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作品名稱 **CONTACTLESS AND NON-DESTRUCTIVE DETECTION OF CHICKEN MEAT CONTAMINATION WITH LASER SPECKLE METHOD**

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關鍵詞 **laser speckle imaging、optics、microorganism**

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## SUMMARY

Harmful microorganisms in food can cause deterioration of human health, poisoning and in some cases even death. Especially fresh meat and chicken products create a suitable environment for the growth of microorganisms in terms of the nutrients it contains, water activity and pH level. For this reason, detection of microorganisms in meat products is an important issue in terms of food safety and human health.

In this project, it is aimed to detect live microorganisms in meat products, especially chicken meat, in a simple, non-destructive, non-contact and fast way using laser speckle method. Laser speckle images of healthy and stale chicken meat were taken, contrast parameter and correlation analysis of the obtained patterns were made. It was observed that the contrast parameter for staled chicken meat increased by approximately 3 times compared to fresh chicken. This increase provides an understanding of the difference between contaminated chicken and fresh chicken. Speckle density changes over time in relation to the movements of living microorganisms. Thus, the correlation in laser speckle density patterns taken from contaminated tissues is disrupted. In the measurements taken with photodiode, by analyzing the change of light intensity of the speckle patterns on fresh and contaminated tissues over time, the detection of microorganisms was made easier and more precisely without the need for image processing.

The proposed measurement system is a new method that detects meat contamination with laser speckle imaging. It can be developed and made portable and can be used easily in homes. Since it is a simple, non-destructive and fast method, it can be used to determine the shelf life of meat in food distribution places and markets. In addition, it has the potential to be calibrated and used for other food products other than meat products. The system developed with this study is cheap and easy to use, and the laser speckle imaging method is used in a different field other than biomedical, contributing to the literature.

## AIM

Detection of microorganisms in the food industry, especially in meat and chicken products, is a critical issue for food safety and human health. Although various methods have been developed to detect microorganisms in foods, the high cost, complex equipment, and the need for skilled technicians limit the widespread use of these methods in the food industry.

In this project, it is aimed to detect live microorganisms that occur in meat products, especially chicken meat, in a simple, non-destructive, non-contact and fast way using laser speckle method. In accordance with this purpose;

- To design a simple and fast measurement system,
- Analyzing the differences between laser speckle patterns taken from healthy and staled chicken meat;
- To detect the presence of microorganisms in the staled chicken by contrast parameter and correlation analysis;
- Upgrading the system for simpler and more precise measurement to make it easier and to make the measuring system portable

is targeted.

# 1.INTRODUCTION

Contamination of food products by microorganisms is very important for food safety and human health. The growth and activity of various types of microorganisms in foods, including bacteria, yeast, and mold, impairs food quality and causes food contamination (Saucier, 2016; Zhang et al., 2012; Rouger et al., 2017). Methods such as microbiological culture methods, high performance liquid chromatography, hyperspectral imaging, Raman spectroscopy, nuclear magnetic resonance technique and mass spectroscopy have been developed to detect and investigate microorganisms and pathogenic agents in food products. These methods contributed to the provision of food safety and quality by providing information about the presence of pathogenic microorganisms. However, these traditional methods require laboratories, costly equipment, and professional technicians. In addition, complex procedures are required for sample preparation and long analysis times. Due to the above limitations, rapid detection and application in markets and homes has restricted their widespread use in food processing, transportation, marketing and preservation in various food industries (Yoon et al., 2016).

Laser speckle imaging (LSI) is a powerful but simple method for visualizing blood flow dynamics in real time. Reasons for choosing LSI for physiological studies; It is due to the ease and low cost of creating a measurement tool, as well as the ability to identify blood flow changes with excellent spatial and temporal resolution (Dunn, 2012). LBG in pre-clinical studies of neurological disorders, clinical applications such as dermatological, neurosurgical, endoscopic studies (Dunn, 2012; Heeman et al., 2019) and seed viability (Braga et al., 2003), shelf life of fruit (Pajuelo et al., 2003) is applied to visualize food conditions such as aging of beef, muscle properties in meat (Amaral et al., 2013). Laser speckle applications are given in more detail in Section 1.3.

