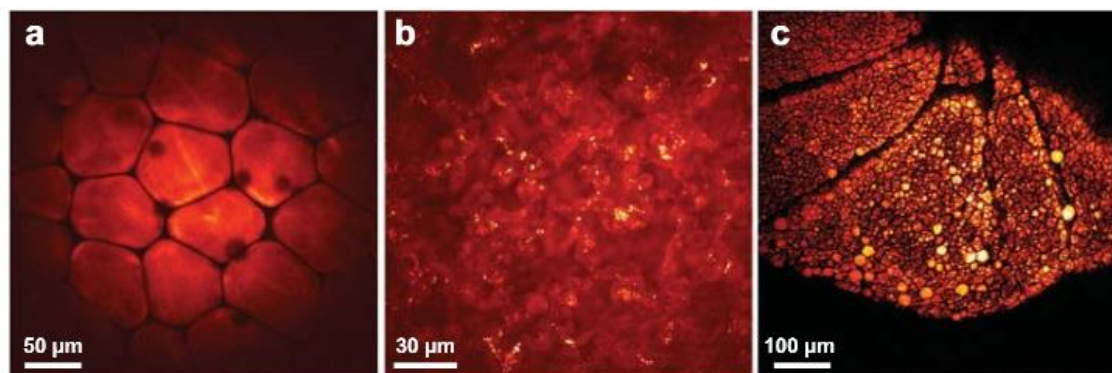
Figure 2.5 CARS imaging of several tissues with CH2 contrast. (a) CARS image of white adipose tissue of mouse omentummajus. The adipose cells are packed with fatty acids and give strong CARS signals. (b) CARS microscopy of mouse lung tissue, showing the individual (Evans & Xie, 2008)

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)1Hev transgene resemble hormonally-stimulated glands from pregnant mice (Li, Hively, & Varmus, 2000).

B6SJL-Tg (Wnt-1)1Hev/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_2 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-μm thickness. After that, the tissue ribbons were transferred to a warm water bath (~ 42 °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 ($AgNO_3$−99.5%) and sodium citrate ($Na_3C_6H_5O_7$−99%) were purchased from Science Fisher (Pittsburgh, PA, US). Deionized (DI) water with a resistivity ~18 MΩ.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 ± 1 ^{o}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 ($HAuCl_4 \cdot 4H_2O$) and sodium citrate ($C_6H_5O_7Na_3$) 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).
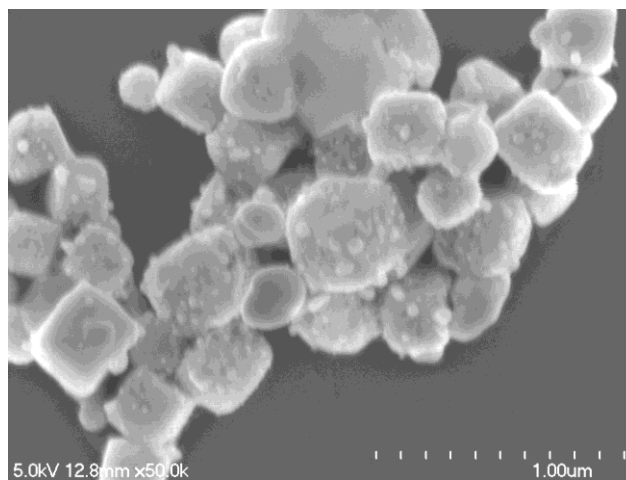
Figure 3.1 SEM image of silver nanoparticles

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

			Diam. (nm)	% Intensity	Width (nm)
Temperature (°C): 25.0				Duration Used (s): 60	
Count Rate (kcps): 344.1				Measurement Position (mm): 4.65	
Cell Description: Disposable sizing cuvette				Attenuator: 8	

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 124.6	Peak 1:	137.1	100.0	41.42
Pdl: 0.076	Peak 2:	0.000	0.0	0.000
Intercept: 0.935	Peak 3:	0.000	0.0	0.000
Result quality : Good				

Size Distribution by Intensity
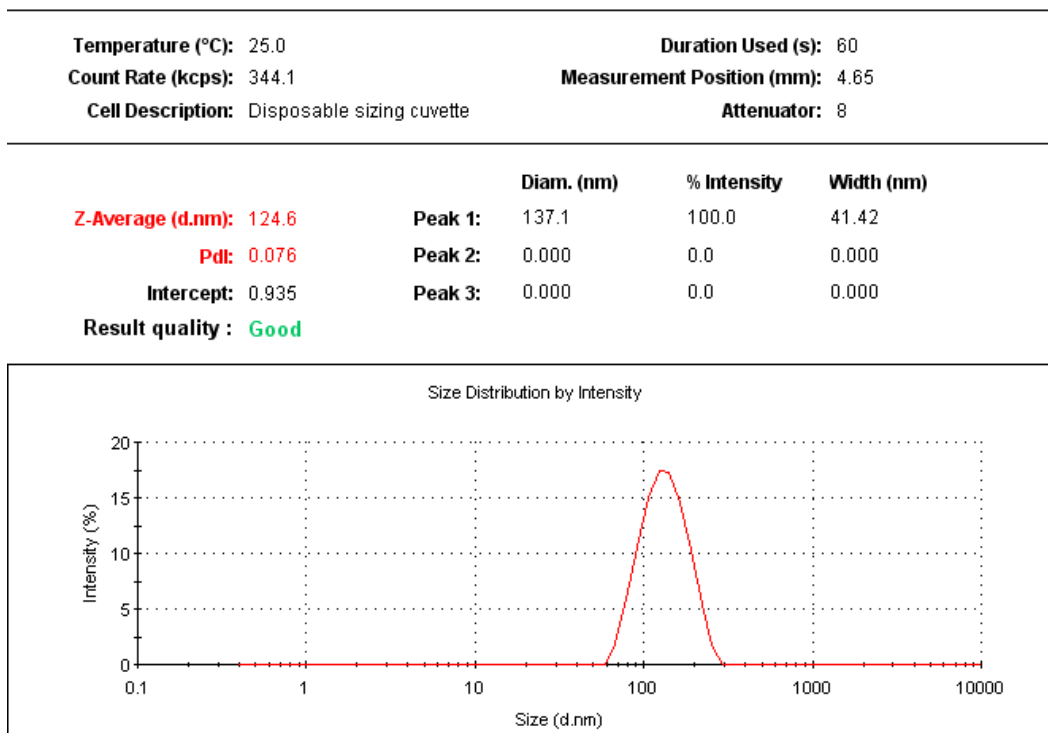
Figure 3.3 Size distribution of gold nanoparticles

3.2.4 Raman Spectrometer Set up

The RS, SERS, and CARS measurements were performed using the Renishaw InVia Reflex Raman microscopy system (Reinshow 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 (Reinshow 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 (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 Reinshow Micro-Raman Specrometer, customer-made Macro-Raman Spectrometer, and a CARS.

Figure 3.4 Reinshow Micro- Raman spectrometer
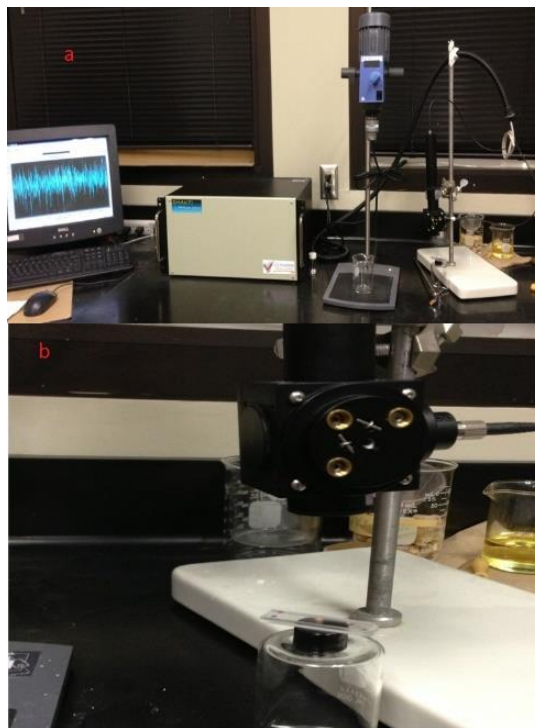
Figure 3.5 (a) Customer- made Macro-Raman spectrometer system. (b) Fresh Mammary gland tissue under the Macro-Raman microscope for testing
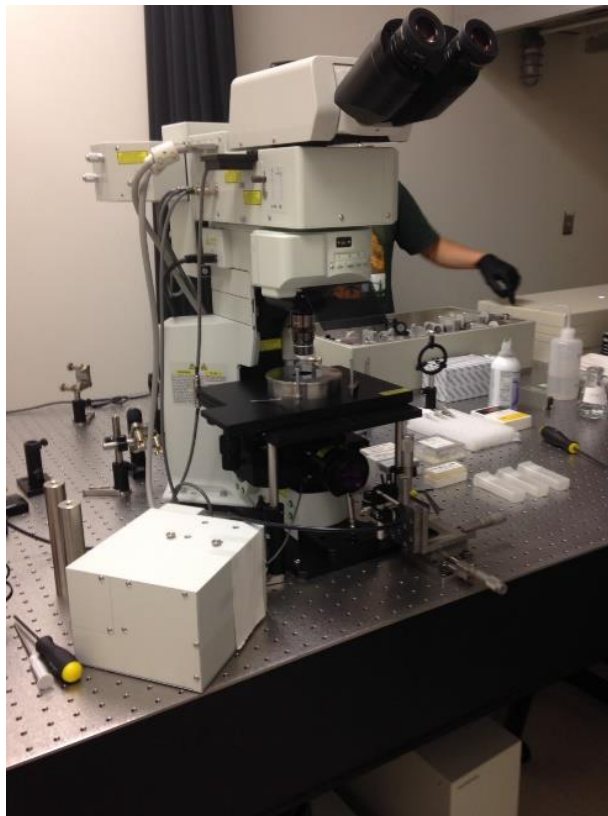
Figure 3.6 CRAS Instrument (most of the optic lens are not shown) used in this study

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 (2800–3000 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 citu (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.
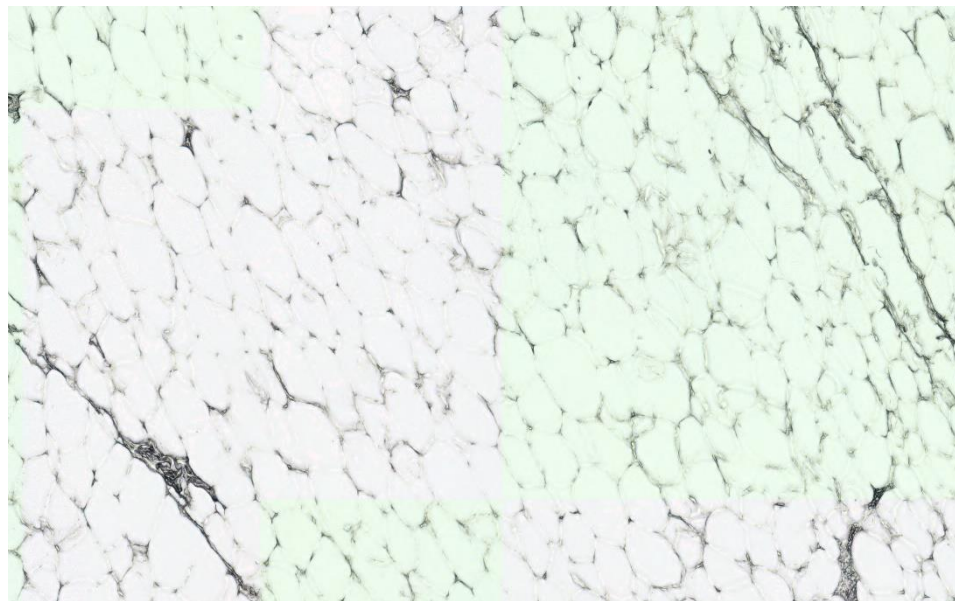
Figure 3.7 microscopic image of a benign breast tissue fixed on glass slide
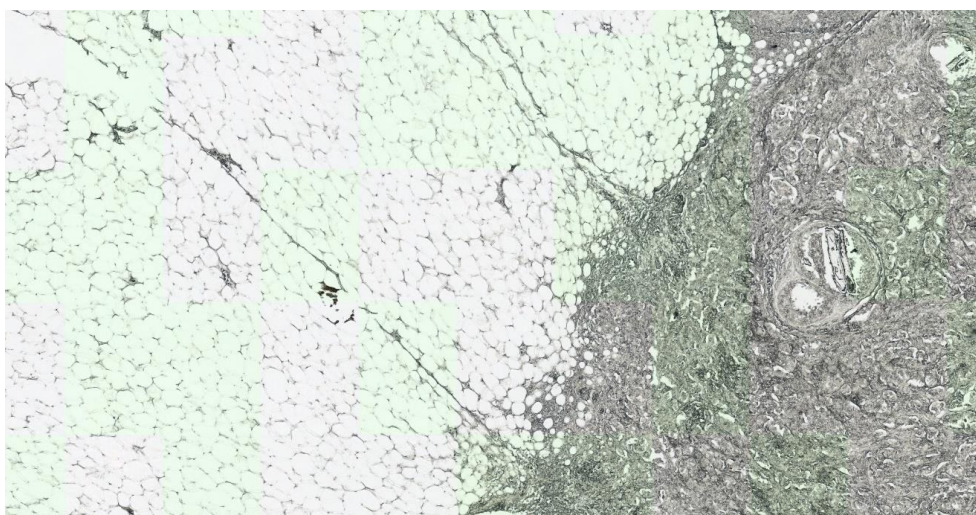
Figure 3.8 A microscopic image of a benign and stage-1 breast tumor tissue fixed on glass
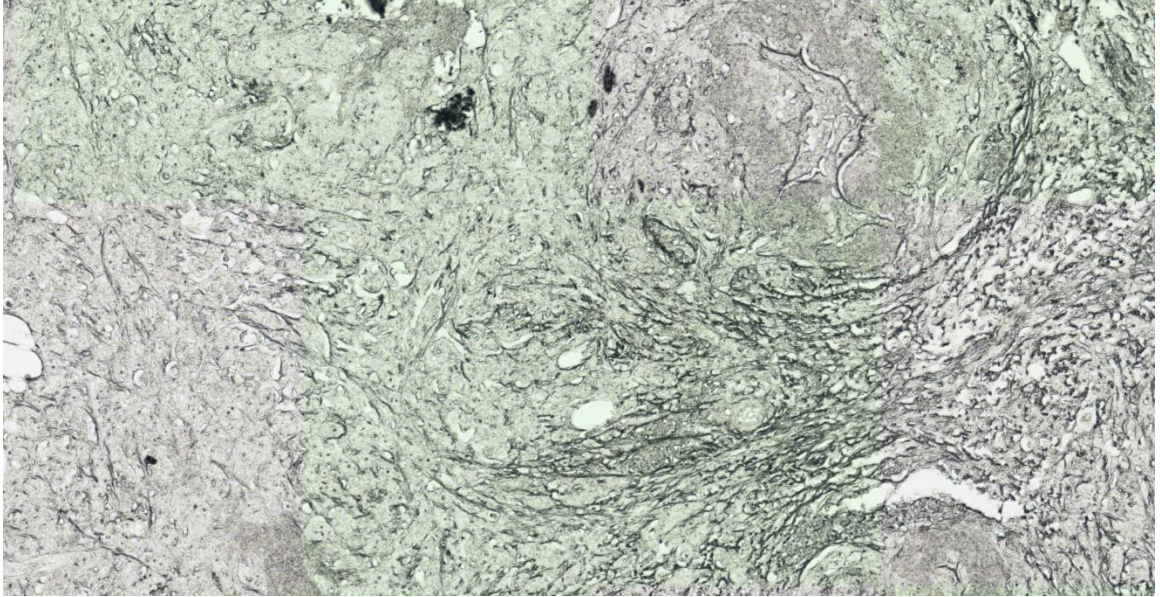
slide

Figure 3.9 A microscopic image of a stage-2 breast tumor tissue fixed on glass slide
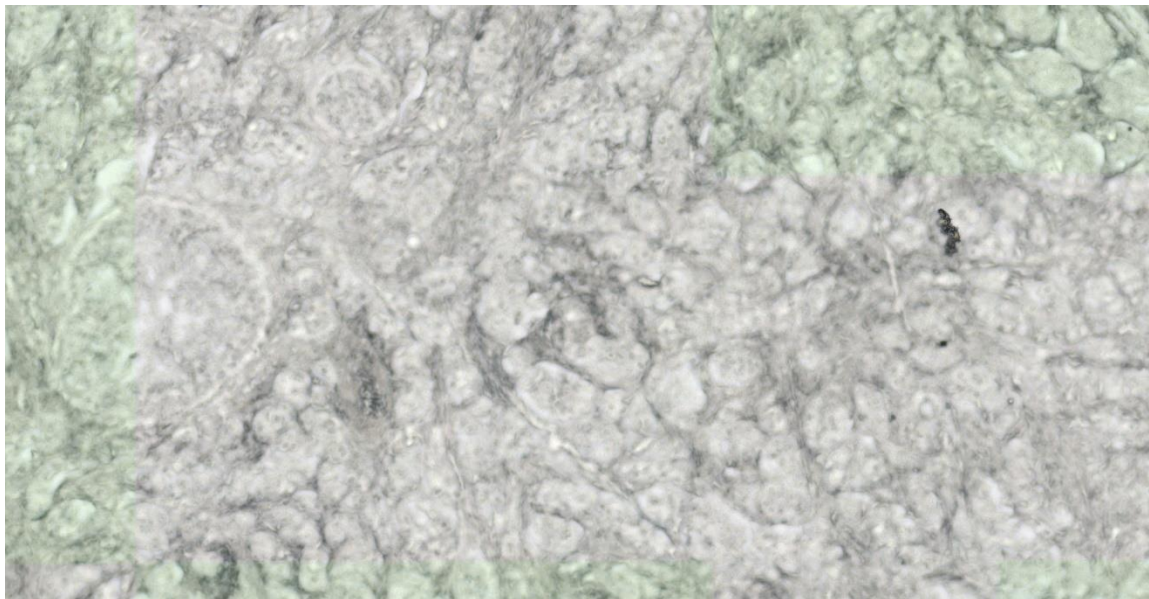
Figure 3.10 A microscopic image of a stage-3 breast tumor tissue fixed on glass slide