Studies in which food safety is ensured with the laser speckle method are quite limited. Other traditional methods cannot be used widely due to high cost and difficulty in application. Detecting food contamination and ensuring food safety with an easy-to-apply, fast-result method will ensure safe use at home, in the food distribution sector, and in storage areas.

In this project, a simple, non-destructive and fast optical method is proposed to measure the quality of meat products, especially chicken meat, using laser speckle imaging. Laser speckle imaging has been used to track moving particles in the environment by analyzing time varying speckle patterns that occur due to deterioration in chicken meat. It has been shown that the analysis of dynamic speckle patterns from chicken meat enables the detection of existing microorganisms. Speckle density changes over time in relation to the movements of living microorganisms. Thus, the correlation in laser speckle density patterns taken from damaged tissues is disrupted. By detecting this deterioration, the living activities of microorganisms can be detected. In addition, the measurement system was developed by using photodiodes instead of cameras, and the detection of microorganisms was made easier and more precisely without the need for image processing.

## 1.1.Laser Speckle Contrast Imaging Method

When a rough surface is illuminated by high-level coherent laser light, the light backscattered from the surface creates an interference pattern consisting of dark and bright areas. This pattern is called speckle. If the illuminated object is not moving and the laser is stationary the interference pattern doesn't change and the pattern is called static speckle pattern. If the illuminated object or the particles in the medium are moving interference pattern changes in time and it is called dynamic speckle pattern. (Briers et al., 2013; Draijer et al., 2009).

Figure (1), explains how laser speckle forms. Incident light coming from the coherent light source scatters from the microstructure of the surface and the reflected waves are at different phases since they reach the imaging sensor from different paths. Light from every point on the illuminated surface combines constructively or destructively to form a single speckle and forms bright, dark spots on the surface (Briers et al., 2013; Draijer et al., 2009).

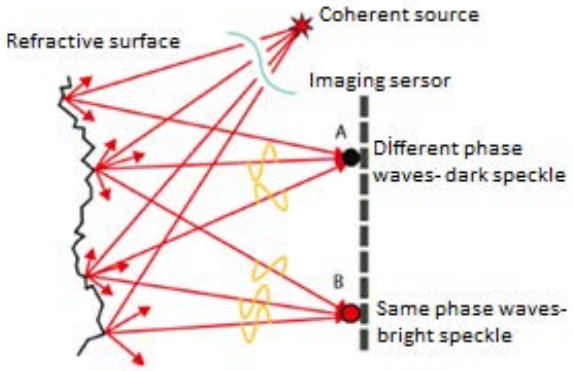



**Figure 1.** Formation of laser speckle.

By using an optic system for imaging of the scattered particles speckle pattern can be observed. Figure (2), show an example of laser speckle imaging. A similar image can be generated by scattering of laser from an opaque medium such as paper or plastic Briers et al., 2013.

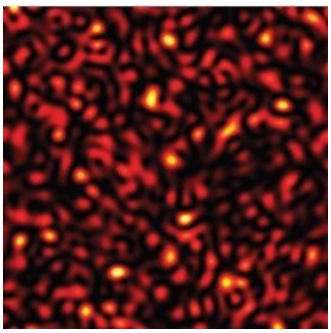


**Figure 2.** Laser speckle pattern.

If the microscopic details of the surface are unknown the only way to characterize the speckle structure is using statistical methods. The amplitude that occurs at an observation point O, as in Figure 3, is determined by the relative phases and amplitudes of each wave and takes values ranging from zero to maximum.

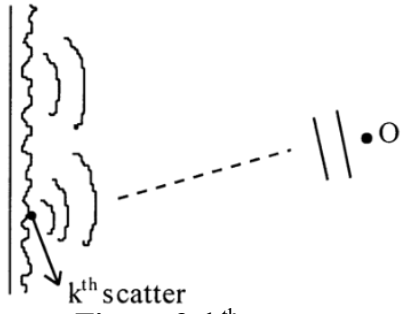



**Figure 3**. k$^{th}$ scatter

Resulted complex amplitude *A(x,y,z)* is given as:

$$A(x,y,z) = \sum_{k=1}^{N} |a_k|\, e^{i\phi_k} \qquad \text{(Equation 1)}$$

*N* is the number of scattered wavelets and $a_k$ and $\phi_k$ are the phase and amplitude factors created by the k$^{th}$ scattered wave, respectively. Intensity of the wawe is given as:

$$I(x,y,z) = |A(x,y,z)|^2 \qquad \text{(Equation 2)}$$

The brightness difference between bright and dark areas in an image is known as the contrast of the image. The ratio of standard deviation in speckle pattern (σ) to the mean intensity (<I>) gives the contrast parameter of the pattern.

$$K = \frac{\sigma}{\langle I \rangle} = \frac{\sqrt{\langle I^2 \rangle - \langle I \rangle^2}}{\frac{I_1 + I_2 + I_3 + \cdots I_N}{N}} \qquad \text{(Equation 3)}$$

If the particle on the observed surface is moving (like the red blood cells) speckle pattern fluctuates and speckles get blurred. This situation decreases speckle contrast. Speckle contrast value changes from 0 to 1 and provides information about the particle movements. 1 stands for the stationary condition and 0 stands for the fastest movement to blur all speckles (Özdemir, 2007; Leonard and Toal, 1998; Draijer et al., 2009; Boas and Dunn, 2010; Pahnvar, 2016; www.bu.edu/neurophotonics/).

High phase coherence of the incident light and the roughness of the surface increases the contrast. A smooth surface doesn't generate any speckle. If such surface was ideally present, contrast would be 0 since it would reflect all the light. If the scattering surface is completely random, C = 1 and a 'fully formed speckle structure' is obtained. For <0 <C <1, it is called the partially speckled speckle structure (Leonard and Toal, 1998; Özdemir, 2007; Khaksari, 2016).

### 1.2.Speckle Pattern That Changes Over Time

For small movements of a solid object, the speckle pattern moves as a whole, that is, the speckles remain related. For larger movements, the speckles become "unrelated" and the speckle pattern changes completely. This decorrelation also occurs when light is scattered from multiple individual moving diffusers, such as particles in a fluid. A single speckle appears to shine like a star. This condition is known as "speckle that changes over time". It was noticed that one of the most important potential applications of speckle fluctuations was first introduced by Stern (1975) when it originated from blood flow. (Briers et al., 2013).

When the particles are moving, the speckle pattern will change over time. When the intensity of the scattered light is measured temporally and at a point in space, it is possible to obtain information about the movement of particles inside the object. This is related to the measured signal between time $t_1$ and time $t_2$ of a signal and is calculated by correlation.

$$C = \frac{\sum_m \sum_n \left(I_{mn}(t_1) - \bar{I}(t_1)\right)\left(I_{mn}(t_2) - \bar{I}(t_2)\right)}{\sqrt{\left(\sum_m \sum_n \left(I_{mn}(t_1) - \bar{I}(t_1)\right)^2\right)\left(\sum_m \sum_n \left(I_{mn}(t_2) - \bar{I}(t_2)\right)^2\right)}} \qquad \text{(Equation 4)}$$

Here, $I_{mn}(t_1)$ and $I_{mn}(t_2)$ are the luminous intensity of the m and n positions for the speckle pattern at time $t_1$ and $t_2$. $\bar{I}$ indicates the average light intensity of the entire speckle pattern. Basically, the process here is to calculate the difference between the light intensity value in that pixel and the average light intensity of the whole image for each pixel location in two images taken at two different times (Draijer et al., 2009; Pahanvar, 2016).