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 $400-1800$ 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 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.
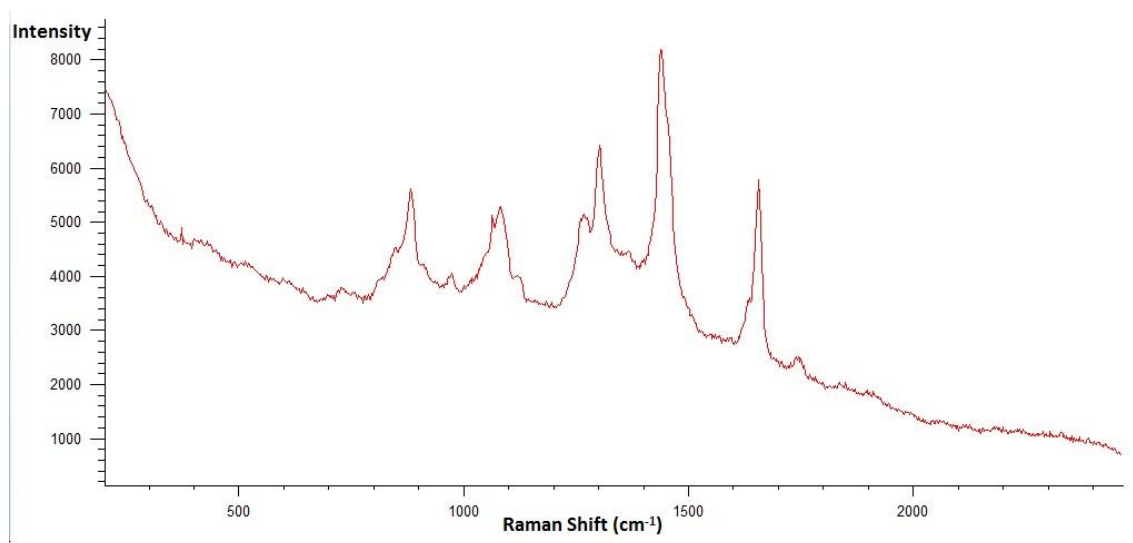
Figure 3.11 Raman spectrum of fixed normal mammary gland tissue using Macro- Raman (SERS-T1)
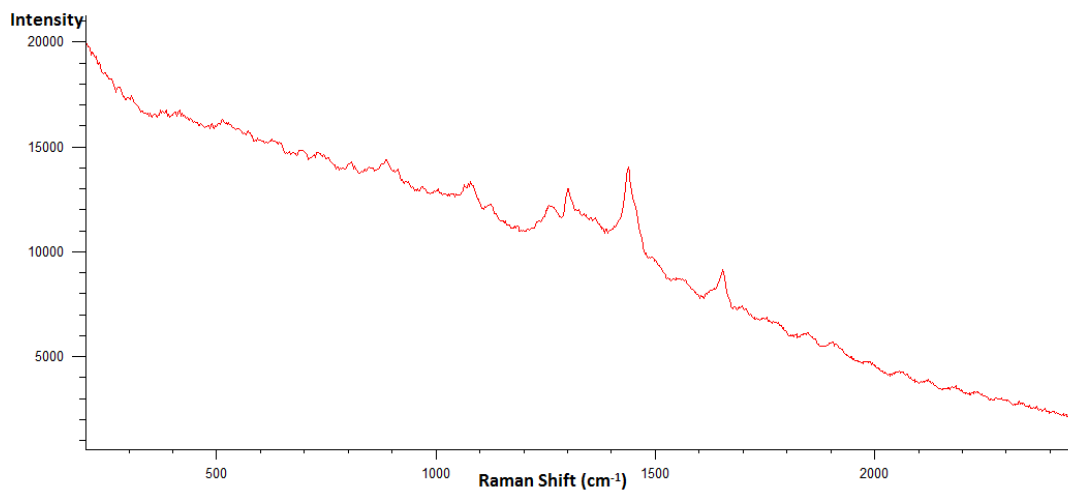
Figure 3.12 Raman spectrum of fresh normal mammary gland tissues using Macro- Raman (SERS-T2)
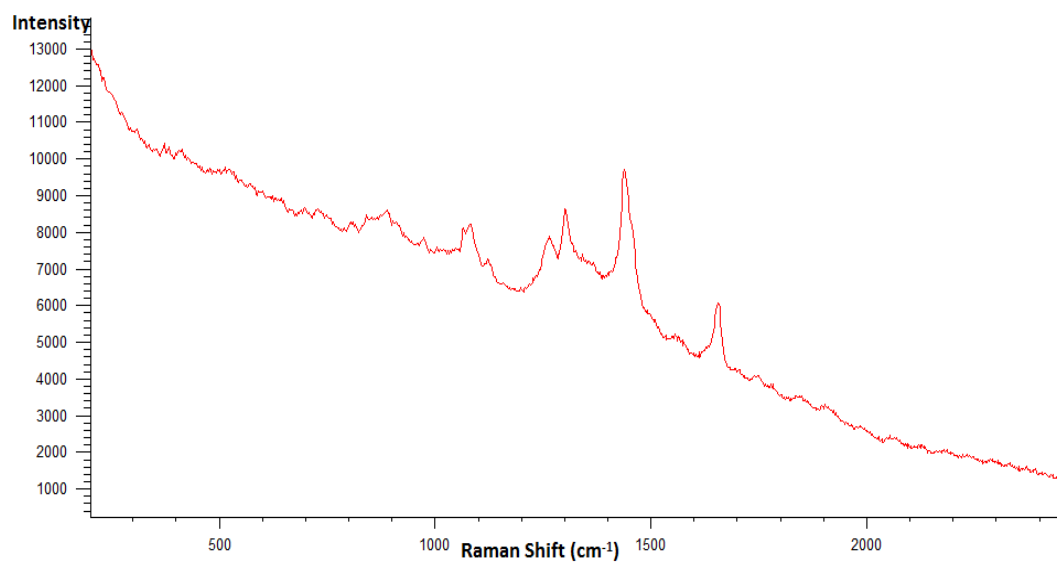
Figure 3.13 Raman spectrum of frozen normal mammary gland tissues using Macro-Raman (SERS-T3)

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.
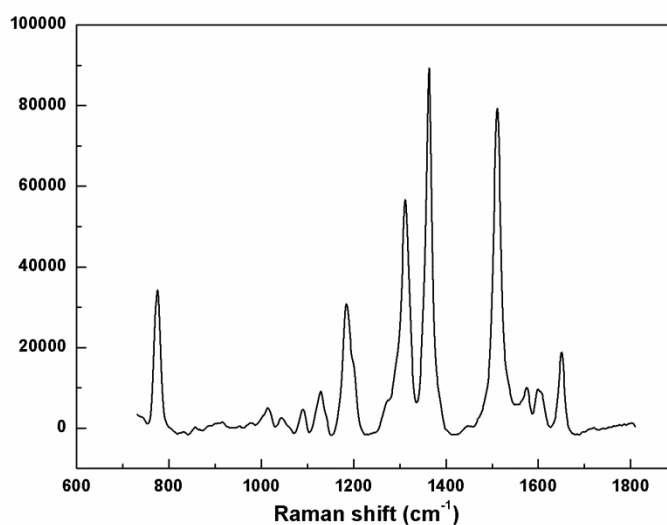
Figure 3.14 Raman spectrum from fresh normal mouse mammary gland tissues using gold nanoparticles (SERS-T4)
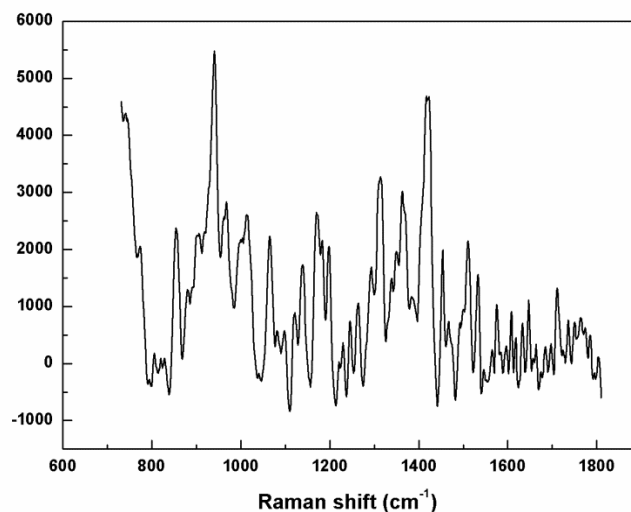
Figure 3.15 Raman spectrum from fresh normal mouse mammary gland tissues using silver nanoparticles (SERS-T5)

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.
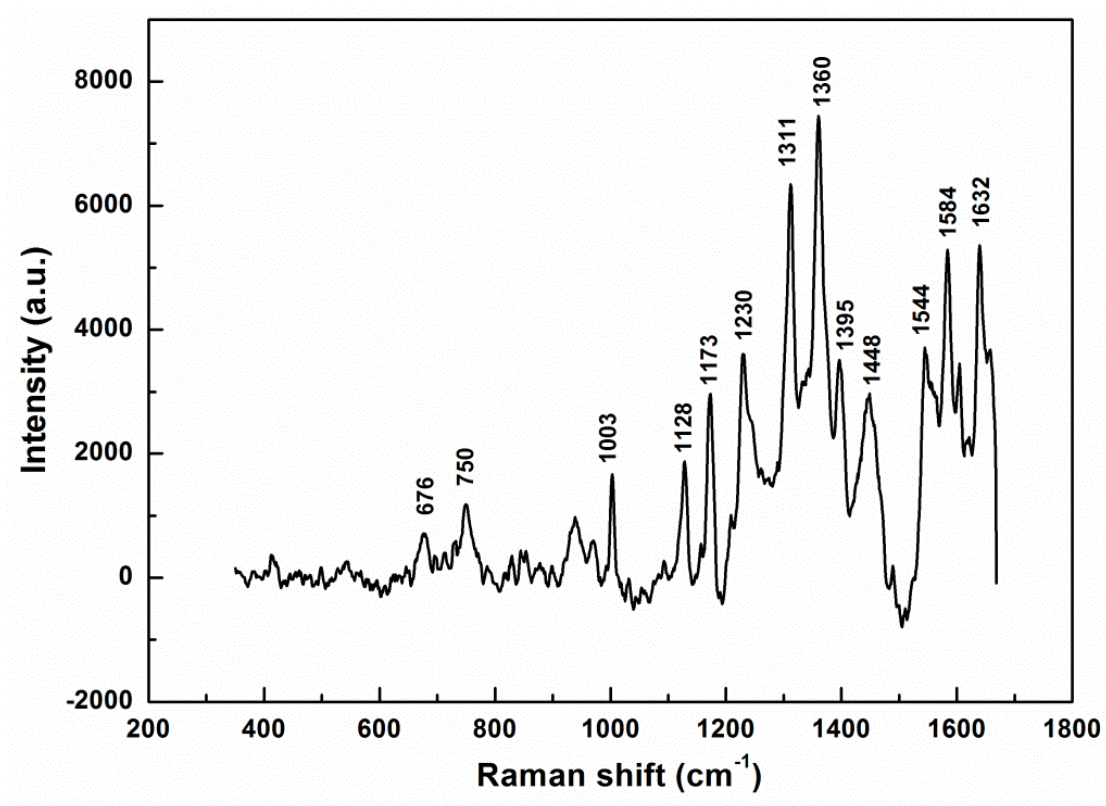
Figure 3.16 Raman spectrum from mouse lung using silver nanoparticles (SERS-T6)
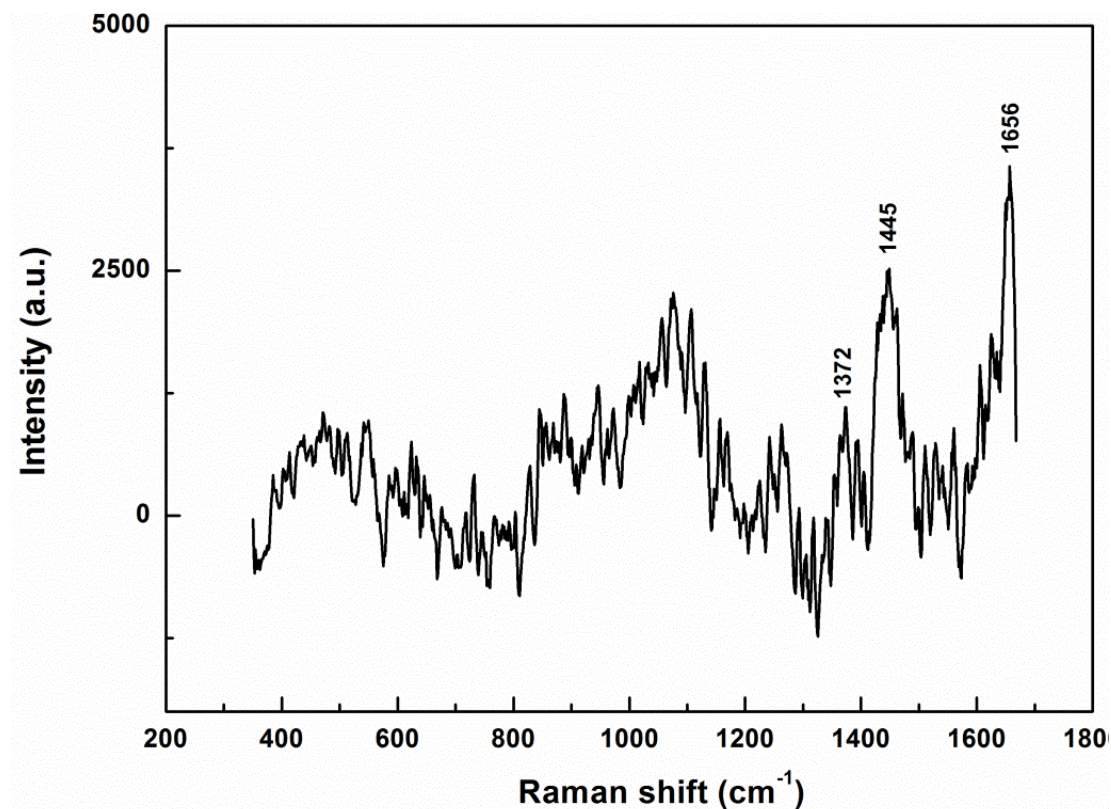
Figure 3.17 Raman spectrum from mouse mammary gland using silver nanoparticles (SERS-T7)

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.
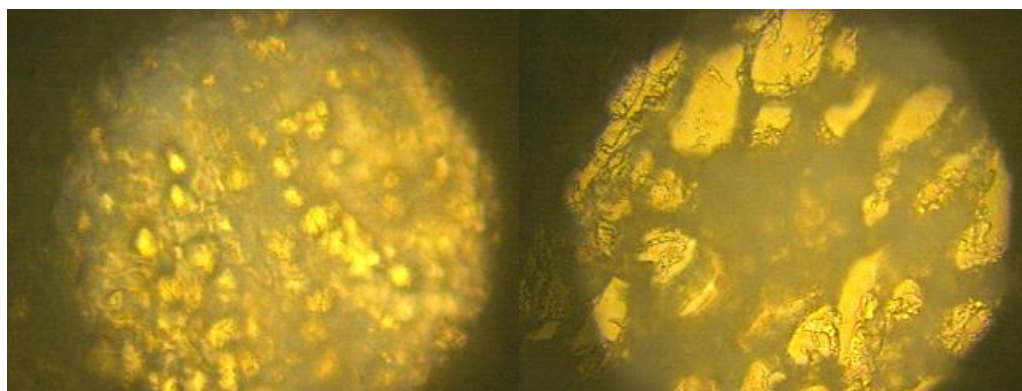
Figure 3.18 cancer (left) and normal (right) mammary gland tissue under Raman microscope
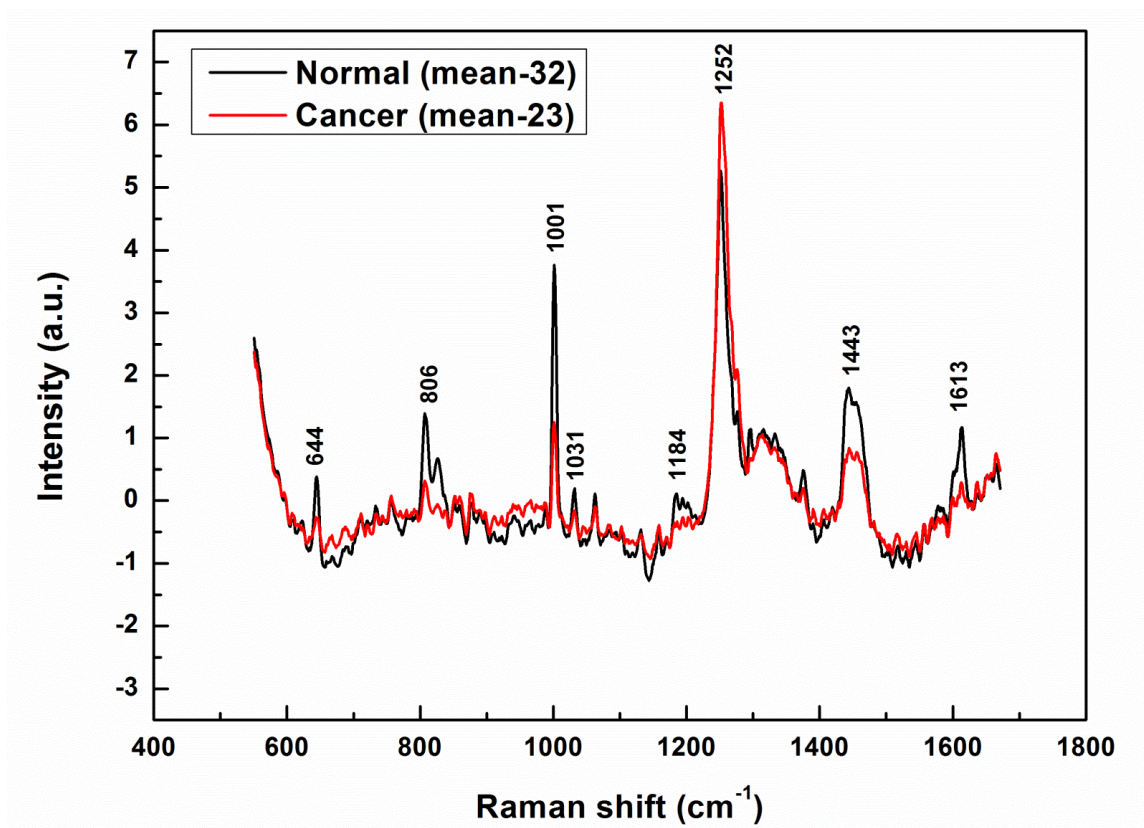
Figure 3.19 Averaged Raman spectrum from normal (black) and cancer (red) of mammary gland tissues (SERS-T8)
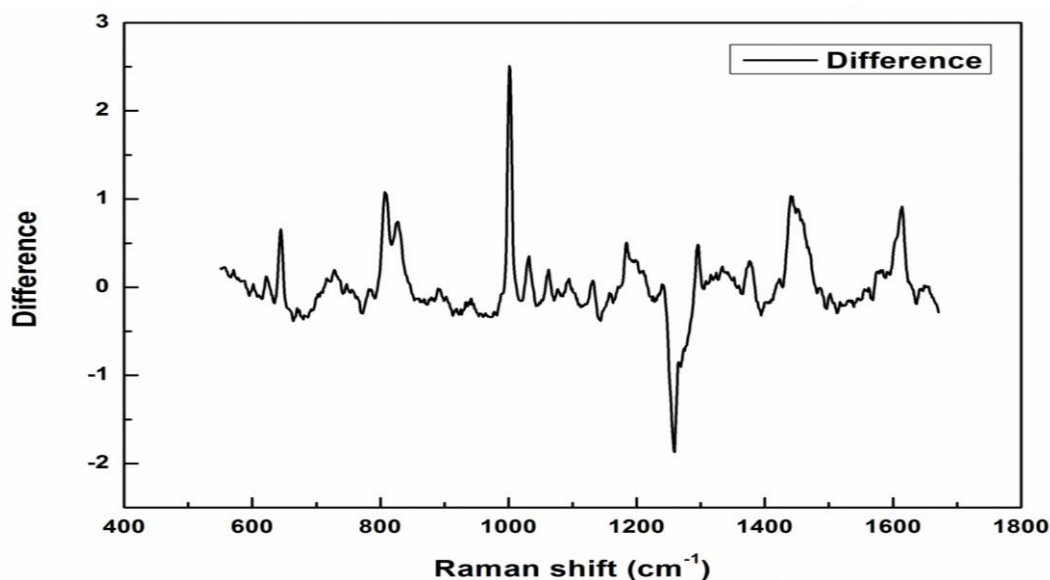
Figure 3.20 Difference graph between Raman spectra of normal and cancer tissues

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 cm^{-1}, 806 cm^{-1}, 1001 cm^{-1}, 1184 cm^{-1}, 1252 cm^{-1}, 1443 cm^{-1}, and 1613 cm^{-1}. The clear difference are in the peaks of 644 cm^{-1}, 806 cm^{-1}, 1001 cm^{-1}, 1252 cm^{-1}, and 1443 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 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 722−725 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 ± 0.37 for normal and 3.11 ± 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 ~ 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.
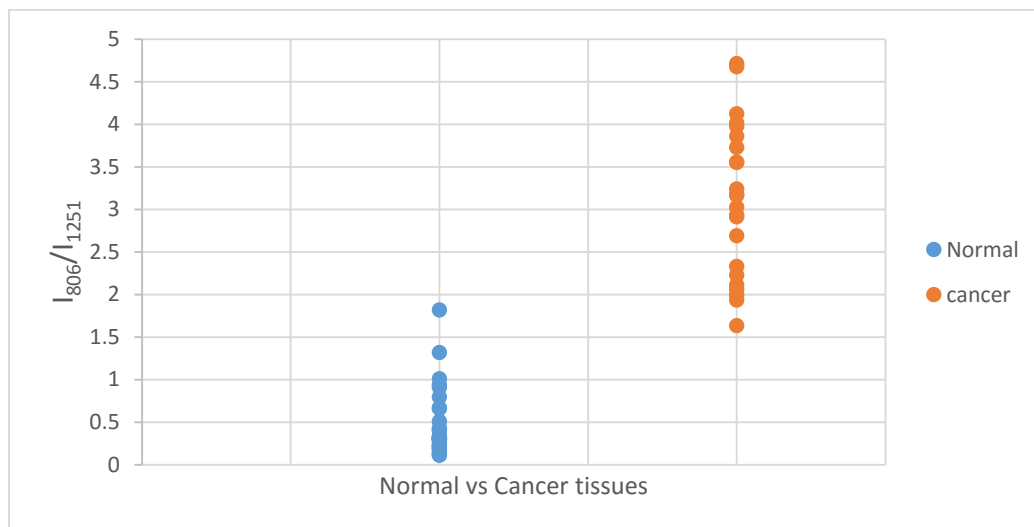
Figure 3.21 Difference between Raman spectrum of normal and cancer mammary gland tissues fixed on gold slides using two band ratio method (SERS-T8)
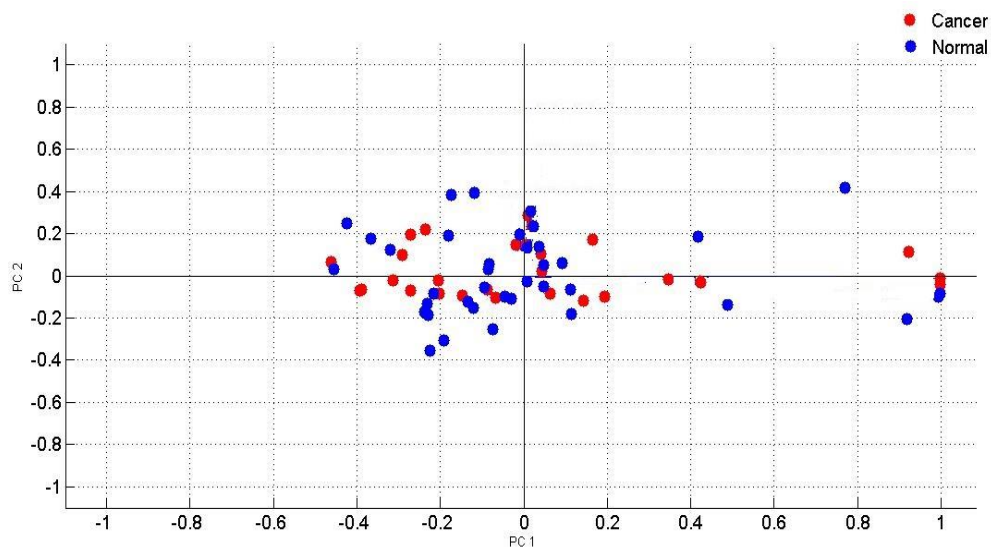
Figure 3.22 PCA results from Raman spectrum of normal and cancer tissues (SERS-T8)

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 50×50 μm^2 tissue area. Results were taken at the wavelength of 1000 cm^{-1} (phenylalanine) and 1600 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).
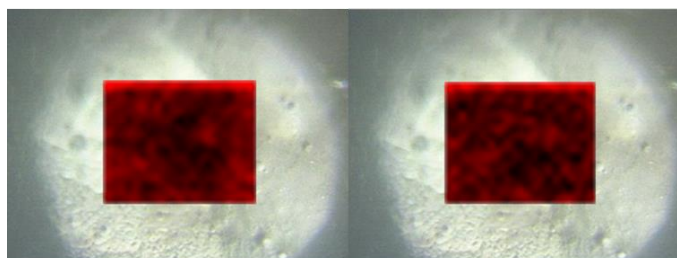
Figure 3.23 Raman imaging from mammary gland tissue at 1600 cm^{-1} (right) and 1000 cm^{-1} (left) (RI-T1)
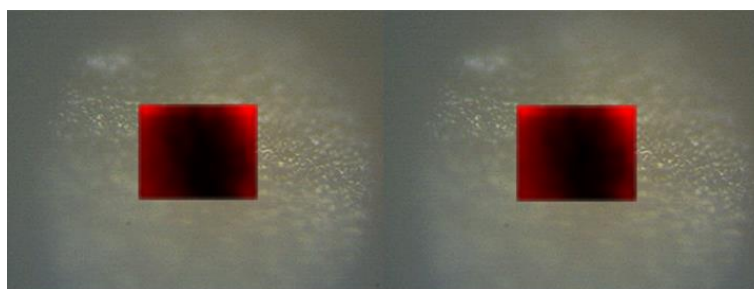
Figure 3.24 Raman imaging from liver tissue at 1600 cm^{-1} (right) and 1000 cm^{-1} (left) (RI-T2)

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.
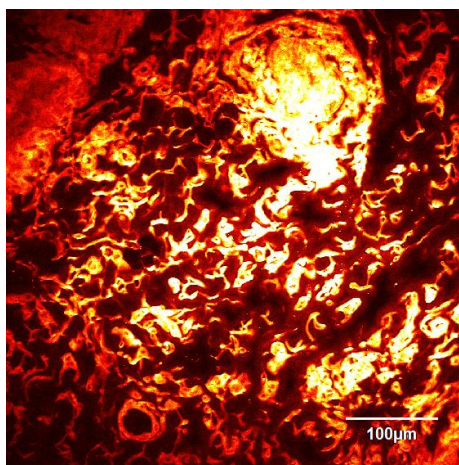
Figure 3.25 CARS imaging of fixed normal mammary gland tissue using lipid filter (CARS-IMG-T1)
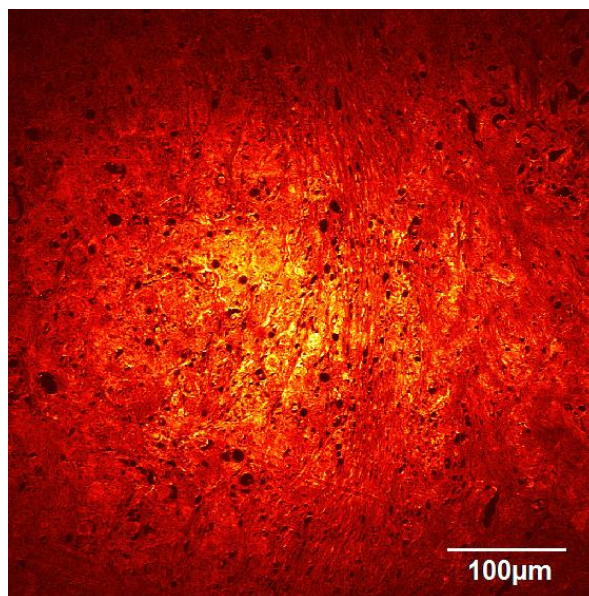
Figure 3.26 CARS imaging of fixed tumor mammary gland tissue using lipid filter (CARS-IMG-T2)

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

Figures 3.27 and 3.28 show the averaged CARS spectra in lipid band (2500 ~3100 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 ~3100 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.
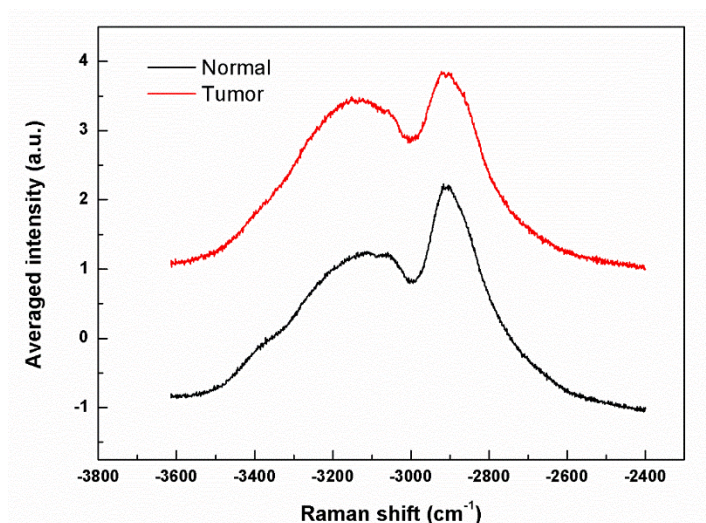
Figure 3.27 Normalized and averaged spectrum for tumor and normal mammary gland tissues fixed on glass slide (CARS-SPT-T1)
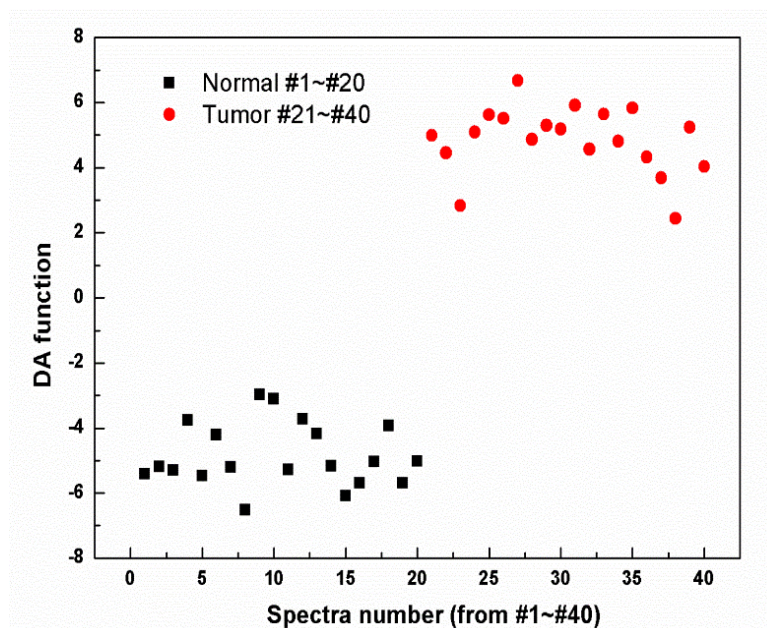
Figure 3.28 PC-DFA analysis of fixed normal and tumor mammary gland tissue using CARS