### 1.3.Laser Speckle Imaging Method Applications

Because of the need of high resolution blood flow screening laser speckle contrast imaging (LSCI) method is widely used in biomedical screening. It is used for screening of bloodstreams in numerous tissues such as retina, skin and brain. These tissues are especially

suitable for LSCI method. Because in these tissues microvessel system is usually superficial. Due to the measurement geometry, LSCI cannot detect the flow of blood in deep tissues (Boas and Dunn, 2010). It is an important parameter that can be used to investigate neurological events such as stroke, cortical spreading depression, and functional activation in the brain. Laser speckle contrast imaging is used on animal models as a tool to better understand the neurological mechanisms behind these situations (https://foil.bme.utexas.edu/).

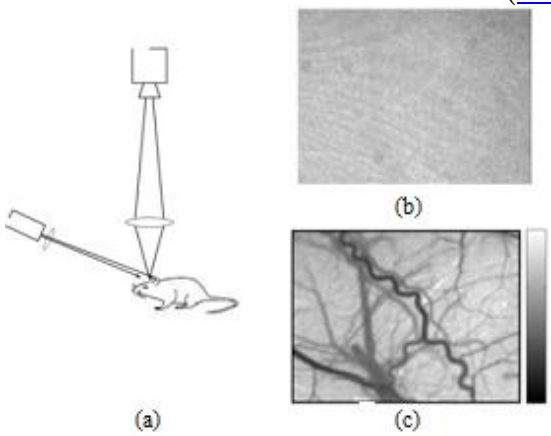



**Figure 4.** a) Basic laser speckle contrast imaging system set up. b) Laser speckle pattern c)

The image resulted from speckle contrast calculations provides much detailed information. Figure 4a shows a basic laser speckle contrast imaging system created with a laser diode and a camera to examine a rat's skull. The raw speckle image of the rat cortex contains little apparent amount of information. (Figure 4b). However, when the contrast of the speckle is calculated, there is a tremendous amount of information about the movement of the scattering particles in the sample (Figure 4c) (Dunn, 2012).

Other medical applications include microcirculation studies, dentistry, cardiovascular research, wound and burn assessments. Laser speckles are used in dentistry and oral surgery to visualize gum blood flow and to classify tooth decay at deeper location. (Heeman et al., 2019).

In addition to medical applications, the laser speckle method has applications in different areas. Some of the studies in these areas are summarized below:

Laser speckle method was used to evaluate seed viability and it was investigated how the water content in seeds affects biospeckle activity. Living and non-living seeds with different specific moisture levels were classified using the same technique (Braga et al., 2003).

Fruits, even hard-shelled ones, show a mottling activity that may be associated with maturity, turgor, damage, aging, and mechanical properties. Laser speckle method is used as a potential methodology for examining the effect on apples and analyzing the caries they produce (Pajuelo et al., 2003).

The laser speckle method has been shown to be an effective method for monitoring and measuring the biological activity of meat during the aging process, which demonstrates the potential for evaluating and predicting the quality of beef (Amaral et al., 2013).
For a self-powered robotic vehicle designed for use in Antarctic Regions, a precision speedometer has been developed using the laser speckle method (Aliverdiev et al., 2001).