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.
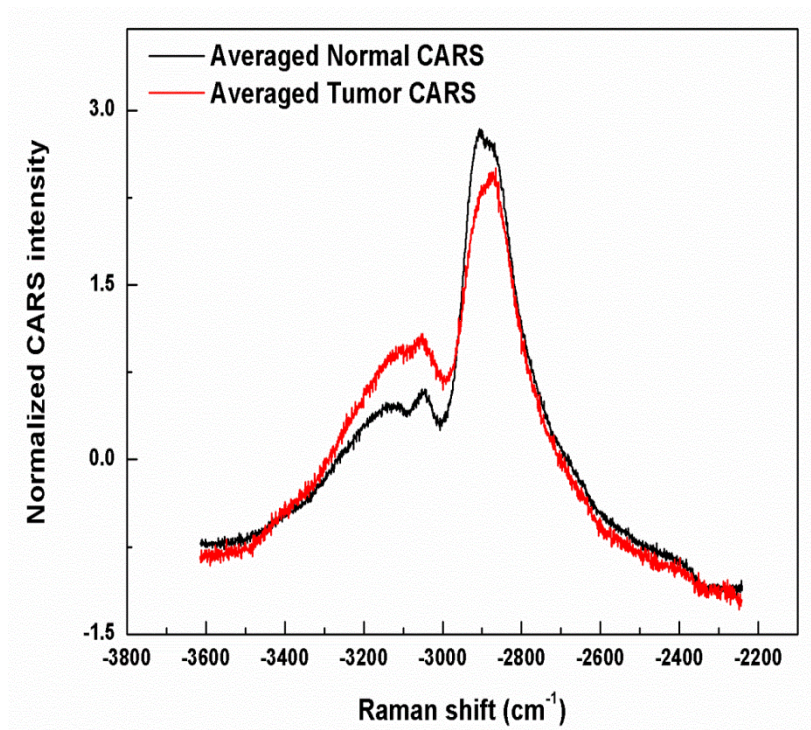
Figure 3.29 Averaged CARS spectrum of normal and tumor tissues (CARS-SPT-T2)
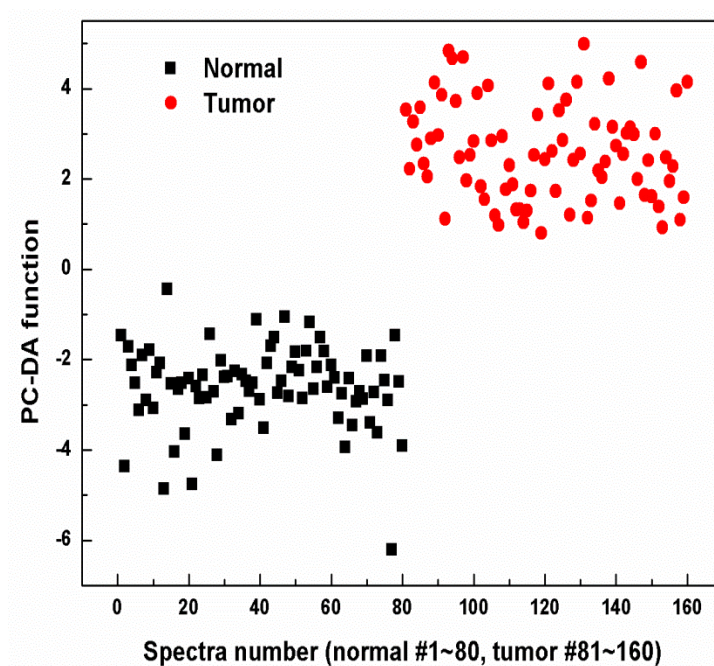
Figure 3.30 PC-DFA analysis of fixed normal and tumor mammary gland tissue from four different mice, using CARS

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 used 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.
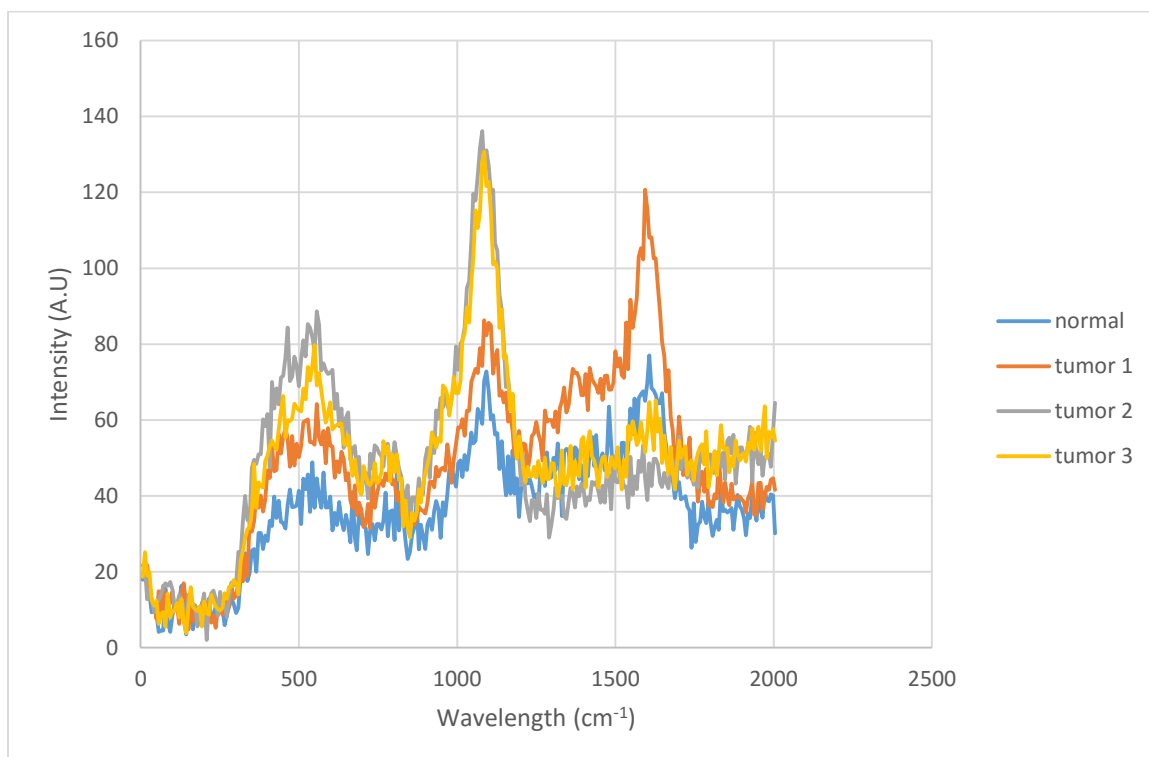
Figure 3.31 The averaged Raman spectrum of ten different Raman spectra from human tissues in each of the different stages using UV-Raman (RS-UV-T1)
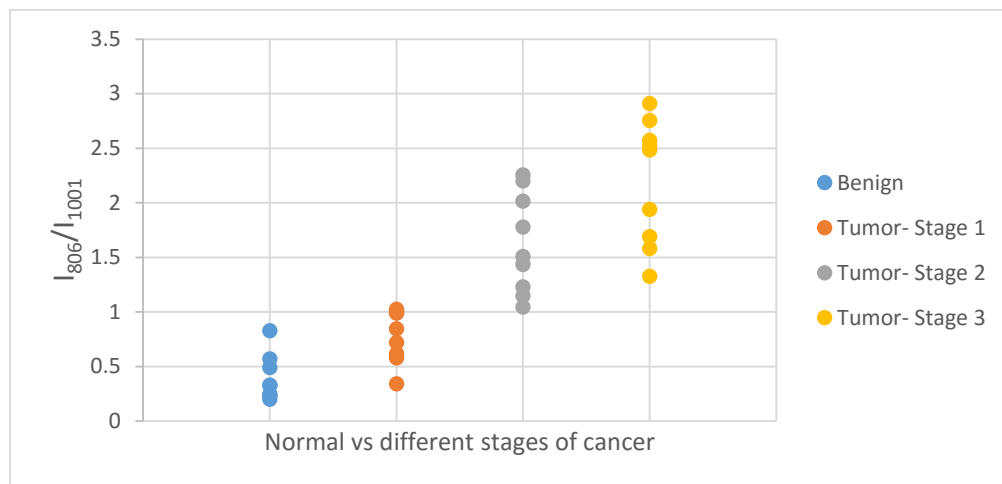
Figure 3.32 Results of using the two-band ratio (I_{806}/I_{1001}) method to differentiate human tissues at different stages. Data of the two ratios is from Figure 3-31
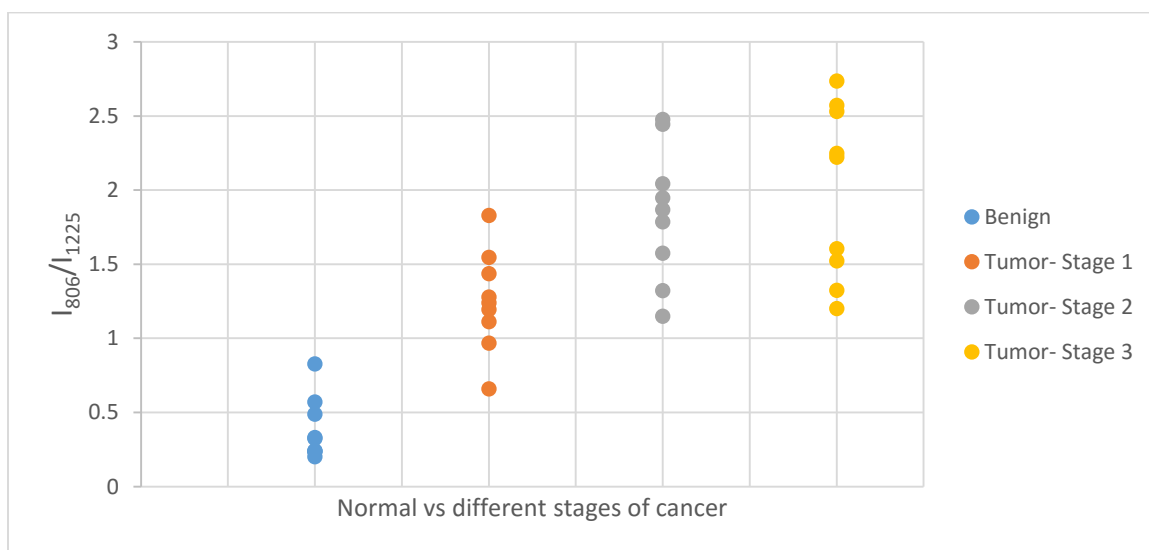
Figure 3.33 Results of using the two-band ratio (I_{806}/I_{1225}) method to differentiate human tissues at different stages.
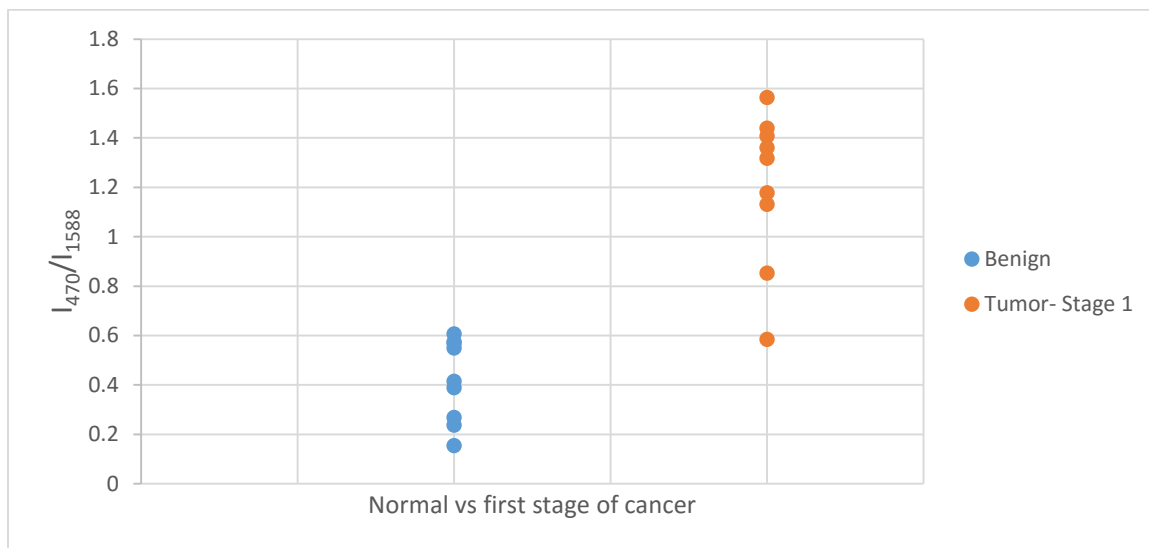
Figure 3.34 Results of using the two-band ratio (I_{470}/I_{1588}) method to differentiate benign and tumor in stage 1

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 $2200-3400$ 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.
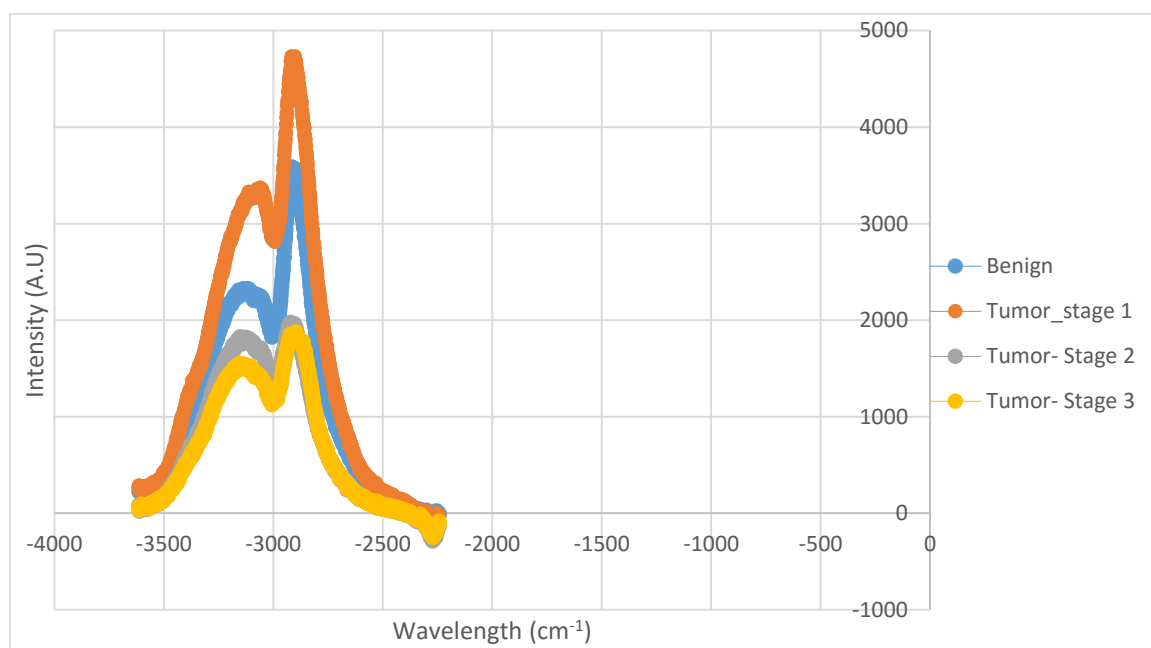
Figure 3.35 Averaged CARS spectrum from benign and tumor tissues in different stages (CARS-SPT-T3)
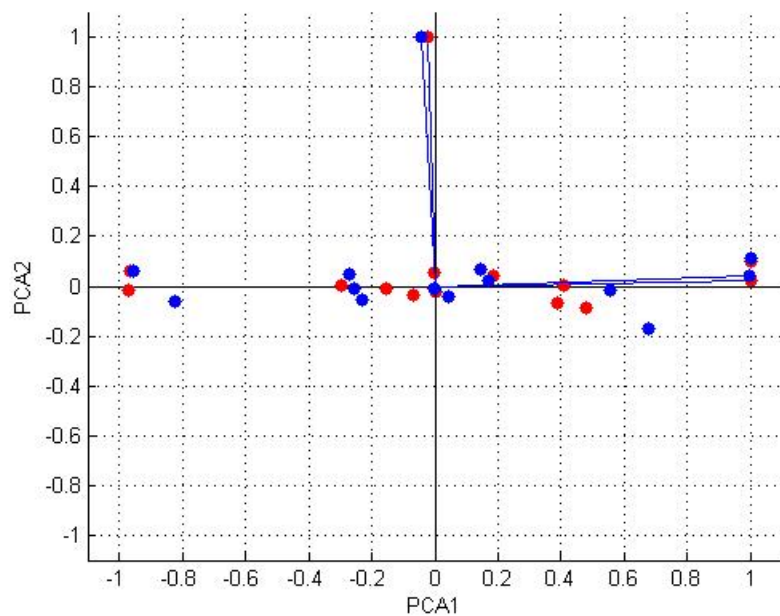
Figure 3.36 PCA results from benign and stage 1 of cancer. Blue dots represent benign and red dots show the tumor tissues
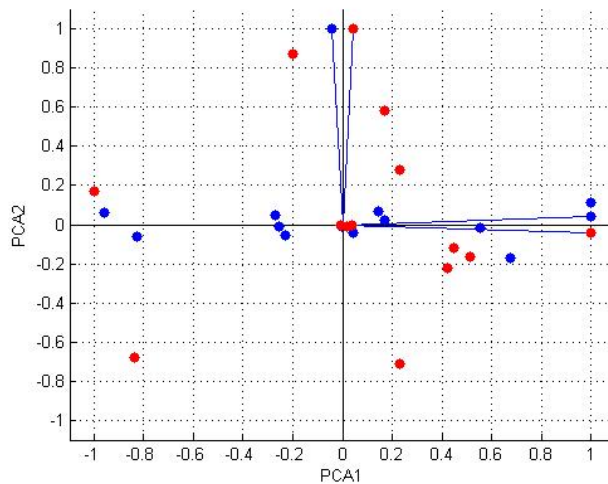
Figure 3.37 PCA results from stage 1 and stage 2 of cancer. Blue dots represent stage 1 and red dots show the stage 2
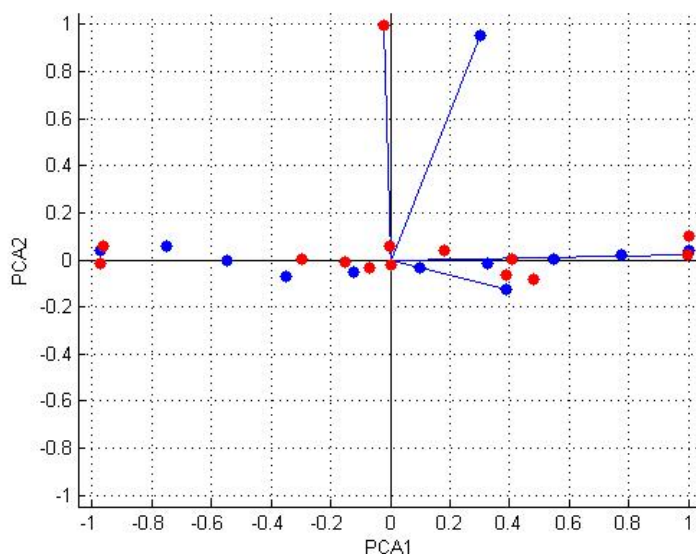
Figure 3.38 PCA results from stage 2 and stage 3 of cancer. Blue dots represent stage 1 and red dots show the stage 2