## 2.METHOD

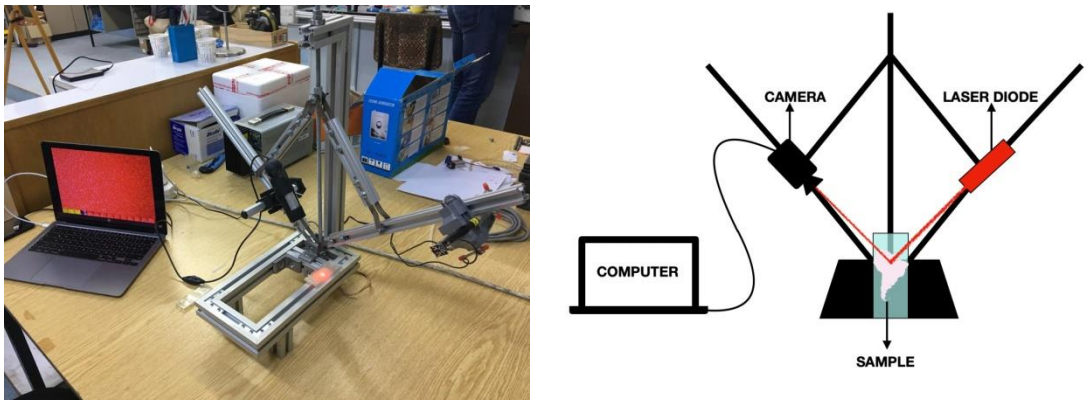
*Tools and Equipments:*

- ✓ 2.0 MP 1000X Optical Zoom Digital Microscope

- ✓ 650 nm Red Laser Diode
- ✓ Photodiode, Breadboard, 1MΩ resistor, 9 Volt Battery
- ✓ System consisting of Optical Rails
- ✓ Computer
- ✓ Oscilloscope
- ✓ Chicken meat

**2.1.Designing the Measurement System**

Laser light was sent on chicken meat to obtain a laser speckle pattern. The optical microscope camera was placed to see the light reflected from the chicken meat. The camera was connected to the computer and the speckle pattern formed by reflection was displayed on the chicken meat. (Figure 5).




(a) (b)

Figure 5. a) Measurement system b) Schematic representation of the measurement system.

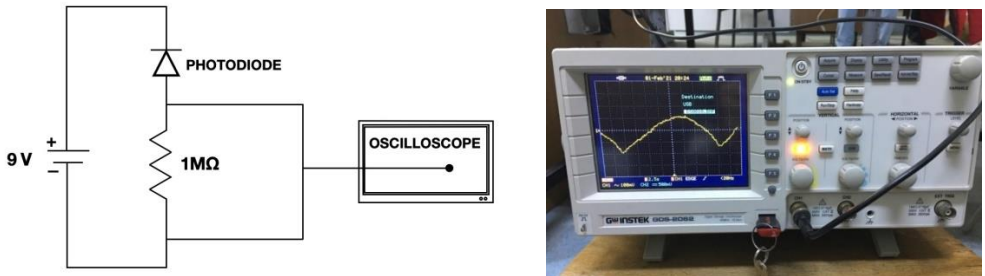
Laser speckle measurements were made using fresh chicken meat and stale chicken meat. When measuring with fresh chicken meat, fresh chicken breast meat was purchased from the market on the date of the experiments. Fresh chicken was kept in a hot and humid environment for 2 days to obtain staled chicken meat. It has been shown in many studies that a chicken kept in this way will be evaluated as staled due to bacterial growth (Saucier, 2016; Zhang et al., 2012; Rouger et al., 2017).

Before laser speckle images were taken, fresh and staled chicken breast meat was cut into slices approximately 2 mm thick, and then the samples were squeezed between two glasses to prevent movement and water drying. Thus, changes during evaporation and drying were prevented from affecting the laser speckle pattern. Laser speckle images of fresh and stale chicken meat samples were taken and recorded. The differences in the speckle patterns between the two samples and the change in the speckle patterns of the obtained images over time were examined.

**2.2.Development of a Measurement System Using Photodiodes**

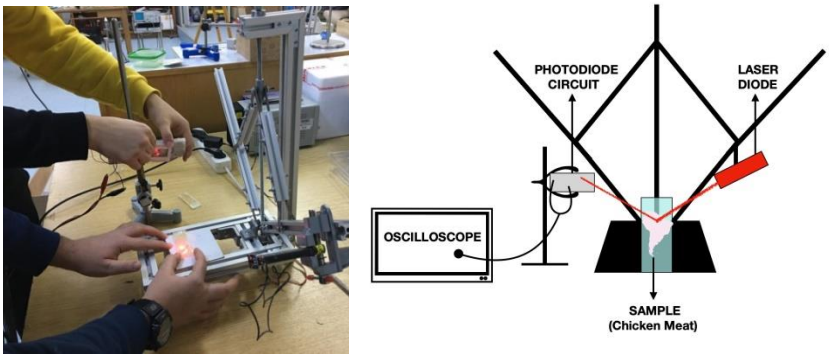
In the measurement system, when the camera is used, the number of photo frames taken by the camera per second is limited and around 24. In this case, changes that are every 1/24 second can be observed. The voltage changes of photodiodes in light can be in the order of nanoseconds. It will also be much easier to read a single photodiode voltage rather than reading the value of each pixel in matrix form like a camera. For this reason, a photodiode was used instead of a camera to observe the changes over time, faster and cheaper than the camera.

A photodiode circuit was formed by connecting a 1MΩ resistor, photodiode and 9V battery in series on the breadboard (Figure 6). Measurement was taken by connecting the ends of the resistance to the oscilloscope.




(a) (b)

**Figure 6.** a) Photodiode circuit, b) Oscilloscope




(a) (b)

**Figure 7.** a) Measurement system formed by photodiode and oscillocope b) Schematic representation of the measurement system.

## 3.FINDINGS

### 3.1.Laser Speckle Image Analysis for Fresh and Staled Chicken Meat Samples

In order to analyze the fresh and stale chicken meat, 6-second videos of laser speckle images were taken and these videos were divided into photo frames with the MATLAB program at 24 times per second.

Figure 13 shows the speckle images formed at t = 0, 3, 5 seconds for fresh and stale chicken meat. In order to see the changes more clearly, certain regions were selected in the speckle patterns and the change of that region over time was examined. As can be seen in Figure 8, when the laser beam is sent, the light reflected from tissues that do not contain living microorganisms exhibits static speckle patterns and do not change over time. However, the presence of living microorganisms dynamically disrupts the light paths in tissues, which causes the formation of contaminated patterns that change over time. Thus, the presence of viable microorganisms can be detected by analyzing dynamic speckle density patterns from meat products.