3.3.10 Summery

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 600−1700cm−1 over the madin 8 wavelengths of 644, 806, 1001, 1031, 1184, 1443 and 1613 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**

PC	Value (%)
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

PC	Value (%)
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.
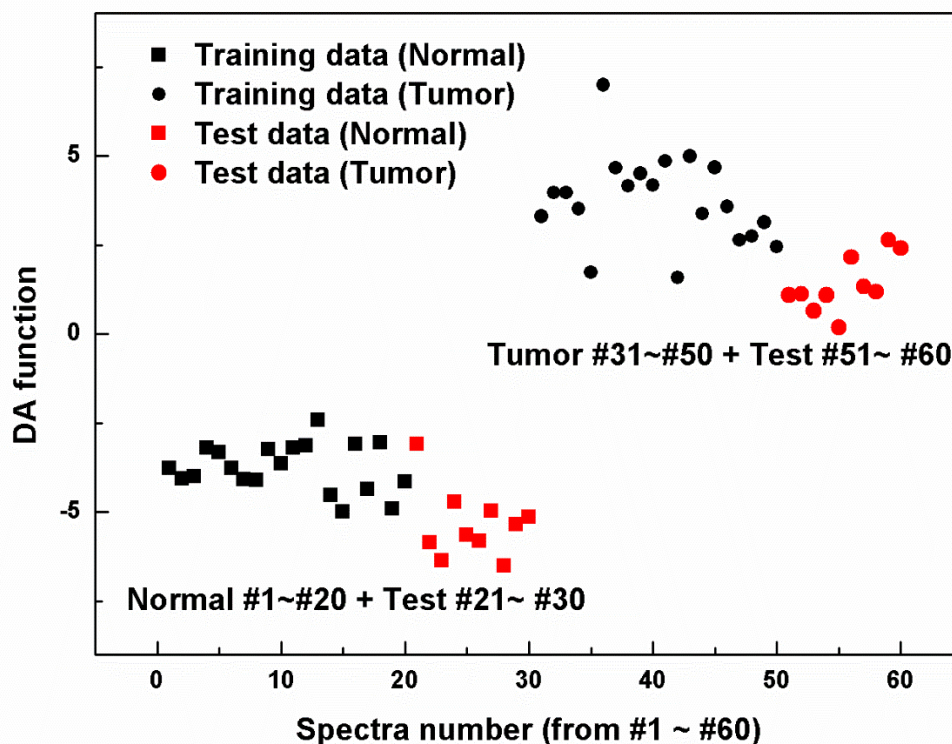
Figure A-1 PC-DFA plot for training and test data

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
-3470.64	599	200	492	304	585	678	436	436	520	695
-3469.5	607	222	485	308	549	680	477	477	530	685
-3468.37	599	227	486	266	574	658	460	460	558	699
-3467.23	664	213	492	300	568	683	475	475	531	717
-3466.1	623	213	472	297	578	726	469	469	540	699
-3464.97	609	211	498	301	562	724	408	408	573	721
-3463.83	640	235	470	315	579	707	459	459	547	718
-3462.7	627	213	491	289	556	704	458	458	563	745
-3461.57	634	246	511	309	588	767	466	466	592	776
-3460.44	624	235	511	328	610	749	520	520	597	792
-3459.3	627	231	508	340	637	747	531	531	541	778

-3458.17	621	257	520	316	610	769	493	493	622	802
-3457.04	654	214	514	325	623	793	495	495	618	779
-3455.91	654	229	515	314	650	812	564	564	601	832
-3454.78	686	269	582	340	673	814	532	532	633	834
-3453.65	664	238	544	315	655	824	546	546	650	871
-3452.51	651	201	518	329	638	812	519	519	611	868
-3451.38	677	263	560	354	695	893	562	562	650	890
-3450.25	631	247	559	323	646	819	538	538	632	903
-3449.12	686	267	586	339	674	901	590	590	725	911
-3447.99	651	237	590	320	670	867	574	574	649	884
-3446.86	646	257	586	323	674	887	567	567	671	919
-3445.73	666	272	600	363	718	934	585	585	726	967
-3444.6	635	276	575	356	711	902	597	597	692	905
-3443.48	722	283	614	389	757	935	636	636	762	975
-3442.35	636	257	623	372	751	920	621	621	737	980
-3441.22	634	292	569	385	741	990	651	651	745	996
-3440.09	662	300	609	352	767	985	624	624	729	1015
-3438.96	633	260	621	375	758	965	619	619	751	1019
-3437.83	692	288	619	358	737	990	640	640	767	1029
-3436.71	690	286	632	370	775	980	663	663	813	1035
-3435.58	673	292	677	396	797	1039	679	679	802	1096
-3434.45	638	282	634	375	777	1049	673	673	791	1071
-3433.32	628	282	623	393	779	1043	652	652	786	1075
-3432.2	686	348	676	422	818	1041	737	737	847	1151
-3431.07	670	307	709	408	815	1103	733	733	831	1117
-3429.94	693	271	666	384	814	1071	740	740	862	1098
-3428.82	696	326	727	394	844	1114	733	733	885	1182
-3427.69	694	316	685	467	856	1133	752	752	889	1175
-3426.57	662	304	688	410	824	1103	748	748	864	1184
-3425.44	678	304	686	402	821	1130	782	782	843	1192
-3424.31	688	335	712	425	873	1128	769	769	896	1161
-3423.19	709	353	731	439	912	1190	812	812	903	1283
-3422.06	692	337	713	425	871	1209	807	807	931	1301
-3420.94	689	345	724	405	878	1140	769	769	920	1242
-3419.82	702	368	725	449	900	1164	840	840	937	1307
-3418.69	689	321	738	438	920	1196	801	801	916	1280
-3417.57	724	332	766	438	910	1233	856	856	987	1343
-3416.44	673	327	740	411	935	1212	804	804	986	1312

-3415.32	727	367	775	481	957	1235	837	837	973	1404
-3414.2	694	294	735	446	920	1254	852	852	1000	1308
-3413.07	716	347	739	450	932	1253	844	844	982	1342
-3411.95	698	365	774	466	963	1258	876	876	1042	1412
-3410.83	701	365	792	472	964	1323	890	890	975	1377
-3409.71	718	356	815	477	1001	1311	859	859	1006	1419
-3408.58	663	364	793	468	998	1305	913	913	1003	1419
-3407.46	757	371	841	492	996	1382	939	939	1038	1479
-3406.34	722	362	827	457	985	1326	935	935	1065	1459
-3405.22	731	390	809	497	1003	1389	930	930	1111	1485
-3404.1	718	371	831	482	1017	1338	955	955	1086	1481
-3402.98	742	402	798	482	1025	1332	900	900	1061	1519
-3401.85	720	383	887	481	1015	1380	911	911	1155	1493
-3400.73	694	379	822	467	1028	1315	949	949	1106	1497
-3399.61	752	415	874	499	1053	1416	955	955	1093	1553
-3398.49	697	401	848	490	1018	1418	963	963	1109	1556
-3397.37	735	430	876	478	1033	1401	991	991	1133	1551
-3396.25	731	379	842	481	1089	1415	956	956	1128	1570
-3395.13	728	408	886	494	1094	1406	996	996	1155	1608
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-3386.18	765	420	933	515	1097	1493	1087	1087	1190	1673
-3385.07	707	411	894	495	1085	1488	1023	1023	1184	1667
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-3382.83	722	439	922	511	1118	1529	1037	1037	1250	1727
-3381.72	747	462	962	574	1152	1585	1087	1087	1278	1728
-3380.6	757	426	951	516	1160	1531	1113	1113	1243	1757
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-3378.37	699	388	953	557	1093	1529	1080	1080	1234	1746
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-3375.02	757	451	978	552	1178	1568	1117	1117	1307	1824
-3373.9	741	446	959	550	1173	1565	1100	1100	1319	1831

-3372.79	762	446	972	510	1177	1576	1123	1123	1266	1835
-3371.67	820	509	1065	599	1234	1656	1182	1182	1318	1866
-3370.56	753	466	992	539	1185	1603	1164	1164	1279	1848
-3369.44	754	416	984	508	1159	1569	1118	1118	1305	1820
-3368.33	759	492	1030	603	1220	1644	1165	1165	1340	1906
-3367.22	744	481	1026	584	1236	1626	1141	1141	1332	1863
-3366.1	762	451	1043	534	1168	1626	1157	1157	1319	1838
-3364.99	734	486	1023	539	1188	1575	1181	1181	1339	1865
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-3362.76	737	444	1033	522	1235	1591	1166	1166	1354	1914
-3361.65	781	475	1076	585	1244	1624	1187	1187	1340	1909
-3360.54	794	483	1034	594	1237	1642	1167	1167	1381	1942
-3359.42	766	447	1080	571	1258	1640	1166	1166	1337	1884
-3358.31	784	491	1042	586	1258	1633	1193	1193	1343	1927
-3357.2	759	473	1038	574	1240	1663	1160	1160	1373	1927
-3356.09	816	507	1062	607	1273	1689	1233	1233	1391	1941
-3354.97	808	514	1096	636	1277	1694	1210	1210	1410	1960
-3353.86	747	475	1040	601	1203	1672	1197	1197	1373	1927
-3352.75	772	507	1078	586	1315	1647	1225	1225	1418	1954
-3351.64	763	496	1088	579	1267	1686	1224	1224	1417	1924
-3350.53	783	483	1059	585	1254	1728	1193	1193	1434	1972
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-3097.51	921	1077	2366	1252	2615	3595	2324	2324	2463	3608
-3096.45	923	1024	2390	1208	2604	3659	2302	2302	2426	3600
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-3094.33	938	1039	2399	1237	2623	3696	2361	2361	2517	3669
-3093.27	903	1052	2354	1227	2608	3649	2277	2277	2453	3651
-3092.21	895	1073	2375	1202	2550	3630	2302	2302	2398	3628
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-3085.85	902	1048	2299	1232	2488	3629	2237	2237	2368	3577
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-3083.73	925	1017	2355	1204	2586	3588	2300	2300	2405	3502
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-3081.61	893	1031	2257	1214	2571	3657	2225	2225	2449	3585
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-3076.32	878	1030	2293	1245	2547	3622	2227	2227	2452	3619
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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
-3022.65	781	965	1926	1118	2151	3471	2047	2047	1952	3193
-3021.6	747	939	1939	1091	2119	3486	2037	2037	1977	3162
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-3019.51	780	916	1917	1119	2105	3508	2016	2016	1921	3147
-3018.46	764	913	1929	1081	2055	3466	2033	2033	1950	3163
-3017.42	745	910	1885	1072	2073	3444	1931	1931	1885	3098
-3016.37	745	926	1897	1077	2074	3455	1980	1980	1912	3132
-3015.33	734	921	1864	1070	2044	3359	1938	1938	1876	3164
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-3011.14	718	887	1807	1069	2035	3410	1980	1980	1892	3086
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-3003.83	700	858	1767	1020	1916	3276	1960	1960	1915	3018
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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
-2990.28	768	864	1778	1033	1920	3212	2034	2034	2108	3159
-2989.24	715	832	1744	1012	1937	3259	2047	2047	2139	3158
-2988.2	733	857	1788	1016	1894	3235	2003	2003	2212	3132
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-2863.73	1258	1011	2442	1184	2931	3716	3286	3286	5094	5833
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-2847.48	1144	898	2279	1143	2713	3519	2966	2966	4460	5161
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-2843.43	1101	865	2217	1096	2601	3383	2883	2883	4267	4957
-2842.42	1090	810	2191	1047	2577	3361	2847	2847	4158	4936
-2841.4	1082	892	2159	1044	2615	3349	2810	2810	4116	4891
-2840.39	1052	834	2119	1046	2502	3325	2745	2745	4077	4820
-2839.38	1072	837	2126	1038	2529	3347	2751	2751	4071	4716
-2838.37	1069	837	2077	1007	2467	3289	2756	2756	4001	4760
-2837.35	1111	863	2138	1091	2565	3300	2774	2774	3958	4709
-2836.34	1035	840	2100	1025	2467	3325	2690	2690	3906	4627
-2835.33	1011	823	2076	1053	2451	3272	2660	2660	3870	4607
-2834.32	1083	810	2055	987	2521	3182	2631	2631	3769	4517
-2833.31	1039	811	2081	1032	2413	3200	2600	2600	3732	4444
-2832.3	1051	805	2070	1017	2388	3155	2520	2520	3744	4452
-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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-2828.25	1029	790	1913	978	2309	3105	2471	2471	3525	4219
-2827.24	1000	781	1930	996	2330	3049	2446	2446	3495	4178
-2826.23	930	741	1946	1004	2241	3052	2399	2399	3432	4080
-2825.22	949	725	1861	914	2182	3059	2349	2349	3338	4040
-2824.21	955	735	1902	955	2217	2883	2330	2330	3345	3970
-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
-2820.18	922	790	1814	945	2201	2880	2279	2279	3194	3882
-2819.17	929	758	1838	956	2152	2868	2247	2247	3118	3807
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-2817.15	891	717	1773	911	2099	2817	2214	2214	3068	3694
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-2812.11	867	737	1746	906	2022	2795	2120	2120	2966	3541
-2811.11	849	676	1701	875	1972	2768	2062	2062	2875	3556
-2810.1	905	684	1734	857	2001	2748	2126	2126	2877	3582

-2809.09	839	686	1737	880	1969	2697	2062	2062	2848	3502
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-2807.08	843	700	1654	799	1922	2688	1993	1993	2769	3385
-2806.08	823	685	1707	826	1958	2643	1957	1957	2708	3447
-2805.07	863	675	1648	824	1898	2712	1992	1992	2737	3376
-2804.06	842	673	1652	791	1877	2579	1922	1922	2666	3337
-2803.06	853	674	1647	831	1900	2647	1933	1933	2669	3317
-2802.05	837	668	1627	864	1839	2587	1906	1906	2640	3245
-2801.05	801	656	1626	817	1879	2561	1915	1915	2607	3265
-2800.04	821	640	1605	818	1799	2539	1945	1945	2557	3216
-2799.04	783	685	1615	782	1795	2516	1917	1917	2591	3174
-2798.03	807	631	1550	771	1768	2531	1821	1821	2534	3088
-2797.03	778	649	1547	789	1710	2513	1813	1813	2481	3089
-2796.02	749	665	1564	786	1763	2542	1823	1823	2509	3043
-2795.02	746	629	1541	774	1787	2481	1754	1754	2454	3086
-2794.02	794	631	1559	765	1719	2474	1811	1811	2442	2978
-2793.01	772	601	1495	784	1729	2406	1757	1757	2407	3000
-2792.01	726	593	1520	772	1708	2373	1717	1717	2347	2929
-2791.01	798	662	1514	774	1704	2429	1769	1769	2360	2973
-2790	780	610	1480	768	1697	2402	1730	1730	2375	2916
-2789	766	607	1529	755	1665	2341	1730	1730	2310	2843
-2788	764	595	1444	745	1654	2380	1674	1674	2264	2797
-2786.99	780	619	1471	752	1665	2322	1661	1661	2256	2870
-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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-2782.99	754	608	1449	719	1656	2320	1632	1632	2222	2696
-2781.99	741	590	1475	728	1628	2296	1591	1591	2167	2727
-2780.98	739	589	1441	714	1570	2183	1584	1584	2153	2720
-2779.98	743	593	1388	708	1614	2205	1591	1591	2157	2666
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-2776.98	724	532	1420	719	1547	2180	1560	1560	2049	2600
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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
-2769.98	656	565	1300	689	1438	2071	1449	1449	1936	2460
-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
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-2762.99	653	523	1254	669	1354	1952	1388	1388	1842	2296
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-2761	629	480	1232	629	1323	1956	1332	1332	1800	2242
-2760	647	487	1242	598	1363	1911	1303	1303	1743	2278
-2759	652	498	1229	638	1343	1978	1310	1310	1755	2241
-2758	607	498	1198	606	1311	1910	1264	1264	1758	2143
-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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-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
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-2743.07	593	477	1098	562	1168	1725	1154	1154	1546	1946
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-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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-2733.14	564	431	1056	533	1120	1627	1082	1082	1418	1797
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-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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-2701.48	505	334	828	403	864	1288	824	824	1058	1366
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-2695.57	496	323	842	424	843	1263	766	766	1059	1310
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-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
-2688.67	500	320	757	440	836	1191	759	759	1008	1273
-2687.69	498	294	759	411	765	1179	706	706	980	1218
-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
-2682.77	514	335	785	393	778	1137	717	717	960	1204
-2681.79	519	301	750	433	804	1176	687	687	954	1173
-2680.81	499	259	727	388	764	1123	691	691	903	1164
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-2673.94	471	294	730	396	758	1092	646	646	908	1146
-2672.95	493	310	706	394	761	1055	630	630	895	1133
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-2670.01	455	242	689	358	714	1032	609	609	838	1093
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-2667.07	435	248	677	343	691	1005	603	603	820	1074
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-2665.11	464	252	693	346	663	1007	646	646	787	1015
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-2644.58	427	269	607	311	605	864	568	568	721	873
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-2634.83	442	213	567	317	578	820	486	486	639	808
-2633.86	412	252	564	311	557	794	472	472	620	779
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-2627.05	422	235	537	296	535	712	445	445	596	713
-2626.08	401	201	495	304	513	741	433	433	567	753
-2625.11	393	186	501	267	504	703	441	441	591	728
-2624.13	385	224	532	295	457	695	440	440	552	715
-2623.16	414	206	521	251	511	711	429	429	524	745
-2622.19	379	206	494	272	503	683	447	447	557	709

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-2619.28	397	191	493	282	474	669	431	431	537	675
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-2617.33	381	224	491	250	455	650	415	415	520	644
-2616.36	382	230	463	264	482	676	416	416	543	682
-2615.39	395	191	468	244	444	644	367	367	518	632
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-2608.61	367	180	448	234	435	644	360	360	501	642
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-2589.26	328	140	367	214	394	478	282	282	382	484
-2588.3	321	150	399	227	379	540	313	313	404	511
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-2586.37	320	108	353	176	332	485	265	265	387	463
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-2579.62	345	134	351	162	326	464	276	276	375	464
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-2532.62	282	105	265	156	215	349	176	176	240	307
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-2528.8	303	100	240	131	232	324	169	169	247	313
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-2523.08	296	94	268	121	224	286	151	151	272	313
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-2518.31	294	94	222	109	226	277	153	153	220	277
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-2506.9	310	92	231	125	209	285	125	125	201	255
-2505.95	312	86	250	146	199	301	147	147	224	278
-2505	296	115	261	141	230	278	168	168	247	255
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-2503.1	263	75	201	106	192	256	142	142	192	264
-2502.15	285	82	212	131	202	276	141	141	203	242
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-2475.64	309	73	248	131	164	252	158	158	205	226
-2474.7	274	89	154	125	150	218	130	130	161	218
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-2466.21	268	79	154	97	139	207	105	105	159	201
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-2460.55	260	61	164	90	159	180	89	89	143	193
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-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	-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	-0.00012	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	-0.99976	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	-0.99976	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
-3590.99	153	230	184	129	98	359	274	514	235	248
-3589.83	213	247	207	116	126	375	334	533	248	319
-3588.67	166	234	219	101	134	360	278	541	255	293
-3587.52	179	236	195	128	73	354	274	502	251	258
-3586.36	187	258	198	102	130	344	280	528	206	295
-3585.2	205	255	244	125	168	373	303	537	273	286
-3584.04	189	243	210	142	130	351	316	525	240	297
-3582.89	202	249	235	128	138	355	288	534	230	271
-3581.73	165	250	232	126	136	376	291	547	286	293
-3580.58	186	255	245	124	146	397	330	558	267	315
-3579.42	194	293	203	130	132	356	314	528	228	300
-3578.26	197	246	229	103	129	368	269	550	212	279
-3577.11	224	254	243	132	146	385	320	562	271	316
-3575.95	171	260	205	89	110	313	274	502	217	270
-3574.8	234	280	231	133	146	340	291	519	251	295
-3573.64	180	237	210	103	111	383	301	523	263	300
-3572.49	200	284	228	103	184	378	324	542	248	313
-3571.33	189	310	240	120	159	363	311	564	255	334
-3570.18	203	230	223	107	128	363	284	549	221	281
-3569.03	215	270	222	94	144	352	298	546	262	281

-3567.87	171	250	224	126	109	355	296	553	246	278
-3566.72	219	243	238	105	131	389	325	527	238	329
-3565.56	213	260	215	87	165	382	305	540	244	311
-3564.41	198	254	236	111	167	376	305	531	231	305
-3563.26	243	275	225	108	158	349	293	541	239	329
-3562.11	178	236	222	89	115	359	248	528	208	298
-3560.95	205	256	258	106	149	377	293	534	221	292
-3559.8	206	296	240	141	178	351	304	569	246	347
-3558.65	229	306	226	116	167	382	322	550	238	339
-3557.5	252	284	242	107	159	380	306	572	249	335
-3556.35	241	279	275	126	188	397	328	558	277	340
-3555.2	223	277	231	114	176	397	312	498	238	328
-3554.04	204	282	227	109	188	408	314	541	236	327
-3552.89	221	284	257	138	160	417	317	555	235	326
-3551.74	212	313	257	119	180	356	330	546	212	343
-3550.59	241	296	251	118	155	415	309	564	234	333
-3549.44	234	299	229	114	171	363	304	573	227	318
-3548.29	263	303	298	141	225	407	360	575	282	362
-3547.14	233	302	241	99	167	395	329	532	230	344
-3545.99	213	290	260	112	187	417	321	565	246	338
-3544.84	226	291	243	89	161	365	314	529	224	339
-3543.7	238	314	276	110	179	398	321	533	212	315
-3542.55	226	326	270	128	199	396	336	567	258	349
-3541.4	260	331	294	144	206	410	359	582	263	397
-3540.25	266	323	234	143	212	417	332	535	243	359
-3539.1	247	314	306	146	202	408	372	542	255	379
-3537.95	232	295	262	78	170	408	259	543	202	336
-3536.81	235	327	266	124	207	441	336	570	257	394
-3535.66	243	343	317	138	226	402	377	567	235	378
-3534.51	271	354	281	164	189	433	322	567	245	383
-3533.37	250	320	294	114	191	421	281	558	234	370
-3532.22	283	353	312	143	234	411	331	571	263	413
-3531.07	244	341	307	150	217	449	341	556	283	418
-3529.93	240	339	273	106	202	427	319	571	209	355
-3528.78	257	343	302	134	222	433	337	532	238	404
-3527.63	320	364	342	143	245	459	344	584	230	428
-3526.49	268	339	288	129	221	430	338	554	228	406
-3525.34	235	315	329	107	208	427	332	573	249	392

-3524.2	305	346	322	124	238	466	343	560	248	420
-3523.05	293	396	328	136	253	440	366	578	266	456
-3521.91	241	343	307	109	234	432	328	537	202	386
-3520.77	279	394	329	129	266	458	361	576	259	455
-3519.62	269	348	328	115	255	455	344	560	242	398
-3518.48	281	348	328	136	231	444	338	559	216	438
-3517.33	292	395	332	119	237	475	338	546	242	428
-3516.19	274	355	340	121	222	494	350	560	229	459
-3515.05	278	412	394	137	259	459	368	600	264	453
-3513.9	344	403	358	157	251	489	367	573	250	470
-3512.76	322	407	357	153	245	501	354	586	281	476
-3511.62	295	414	400	121	311	504	354	599	254	460
-3510.48	282	401	368	123	261	467	368	571	221	474
-3509.33	309	400	342	113	284	477	379	617	241	460
-3508.19	318	397	371	136	287	492	379	560	257	491
-3507.05	319	405	390	137	292	500	365	593	253	483
-3505.91	307	403	367	153	255	494	360	607	231	470
-3504.77	372	452	410	126	305	555	394	620	268	502
-3503.63	340	464	428	167	340	540	431	682	283	513
-3502.49	331	437	388	140	269	515	372	586	238	511
-3501.35	404	460	392	178	341	546	379	635	251	524
-3500.2	337	425	408	135	282	530	378	619	249	515
-3499.06	336	430	394	156	317	506	408	601	253	512
-3497.93	351	446	412	126	324	544	394	599	216	548
-3496.79	367	458	418	137	278	560	413	632	257	555
-3495.65	380	510	459	148	348	593	411	695	292	593
-3494.51	343	489	444	140	333	577	381	574	250	541
-3493.37	409	516	484	170	345	577	427	665	244	558
-3492.23	400	518	439	168	343	590	436	657	296	611
-3491.09	392	533	465	153	366	588	419	678	258	612
-3489.95	407	526	462	152	376	601	425	644	253	594
-3488.81	407	484	449	154	329	591	425	634	272	606
-3487.68	391	533	496	130	393	585	451	625	249	614
-3486.54	383	534	480	167	377	601	423	652	258	619
-3485.4	401	530	479	146	407	592	461	656	228	626
-3484.26	401	522	480	131	378	638	398	638	230	629
-3483.13	426	547	520	171	380	616	425	687	273	650
-3481.99	473	571	533	178	405	621	451	678	271	668

-3480.85	434	565	519	153	415	678	424	693	277	669
-3479.72	434	559	552	158	421	657	458	689	231	677
-3478.58	459	630	537	171	436	653	474	696	238	707
-3477.45	437	618	535	131	437	694	448	666	256	695
-3476.31	454	604	530	139	425	673	427	669	233	741
-3475.18	458	598	532	142	446	687	459	696	250	699
-3474.04	485	635	572	153	459	714	461	681	248	725
-3472.91	490	620	585	155	463	705	454	719	273	749
-3471.77	547	671	583	193	486	730	524	712	289	768
-3470.64	524	683	631	189	493	722	509	737	288	800
-3469.5	504	701	593	172	486	774	497	741	284	764
-3468.37	524	693	671	159	488	740	537	723	259	794
-3467.23	552	720	657	173	537	740	518	729	275	839
-3466.1	537	695	612	188	480	774	493	717	270	836
-3464.97	539	707	622	151	526	788	520	734	275	842
-3463.83	547	720	636	173	555	834	516	727	284	839
-3462.7	527	717	662	194	543	802	494	766	250	845
-3461.57	566	746	692	181	573	854	542	775	270	906
-3460.44	612	774	730	189	550	824	561	791	259	893
-3459.3	587	775	670	161	589	825	527	789	291	917
-3458.17	579	774	729	187	586	868	579	757	282	940
-3457.04	622	776	697	175	575	837	519	744	251	921
-3455.91	632	809	743	193	614	900	552	769	306	999
-3454.78	622	830	819	201	643	921	592	802	300	1031
-3453.65	617	815	757	203	607	902	560	821	301	1005
-3452.51	650	828	754	207	616	932	547	819	270	964
-3451.38	688	850	848	191	668	922	583	815	299	1021
-3450.25	642	844	766	187	623	902	566	794	246	991
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-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
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-2708.39	974	1356	1753	244	1268	1632	904	1066	354	2086
-2707.4	945	1326	1714	195	1271	1653	871	1023	350	2049
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-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
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-2697.54	870	1215	1582	223	1158	1463	819	981	332	1874
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-2688.67	880	1168	1537	237	1067	1385	762	958	315	1763
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-2675.9	762	1044	1311	200	974	1237	661	843	280	1600
-2674.92	764	1039	1312	208	957	1213	704	852	306	1621
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-2667.07	686	974	1224	168	893	1192	612	787	280	1520
-2666.09	682	993	1222	178	891	1163	606	803	301	1467
-2665.11	690	975	1213	155	891	1110	661	777	298	1459
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-2663.15	711	954	1182	161	879	1143	619	804	321	1469
-2662.17	683	963	1183	183	854	1173	597	780	292	1423
-2661.2	684	931	1193	164	893	1092	608	764	309	1394
-2660.22	689	942	1209	188	869	1103	651	763	297	1437
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-2658.26	635	904	1133	156	819	1076	588	733	281	1354
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-2652.39	567	831	1036	125	747	943	544	732	192	1252
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-2647.51	607	805	1044	166	740	987	583	725	296	1264
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-2644.58	593	797	1017	142	751	994	575	699	267	1226
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-2642.63	558	769	985	172	679	932	542	675	233	1187
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-2640.68	535	772	983	159	692	929	515	693	263	1193
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-2615.39	433	591	739	112	559	698	418	576	204	905
-2614.42	392	588	715	125	517	700	394	595	186	884
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-2611.51	387	556	692	122	493	659	395	546	204	829
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-2607.64	387	547	692	126	508	673	404	564	195	834
-2606.67	374	547	625	70	438	626	365	489	157	806
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-2604.73	363	522	647	108	443	623	366	517	196	799

-2603.76	413	501	658	117	496	624	381	529	189	808
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-2587.33	330	463	548	93	405	505	347	465	158	661
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-2530.71	252	353	391	119	285	413	271	392	187	473
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-2527.84	238	307	327	77	246	364	248	363	163	466
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-2494.56	197	257	226	100	192	300	234	336	151	326
-2493.62	175	218	236	73	160	272	218	317	127	297
-2492.67	156	239	246	63	154	290	178	327	114	314
-2491.72	154	224	244	74	142	271	163	284	111	299
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-2440.82	123	161	158	50	109	190	151	227	129	193
-2439.88	86	141	146	74	73	186	123	241	91	197
-2438.94	117	139	131	52	96	211	145	279	111	188
-2438.01	101	179	151	43	99	198	164	218	107	192
-2437.07	101	129	128	45	92	173	147	230	83	173
-2436.13	117	113	99	59	80	194	142	241	94	182
-2435.2	92	126	143	42	71	183	112	222	81	152
-2434.26	89	133	119	65	92	180	135	211	120	181
-2433.32	123	147	165	78	113	184	139	220	109	182
-2432.39	93	149	127	30	100	147	127	245	91	177
-2431.45	109	134	143	79	83	188	116	219	97	180
-2430.51	124	137	157	38	104	167	139	236	119	190
-2429.58	102	134	132	57	77	203	142	215	96	171
-2428.64	107	132	121	69	94	166	140	249	98	161
-2427.71	116	136	107	42	79	145	139	202	112	150
-2426.77	83	117	133	43	83	163	132	206	73	170
-2425.84	70	124	100	32	50	140	95	235	70	145
-2424.9	104	114	123	44	91	178	121	222	88	168
-2423.97	87	120	96	20	59	121	124	204	77	136

-2423.03	111	117	112	44	92	187	133	235	110	156
-2422.1	121	141	118	71	78	177	133	235	85	167
-2421.16	86	106	80	33	71	117	114	195	83	134
-2420.23	112	141	155	98	99	176	147	260	142	191
-2419.29	90	136	128	75	84	137	119	245	94	164
-2418.36	106	127	126	76	65	155	107	215	81	162
-2417.43	94	128	100	6.0002	70	145	137	182	85	142
-2416.49	109	135	132	58	93	149	142	227	78	180
-2415.56	104	107	87	54	59	156	121	200	91	154
-2414.63	102	129	93	58	74	174	130	236	111	159
-2413.69	100	137	168	89	107	164	141	236	127	170
-2412.76	90	122	103	26	44	142	132	201	51	144
-2411.83	93	103	90	39	70	175	126	208	79	120
-2410.9	97	133	124	29	80	143	142	210	102	185
-2409.96	91	105	110	57	91	168	122	232	98	158
-2409.03	47	104	66	46	57	126	105	193	79	110
-2408.1	105	100	88	17	60	169	116	188	94	152
-2407.17	94	131	126	68	101	140	132	242	90	152
-2406.24	81	100	63	57	57	129	123	172	63	147
-2405.3	81	92	102	43	67	136	121	194	58	125
-2404.37	53	81	89	13	24	108	110	164	81	105
-2403.44	101	155	128	84	103	203	159	209	94	131
-2402.51	64	100	75	37	69	124	96	189	53	136
-2401.58	86	86	60	51	45	161	96	181	78	126
-2400.65	95	130	127	76	98	141	143	226	127	153
-2399.72	84	132	74	56	69	123	127	184	78	132
-2398.79	78	97	77	44	47	116	108	181	73	113
-2397.86	61	71	70	22	32	123	103	191	61	110
-2396.93	91	134	91	45	80	156	115	174	60	134
-2396	78	102	62	15	40	100	72	171	62	104
-2395.07	74	62	65	37	35	120	104	189	70	119
-2394.14	105	120	104	50	94	114	140	201	92	132
-2393.21	73	100	81	51	59	132	93	145	82	95
-2392.28	131	104	106	60	69	133	122	182	90	133
-2391.35	66	99	58	33	61	108	94	165	77	117
-2390.42	59	97	89	51	37	125	75	147	55	104
-2389.5	51	85	75	28	29	107	99	182	74	100
-2388.57	81	105	95	66	44	152	85	182	70	100

-2387.64	85	81	89	41	43	84	83	146	49	108
-2386.71	72	80	63	44	52	107	88	146	68	107
-2385.78	65	44	40	19	34	88	56	152	50	103
-2384.86	71	108	67	24	59	75	90	151	81	96
-2383.93	82	84	81	41	35	109	106	140	51	95
-2383	72	131	89	26	62	112	119	157	71	125
-2382.07	70	90	83	34	37	103	79	171	65	110
-2381.15	54	69	55	36	4	60	89	114	69	67
-2380.22	60	101	54	56	70	124	85	131	78	110
-2379.29	40	60	57	44	36	89	55	186	66	85
-2378.37	64	75	17	32	7	47	54	118	42	56
-2377.44	45	84	50	27	47	94	72	125	37	88
-2376.51	27	75	62	13	23	75	71	111	44	91
-2375.59	77	78	49	38	39	101	82	135	49	82
-2374.66	51	68	74	30	19	91	78	92	73	94
-2373.74	43	81	57	50	51	124	94	120	47	80
-2372.81	85	88	73	63	67	105	86	165	73	108
-2371.89	43	54	44	32	11	71	75	101	45	66
-2370.96	26	58	73	28	42	60	72	129	44	81
-2370.04	41	57	22	27	13	77	73	107	31	96
-2369.11	62	75	72	17	49	98	95	132	42	54
-2368.19	43	47	19	18	29	70	81	116	44	37
-2367.26	80	63	51	37	59	88	101	131	55	85
-2366.34	57	45	36	33	32	81	56	125	35	67
-2365.41	21	53	13	20	2.0001	53	63	76	15	46
-2364.49	55	51	70	25	59	65	69	123	38	73
-2363.57	10	56	47	17	7	71	57	93	29	33
-2362.64	5.9999	37	20	15	1.9999	78	56	62	-1.0001	39
-2361.72	52	38	24	11	46	82	46	75	26	53
-2360.79	27	26	24	8.9998	-4.0002	61	22	46	3.9998	26
-2359.87	28	41	25	18	17	20	20	90	16	22
-2358.95	18	25	-0.0002	-4.0002	2.9998	35	37	43	14	33
-2358.03	39	42	57	20	33	42	56	86	36	38
-2357.1	23	42	6	40	22	44	44	59	40	47
-2356.18	32	43	30	12	26	66	39	40	7.9999	55
-2355.26	39	48	37	26	27	55	39	65	41	60
-2354.34	27	63	26	31	44	49	51	58	46	42
-2353.41	42	69	41	27	32	48	52	61	33	59

-2352.49	12	9.9999	-12	-3.0001	-12	23	-8.0001	17	-2.0001	-2.0001
-2351.57	19	21	55	14	32	28	42	41	34	47
-2350.65	11	30	26	27	35	19	37	29	20	39
-2349.73	6	-1	-5	2	-16	35	20	22	-20	10
-2348.81	35	41	10	0	5	8	19	33	-3	29
-2347.89	38	57	47	27	24	72	50	60	30	59
-2346.97	-1.9999	6.0001	-14	-13	-56	-21	-12	-32	-55	1.0001
-2346.05	13	3.9999	-1.0001	14	17	7.9999	13	15	9.9999	25
-2345.12	44	16	61	25	31	27	20	63	23	56
-2344.2	-5.9998	18	12	3.0002	-19	-22	12	7.0002	-20	-7.9998
-2343.28	-4.0001	4.9999	-3.0001	7.9999	6.9999	20	9.9999	9.9999	20	2.9999
-2342.36	22	2.0001	1.0001	-38	-8.9999	2.0001	16	3.0001	-37	13
-2341.44	19	27	28	19	5.0002	22	39	32	-7.9998	27
-2340.53	4.0001	35	14	0.00012	-44	22	-12	-9.9999	-12	12
-2339.61	4.9999	14	-0.0001	4.9999	-10	20	-11	19	-2.0001	15
-2338.69	37	29	9.9999	19	-2.0001	2.9999	6.9999	28	27	31
-2337.77	5.9998	31	-22	24	-8.0002	3.9998	33	-2.0002	-10	1.9998
-2336.85	-36	-8	-13	-20	-7	-20	-19	1	-15	-19
-2335.93	-18	-12	17	-10	-14	-16	9.9999	-15	-30	-9.0001
-2335.01	24	2.0002	26	19	20	22	32	39	-0.9998	20
-2334.09	-35	15	-12	-12	-23	-13	4.9998	8.9998	-33	1.9998
-2333.17	16	-6.0001	-1.0001	-8.0001	3.9999	12	11	3.9999	-9.0001	8.9999
-2332.26	41	21	5.9998	13	5.9998	7.9998	9.9998	5.9998	20	27
-2331.34	-3.0002	1.9998	-12	-7.0002	4.9998	-6.0002	21	-31	-27	2.9998
-2330.42	-7.9998	9.0002	12	-9.9998	0.00024	5.0002	-5.9998	38	-17	6.0002
-2329.5	-4.0002	-11	-27	3.9998	-30	-6.0002	-7.0002	-14	-32	-16
-2328.59	29	58	48	31	58	41	40	33	-2	27
-2327.67	-4.9998	-7.9998	-45	-37	-37	-27	-28	-26	-34	-33
-2326.75	-24	-35	-23	-11	-48	-16	-2.9998	-7.9998	-34	-18
-2325.83	6.9999	0.99988	7.9999	-12	9.9999	-28	-9.0001	-3.0001	-8.0001	19
-2324.92	-23	-15	28	25	3.0002	-5.9998	4.0002	2.0002	8.0002	-11
-2324	-6.0001	7.9999	3.9999	13	11	7.9999	-2.0001	19	4.9999	13
-2323.09	8	17	-23	2	5	10	-2	5	-25	3
-2322.17	0.00012	-0.99988	2.0001	-3.9999	5.0001	7.0001	29	-2.9999	-9.9999	-4.9999
-2321.25	7.0002	18	2.0002	-8.9998	-4.9998	1.0002	25	-18	7.0002	3.0002
-2320.34	4.0002	-4.9998	6.0002	1.0002	-6.9998	23	-12	1.0002	-32	-0.9998
-2319.42	13	-2.9999	-1.9999	1.0001	-15	-48	-0.9999	-12	8.0001	0.00012

-2318.51	20	27	3	28	9	24	33	17	28	3
-2317.59	-1.0002	-20	24	-24	-9.0002	-3.0002	6.9998	35	-16	17
-2316.68	-12	-1.0001	-3.0001	-14	-19	-5.0001	-21	-2.0001	-26	-15
-2315.76	6.0001	14	26	-8.9999	15	16	1.0001	-0.9999	13	3.0001
-2314.85	-29	27	-3.0002	-3.0002	-4.0002	4.9998	-7.0002	-12	-38	16
-2313.93	-3.0001	-5.0001	-16	11	-34	-3.0001	-20	18	-34	-17
-2313.02	25	27	-11	7.0002	-14	-13	23	-5.9998	-8.9998	18
-2312.1	-6.0001	4.9999	-9.0001	-14	-9.0001	8.9999	-17	-2.0001	-24	0.99988
-2311.19	-36	-21	-6	-12	-30	-27	-25	-1	-21	-10
-2310.27	-1.9998	14	-27	-15	-22	-18	5.0002	-20	-29	-38
-2309.36	-34	-24	1.9998	-18	-3.0002	-14	-50	-22	-13	-19
-2308.45	-2.9999	4.0001	5.0001	8.0001	-0.9999	17	10	24	-16	3.0001
-2307.53	27	19	-16	-8	17	14	7	4	-12	-2
-2306.62	-31	7	-27	0	-27	-28	-3	-49	-17	-13
-2305.71	34	17	26	41	18	23	7.9998	40	5.9998	1.9998
-2304.79	4.0001	12	7.0001	-1.9999	-2.9999	-15	20	3.0001	-9.9999	4.0001
-2303.88	10	21	-14	-4.9999	-1.9999	-6.9999	-11	-19	-33	-38
-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
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-2793.01	750	840	621	1025	954	717	891	848	877	989
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-2791.01	743	834	631	1010	921	707	861	842	839	982
-2790	741	824	620	1015	918	689	844	835	870	955
-2789	756	814	604	962	904	715	813	793	812	938
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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
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-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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-2771.98	637	718	518	860	779	612	725	691	732	806
-2770.98	655	718	530	879	771	610	708	716	672	812
-2769.98	642	711	534	897	781	609	724	705	720	799
-2768.98	619	670	528	862	772	610	736	706	677	831
-2767.98	621	695	543	841	770	596	706	689	714	776
-2766.98	637	702	551	866	730	597	697	686	712	774
-2765.99	645	680	504	863	734	582	696	660	712	759
-2764.99	558	660	499	808	706	559	675	693	646	758
-2763.99	639	704	554	863	771	607	705	686	719	775
-2762.99	612	672	494	843	699	582	706	635	701	731
-2761.99	633	660	553	824	715	579	696	677	690	763
-2761	568	634	499	793	686	538	635	617	634	693
-2760	582	658	504	805	701	578	670	638	666	745
-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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-2754.02	547	613	459	721	661	536	599	603	624	674
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-2748.04	530	563	445	730	648	484	561	557	574	653
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-2746.05	558	570	458	714	588	483	564	556	593	620
-2745.06	477	530	393	700	627	478	550	541	544	605
-2744.07	503	534	415	665	602	477	557	533	582	626
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-2742.08	483	539	392	719	621	489	560	552	533	620
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-2740.09	462	526	397	676	590	478	557	534	529	615
-2739.1	486	508	427	690	559	458	533	538	562	600
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-2737.11	492	569	417	669	589	493	544	558	569	651
-2736.12	486	492	400	631	561	454	518	488	534	568
-2735.12	489	543	400	626	550	458	563	520	549	603
-2734.13	482	496	364	625	541	418	489	501	528	531
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-2732.15	473	550	403	629	531	463	515	511	523	547
-2731.15	457	489	401	649	564	469	511	500	542	556
-2730.16	480	537	397	637	592	490	502	517	500	557
-2729.17	444	481	373	635	534	415	491	483	502	553
-2728.18	432	477	398	631	523	422	518	444	524	546
-2727.19	451	465	364	617	560	433	448	483	478	537
-2726.2	421	475	345	623	555	440	519	481	507	599
-2725.21	447	470	381	601	526	400	483	452	482	546
-2724.22	422	475	315	568	547	430	480	481	465	525
-2723.23	443	494	366	614	509	421	483	493	517	557
-2722.23	408	453	324	576	528	389	462	429	474	486
-2721.24	453	491	396	586	522	396	482	475	483	523
-2720.25	406	445	311	573	490	385	469	464	465	514
-2719.26	392	446	313	538	492	362	445	449	454	494
-2718.28	425	461	350	544	499	389	428	425	455	494
-2717.29	392	451	353	550	462	409	439	433	449	456
-2716.3	412	437	318	576	476	379	420	432	461	497
-2715.31	365	398	327	551	464	365	437	418	421	500
-2714.32	401	436	360	552	431	358	410	416	434	481
-2713.33	400	445	319	573	487	398	413	472	457	511
-2712.34	416	468	349	576	534	414	459	454	450	509
-2711.35	382	422	374	566	442	391	401	396	450	457
-2710.37	367	416	329	498	469	373	434	398	438	483
-2709.38	412	414	321	552	459	378	434	409	445	491
-2708.39	365	422	338	532	470	359	434	412	437	480
-2707.4	388	436	334	530	466	379	409	419	433	474
-2706.41	372	413	294	531	438	351	415	386	400	416
-2705.43	385	412	313	521	467	371	433	404	418	476

-2704.44	391	400	308	515	436	351	405	389	408	456
-2703.45	382	404	291	480	452	343	406	387	405	413
-2702.47	388	386	295	528	456	367	402	400	439	454
-2701.48	360	389	313	497	458	323	379	389	424	462
-2700.49	385	440	338	531	432	390	427	430	426	469
-2699.51	369	369	287	513	420	348	396	405	415	439
-2698.52	350	405	306	504	455	369	427	402	415	471
-2697.54	335	344	262	432	405	306	322	344	358	402
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-2695.57	380	374	261	495	414	331	401	379	387	427
-2694.58	325	366	264	468	420	332	367	363	398	411
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-2691.63	342	374	285	475	432	321	362	380	389	402
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-2689.66	332	346	276	457	393	329	379	344	374	420
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-2686.71	339	379	273	463	399	324	366	361	368	409
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-2684.74	330	339	279	452	356	305	357	349	370	410
-2683.76	297	324	238	425	361	301	362	355	342	394
-2682.77	283	318	259	415	344	271	343	320	349	364
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-2680.81	299	369	275	439	368	304	344	334	312	393
-2679.83	313	350	275	430	358	309	337	335	375	392
-2678.84	287	309	254	409	353	278	354	337	334	376
-2677.86	317	370	252	423	350	314	316	365	372	374
-2676.88	295	312	204	401	329	305	327	318	321	379
-2675.9	299	329	260	428	332	319	328	327	321	353
-2674.92	279	311	206	396	320	261	293	298	297	331
-2673.94	280	303	217	392	300	283	317	297	321	349
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-2671.97	310	300	234	404	343	291	318	315	321	338
-2670.99	282	306	223	385	316	273	271	312	344	351
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-2667.07	262	290	200	370	346	264	291	292	298	325
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-2646.53	254	283	167	350	279	242	252	243	276	309
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-2642.63	226	228	191	303	255	247	245	229	235	274
-2641.66	231	213	203	297	273	205	210	216	255	275
-2640.68	241	254	162	303	279	230	233	252	262	283
-2639.71	237	242	159	298	267	225	219	236	246	260
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-2636.78	214	209	148	311	236	193	200	212	237	237
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-2629.97	188	183	155	266	237	159	201	212	225	247
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-2627.05	197	205	153	296	237	199	190	215	229	234
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-2614.42	161	178	152	259	218	168	167	175	188	227
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-2603.76	143	162	119	194	170	122	146	130	148	173
-2602.8	180	155	138	205	180	169	176	165	164	194
-2601.83	154	156	105	220	159	162	163	165	193	175
-2600.86	126	133	102	203	180	122	137	120	148	192
-2599.89	135	163	145	223	197	179	167	174	171	217
-2598.93	149	144	118	208	170	154	125	159	187	181
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-2595.06	161	125	94	197	152	130	135	143	138	185
-2594.09	110	130	109	185	168	131	119	135	147	162

-2593.13	159	155	151	203	165	157	169	172	186	195
-2592.16	137	151	133	200	166	135	152	148	138	194
-2591.19	129	162	98	193	147	143	136	150	128	170
-2590.23	124	88	117	194	155	113	141	122	138	153
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-2586.37	162	160	155	183	162	131	142	167	164	173
-2585.4	120	142	92	190	140	123	118	127	118	139
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-2583.47	103	123	117	170	177	118	123	153	145	136
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-2573.84	118	133	95	175	119	122	144	135	144	166
-2572.88	127	115	136	158	150	131	136	110	118	152
-2571.91	113	83	71	141	126	92	104	114	124	145
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-2556.54	98	79	89	147	126	114	93	87	88	147
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-2549.83	64	83	74	136	107	78	69	81	76	133
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-2544.09	98	103	75	129	150	102	79	110	113	131
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-2282.02	-104	-166	-180	-232	-223	-238	-252	-293	-347	-242
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-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
-3484.26	262	228	309	277	215	151	82	176	234	224
-3483.13	194	181	265	199	166	94	42	152	205	194
-3481.99	259	223	255	257	190	127	61	179	261	241
-3480.85	243	228	294	239	212	105	49	158	211	216
-3479.72	240	252	332	269	205	140	85	181	232	241
-3478.58	228	213	313	301	218	119	70	194	253	241
-3477.45	253	209	261	224	173	112	20	167	221	211
-3476.31	277	244	309	299	227	132	79	177	285	273
-3475.18	238	231	325	239	204	129	49	171	245	247
-3474.04	298	269	303	304	234	154	95	202	281	294
-3472.91	222	239	279	282	229	104	47	185	282	227
-3471.77	293	251	360	311	203	152	66	209	288	262
-3470.64	279	263	326	295	257	140	75	214	282	243
-3469.5	292	246	355	295	248	154	61	182	274	278
-3468.37	325	289	365	337	243	151	95	214	309	296
-3467.23	289	262	337	281	218	136	67	205	270	256
-3466.1	313	245	351	321	244	141	74	238	298	284
-3464.97	298	258	322	305	263	124	68	196	300	277
-3463.83	342	281	385	342	286	154	73	211	336	329
-3462.7	296	281	336	311	247	114	80	216	291	278
-3461.57	331	279	370	349	262	131	45	216	367	288
-3460.44	311	296	419	340	272	153	73	233	315	319
-3459.3	284	254	335	330	261	153	65	222	296	269
-3458.17	351	290	406	370	294	145	79	247	350	351
-3457.04	334	331	396	344	287	176	76	275	332	335
-3455.91	342	313	426	364	283	168	90	248	329	333
-3454.78	329	296	402	352	281	120	90	232	307	294
-3453.65	386	330	401	384	336	175	63	260	389	343
-3452.51	329	311	460	382	326	149	93	248	364	370
-3451.38	379	299	401	365	282	161	83	226	345	316
-3450.25	376	311	405	393	313	148	59	238	362	338
-3449.12	345	303	400	337	313	168	56	255	333	322
-3447.99	384	378	461	437	328	163	105	280	388	367

-3446.86	373	360	436	376	312	165	83	256	383	343
-3445.73	363	340	423	394	332	171	71	250	366	355
-3444.6	342	343	453	383	299	126	67	224	367	328
-3443.48	419	361	465	405	341	160	94	274	380	365
-3442.35	441	357	497	432	340	190	83	276	392	401
-3441.22	418	349	486	413	358	191	91	287	392	353
-3440.09	452	363	492	449	373	190	113	305	392	377
-3438.96	411	328	445	402	315	130	83	269	382	367
-3437.83	405	335	506	419	375	163	70	290	392	367
-3436.71	404	362	497	461	348	160	102	264	403	376
-3435.58	450	395	521	451	357	193	85	301	422	385
-3434.45	453	370	501	457	367	150	89	291	413	396
-3433.32	449	372	559	436	387	184	85	309	472	406
-3432.2	484	415	517	462	408	206	123	313	415	404
-3431.07	433	380	528	434	374	162	67	275	420	385
-3429.94	452	359	553	476	394	188	83	301	471	406
-3428.82	487	421	501	508	405	178	110	329	461	407
-3427.69	489	414	570	526	403	181	108	322	460	447
-3426.57	492	434	546	497	395	192	118	329	460	439
-3425.44	499	414	564	490	412	200	92	318	491	447
-3424.31	503	430	564	512	427	192	112	351	469	428
-3423.19	529	449	591	504	434	211	96	348	479	429
-3422.06	504	450	585	522	440	220	94	339	500	471
-3420.94	509	449	586	532	442	208	126	344	499	453
-3419.82	546	488	647	528	444	174	108	348	538	460
-3418.69	541	446	636	557	451	221	125	395	518	477
-3417.57	550	432	622	532	461	206	92	338	544	458
-3416.44	532	465	639	520	445	184	91	339	490	480
-3415.32	584	493	684	555	489	228	133	357	536	478
-3414.2	509	482	643	549	488	186	107	370	519	488
-3413.07	581	489	653	592	490	200	132	391	559	474
-3411.95	575	497	690	599	503	230	104	405	552	483
-3410.83	565	464	677	547	473	196	119	378	549	491
-3409.71	601	507	677	604	508	219	133	386	549	513
-3408.58	561	519	717	625	499	231	119	400	576	520
-3407.46	597	519	725	562	531	229	101	376	569	490
-3406.34	575	525	683	626	517	226	110	405	576	493
-3405.22	639	545	690	631	564	229	137	406	579	544

-3404.1	625	541	710	627	565	232	131	403	603	533
-3402.98	614	505	707	626	474	189	121	399	563	501
-3401.85	670	533	751	683	586	272	165	412	603	546
-3400.73	587	524	735	623	547	226	99	376	607	538
-3399.61	630	583	748	622	554	250	116	434	611	521
-3398.49	639	525	726	659	573	246	148	426	614	550
-3397.37	666	581	701	617	557	246	104	405	595	529
-3396.25	682	581	772	677	580	269	141	434	631	585
-3395.13	663	556	769	672	558	228	131	407	619	536
-3394.01	646	544	743	656	573	236	116	418	629	529
-3392.9	642	564	727	718	592	241	108	399	652	576
-3391.78	668	548	792	642	555	227	131	408	645	573
-3390.66	691	575	779	686	577	234	143	431	615	585
-3389.54	691	583	797	663	616	258	118	423	638	574
-3388.42	671	572	820	701	576	252	154	414	657	607
-3387.3	690	592	822	708	597	231	146	453	636	579
-3386.18	719	575	802	721	611	268	152	462	697	656
-3385.07	728	618	837	730	661	271	139	447	669	626
-3383.95	698	583	810	685	591	251	129	429	666	585
-3382.83	709	601	824	727	617	255	149	460	691	600
-3381.72	733	623	785	737	636	270	113	441	697	608
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-3378.37	768	614	861	784	659	288	159	499	748	666
-3377.25	744	635	845	770	662	287	170	457	710	636
-3376.13	760	651	867	757	650	297	166	500	729	617
-3375.02	747	659	895	795	648	267	145	516	746	628
-3373.9	752	649	824	761	672	268	118	475	727	618
-3372.79	753	608	858	772	643	230	157	482	732	625
-3371.67	817	656	914	810	669	258	165	489	702	608
-3370.56	832	653	1027	829	697	284	144	522	745	654
-3369.44	782	684	900	808	677	268	159	508	737	642
-3368.33	818	681	920	814	676	268	158	510	734	653
-3367.22	755	636	887	803	677	242	130	476	722	639
-3366.1	778	682	880	775	689	287	144	485	742	638
-3364.99	783	666	922	810	680	285	168	474	751	638
-3363.87	811	678	927	801	709	267	172	501	765	653
-3362.76	801	654	900	793	716	256	168	515	742	665

-3361.65	798	715	931	840	703	250	150	520	762	676
-3360.54	806	719	922	805	725	286	160	539	812	687
-3359.42	842	664	907	842	704	278	148	520	767	653
-3358.31	868	731	984	823	761	323	169	562	824	689
-3357.2	852	713	910	846	707	282	175	536	789	682
-3356.09	849	721	964	827	750	288	145	542	783	694
-3354.97	862	713	954	871	750	296	195	547	838	717
-3353.86	834	745	934	870	753	277	179	580	803	693
-3352.75	912	744	961	899	738	328	219	589	825	751
-3351.64	869	684	984	842	753	270	176	559	773	765
-3350.53	891	740	998	915	776	315	159	572	863	764
-3349.42	884	763	955	906	782	317	187	564	825	730
-3348.31	915	774	1020	889	819	292	161	565	876	721
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-3344.98	893	760	1049	933	822	307	182	574	890	732
-3343.87	916	742	987	927	782	311	189	567	887	789
-3342.76	936	753	1046	930	850	340	212	582	933	757
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-3322.83	1043	895	1137	1043	915	391	235	686	1053	872
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-3307.38	1128	952	1213	1084	1033	391	232	732	1160	965
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-3083.73	1956	1575	2069	2009	1669	603	378	1227	1647	1413
-3082.67	1925	1611	2120	1986	1684	592	369	1209	1611	1451
-3081.61	1914	1561	2017	1936	1687	595	340	1195	1631	1470
-3080.55	1958	1616	2011	2004	1689	594	349	1214	1652	1458
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-3075.26	1896	1548	2037	1942	1650	514	346	1185	1617	1463
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-2842.42	1393	1184	2108	1197	1364	571	577	1837	2770	1918
-2841.4	1404	1180	2107	1211	1352	607	569	1840	2745	1881
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-2836.34	1302	1123	2029	1198	1284	585	554	1758	2582	1826
-2835.33	1320	1162	2063	1130	1289	543	501	1730	2625	1793
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-2831.29	1221	1071	2027	1119	1201	512	498	1614	2499	1713
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-2829.26	1220	1070	1974	1172	1240	521	515	1637	2429	1683
-2828.25	1252	1094	2004	1067	1207	524	455	1561	2433	1641
-2827.24	1196	1058	1962	1115	1141	479	515	1574	2361	1619
-2826.23	1230	1058	1933	1103	1208	511	504	1561	2374	1642
-2825.22	1184	1006	1915	1063	1113	494	440	1504	2270	1590
-2824.21	1225	1065	1944	1093	1140	520	518	1511	2269	1611
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-2820.18	1079	981	1867	1007	1105	469	441	1395	2142	1466
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-2815.14	1105	939	1717	1001	1052	479	433	1234	2016	1430
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-2813.12	1048	896	1653	990	1006	389	411	1200	1941	1351
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-2793.01	904	769	1292	822	799	369	287	865	1446	1074
-2792.01	929	807	1320	861	847	346	341	923	1475	1112
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-2790	856	767	1212	805	801	364	294	891	1444	1080
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-2786.99	884	765	1234	796	800	351	326	837	1396	1032
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-2772.98	784	671	1062	718	689	321	270	714	1186	897
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-2769.98	788	664	1040	694	670	336	270	710	1165	903
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-2767.98	759	646	995	645	670	303	247	695	1133	894
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-2765.99	743	650	1008	678	662	299	255	678	1157	906
-2764.99	727	616	939	639	647	300	218	656	1095	852
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-2761	684	580	957	619	617	286	176	621	1040	822
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-2720.25	497	437	664	464	441	199	162	450	709	609
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-2718.28	495	445	627	461	456	209	120	465	736	565
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-2715.31	478	444	589	451	413	195	131	422	664	569
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-2713.33	462	452	647	446	427	213	158	441	705	590
-2712.34	479	443	620	454	417	226	186	444	701	569
-2711.35	471	436	588	439	453	195	123	425	689	557
-2710.37	449	435	629	442	400	204	153	425	694	545
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-2707.4	457	412	648	419	416	205	114	421	701	553
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-2692.61	368	360	521	326	334	151	92	361	550	476
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-2685.72	363	356	496	380	335	166	119	343	533	461
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-2683.76	354	315	487	330	321	168	92	355	523	457
-2682.77	354	310	478	339	288	166	120	321	501	404
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-2674.92	334	293	457	315	301	140	89	287	487	402
-2673.94	308	307	462	315	293	145	118	280	459	409
-2672.95	334	295	454	316	304	149	106	326	484	394
-2671.97	321	298	465	345	310	180	114	321	473	428
-2670.99	305	285	445	295	296	137	103	315	490	408
-2670.01	338	286	402	326	284	142	106	289	433	373
-2669.03	330	312	429	313	304	168	120	318	474	416
-2668.05	356	307	454	304	286	175	124	328	468	419
-2667.07	303	298	433	310	285	155	105	289	442	352
-2666.09	316	281	400	294	271	131	81	278	452	364
-2665.11	359	301	418	345	299	207	108	331	499	405
-2664.13	294	290	461	299	277	134	109	292	458	350
-2663.15	287	228	374	251	234	108	67	263	406	338
-2662.17	335	325	442	312	305	177	126	293	464	389
-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
-2657.28	325	285	416	308	267	151	84	291	439	351
-2656.3	303	248	397	293	237	150	96	254	420	345
-2655.33	314	271	390	283	290	190	117	289	460	383
-2654.35	274	221	396	270	273	126	103	234	408	346
-2653.37	315	295	375	275	288	158	124	265	400	348
-2652.39	287	254	377	263	246	141	63	274	376	352
-2651.42	301	271	412	309	270	161	120	293	434	362
-2650.44	303	257	376	287	223	137	117	277	404	362
-2649.46	279	239	365	250	227	109	91	243	359	342

-2648.49	310	277	369	269	264	150	93	272	431	365
-2647.51	275	285	413	300	263	157	109	251	408	367
-2646.53	259	277	377	247	256	127	112	239	411	321
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-2644.58	263	243	368	284	227	152	98	255	394	343
-2643.61	279	247	319	217	216	114	66	227	355	263
-2642.63	266	235	363	249	224	120	77	248	336	306
-2641.66	281	245	340	249	217	152	83	215	397	286
-2640.68	264	241	387	246	243	148	108	252	342	313
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-2638.73	266	223	329	238	240	129	61	215	342	276
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-2626.08	211	214	268	191	191	158	72	191	308	244
-2625.11	185	159	270	166	159	103	63	194	275	254
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-2611.51	189	174	256	193	159	109	52	154	268	219
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-2323.09	-60	-48	-80	-42	-53	-56	-74	-53	-86	-50
-2322.17	-77	-40	-76	-70	-67	-90	-97	-70	-80	-91
-2321.25	-63	-59	-48	-41	-59	-52	-34	-82	-63	-57
-2320.34	-63	-55	-68	-49	-38	-32	-72	-57	-79	-89
-2319.42	-32	-25	-38	-23	-30	-20	-28	-30	-59	-51
-2318.51	-53	-43	-55	-32	-67	-31	-68	-79	-81	-54
-2317.59	-66	-68	-65	-60	-41	-47	-47	-42	-82	-63
-2316.68	-124	-71	-69	-56	-80	-90	-74	-66	-96	-86
-2315.76	-58	-42	-68	-62	-56	-61	-75	-45	-79	-62
-2314.85	-105	-108	-81	-71	-90	-68	-106	-76	-95	-93
-2313.93	-117	-90	-92	-79	-77	-67	-82	-72	-93	-113
-2313.02	-76	-73	-85	-74	-80	-79	-84	-94	-85	-96
-2312.1	-68	-54	-62	-59	-63	-24	-79	-69	-86	-97
-2311.19	-98	-74	-92	-64	-77	-61	-80	-69	-108	-100
-2310.27	-98	-109	-114	-66	-85	-93	-91	-117	-112	-100
-2309.36	-103	-82	-112	-83	-74	-50	-87	-83	-110	-96
-2308.45	-119	-98	-83	-83	-88	-84	-105	-110	-117	-81
-2307.53	-135	-131	-139	-113	-111	-126	-117	-108	-159	-126
-2306.62	-113	-88	-109	-76	-78	-92	-114	-81	-114	-127
-2305.71	-119	-96	-85	-89	-103	-99	-108	-106	-135	-114
-2304.79	-86	-112	-97	-64	-113	-64	-92	-90	-124	-102
-2303.88	-117	-90	-93	-89	-91	-87	-101	-98	-126	-94
-2302.97	-81	-65	-88	-97	-91	-80	-86	-115	-117	-96
-2302.05	-119	-121	-88	-103	-128	-106	-117	-137	-148	-131
-2301.14	-147	-155	-148	-148	-123	-115	-144	-119	-145	-149
-2300.23	-130	-117	-115	-88	-80	-90	-97	-124	-132	-134
-2299.32	-116	-116	-109	-107	-116	-113	-128	-151	-150	-143
-2298.41	-83	-102	-118	-63	-95	-78	-94	-85	-122	-121
-2297.49	-119	-115	-115	-96	-117	-115	-141	-146	-150	-143
-2296.58	-136	-139	-122	-118	-112	-112	-133	-141	-154	-164
-2295.67	-158	-145	-100	-79	-128	-121	-114	-115	-174	-155
-2294.76	-137	-140	-158	-130	-136	-135	-134	-161	-172	-184
-2293.85	-147	-148	-171	-107	-118	-135	-140	-147	-173	-169
-2292.94	-159	-114	-96	-109	-116	-114	-143	-144	-158	-151
-2292.03	-113	-139	-131	-123	-120	-92	-118	-142	-179	-160
-2291.12	-173	-179	-157	-119	-134	-167	-183	-172	-203	-182

-2290.21	-180	-159	-138	-153	-165	-149	-181	-169	-191	-210
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-2288.39	-164	-156	-174	-147	-134	-133	-171	-176	-189	-187
-2287.48	-174	-164	-152	-112	-151	-117	-158	-167	-186	-178
-2286.57	-204	-154	-160	-154	-173	-176	-144	-188	-208	-223
-2285.66	-182	-170	-147	-136	-172	-144	-179	-163	-204	-212
-2284.75	-161	-191	-185	-159	-133	-128	-209	-206	-210	-194
-2283.84	-211	-174	-186	-179	-173	-167	-201	-223	-213	-225
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-2281.11	-231	-222	-180	-185	-191	-184	-203	-223	-240	-228
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-2277.48	-225	-170	-181	-163	-155	-181	-191	-226	-268	-241
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-2275.66	-190	-187	-193	-171	-169	-165	-219	-220	-235	-231
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-2273.85	-243	-226	-224	-197	-226	-207	-228	-269	-292	-291
-2272.94	-226	-219	-212	-178	-185	-205	-237	-272	-272	-254
-2272.03	-208	-208	-181	-159	-177	-166	-214	-226	-277	-255
-2271.13	-274	-235	-241	-189	-228	-215	-233	-268	-295	-319
-2270.22	-238	-211	-223	-226	-221	-216	-233	-264	-292	-277
-2269.31	-218	-223	-232	-208	-213	-201	-227	-263	-278	-272
-2268.41	-226	-223	-206	-218	-223	-209	-247	-259	-275	-266
-2267.5	-214	-196	-205	-193	-178	-162	-197	-228	-263	-241
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-2264.78	-211	-179	-198	-166	-188	-165	-202	-228	-243	-248
-2263.88	-200	-180	-180	-187	-190	-171	-211	-231	-274	-221
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-2262.07	-180	-171	-166	-158	-149	-163	-194	-229	-258	-253
-2261.16	-219	-179	-201	-188	-171	-167	-206	-217	-248	-259
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-2259.35	-193	-137	-134	-147	-154	-122	-205	-185	-241	-207
-2258.45	-199	-176	-194	-172	-157	-161	-193	-209	-256	-247
-2257.54	-216	-184	-180	-152	-209	-172	-228	-227	-245	-242
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-2255.73	-183	-196	-168	-181	-135	-141	-193	-187	-236	-219
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-2253.93	-157	-139	-140	-112	-164	-128	-152	-168	-191	-197
-2253.02	-156	-150	-158	-135	-132	-98	-169	-171	-210	-189
-2252.12	-192	-170	-178	-140	-146	-127	-162	-157	-198	-209
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-2248.51	-89	-61	-94	-95	-95	-88	-104	-149	-139	-122
-2247.6	-118	-113	-107	-95	-94	-78	-109	-126	-161	-149
-2246.7	-124	-124	-110	-69	-100	-58	-97	-115	-129	-154
-2245.8	-107	-106	-78	-77	-86	-86	-114	-114	-129	-139
-2244.9	-91	-104	-92	-74	-66	-41	-123	-96	-148	-145
-2244	-126	-75	-89	-56	-87	-76	-84	-124	-108	-114
-2243.09	-86	-101	-107	-86	-110	-87	-112	-99	-150	-118
-2242.19	-70	-81	-98	-66	-42	-59	-68	-101	-116	-100