Fresh Staled

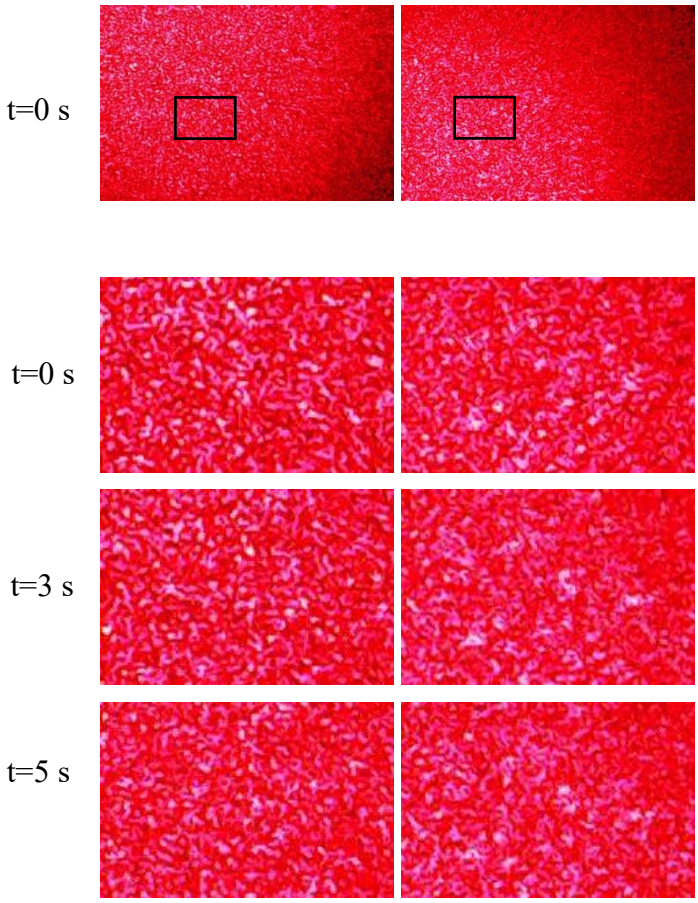



**Figure 8.** Speckle images formed at t = 0th, t = 3rd, t = 5th second for fresh and stale chicken.

Laser speckle images taken from fresh chicken meat and chicken meat kept in warm environment for 2 days were compared with 3 different methods. The first of these methods is the contrast parameter analysis. For the laser speckle patterns obtained from the recorded camera images, the contrast parameters given in equation (3) were calculated and the time dependent graph of the contrast parameters was drawn. Graphs obtained for fresh and staled chicken meat were drawn and compared.

Figure 9 shows the time dependent variation of contrast parameters for fresh and stale chicken. It is seen that the growth of microorganisms in the staled chicken meat causes an increase in the contrast parameter. With the spread of microorganisms on the surface, the reflection and scattering increase, the contrast parameter also increases. At the same time, short-term changes in the contrast parameter of the laser speckle are more. In order to examine the contrast change in sequent patterns, the points were determined and averaged on the graph, as shown by the red dots in Figure 9, and it was found that the contrast change was 0.003 in the staled chicken and 0.001 in the fresh chicken. This approximately 3-fold increase provides the difference between staled chicken and fresh chicken.

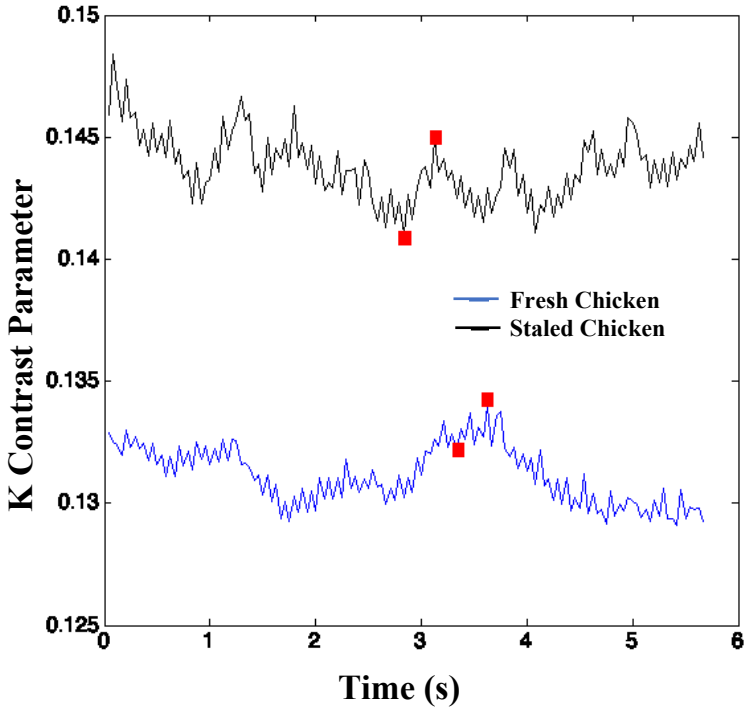



**Figure 9.** Time depending change of Contrast parameter for fresh and staled chicken meat.

As a second method, correlation calculation of speckle patterns was performed. When the particles are mobile, the speckle pattern changes over time. In order to examine the time-dependent change of the speckle pattern, by calculating the correlation connection given in equation 4, a graph of the change over time of the correlation function was drawn for fresh and staled meat. (Figure 10)

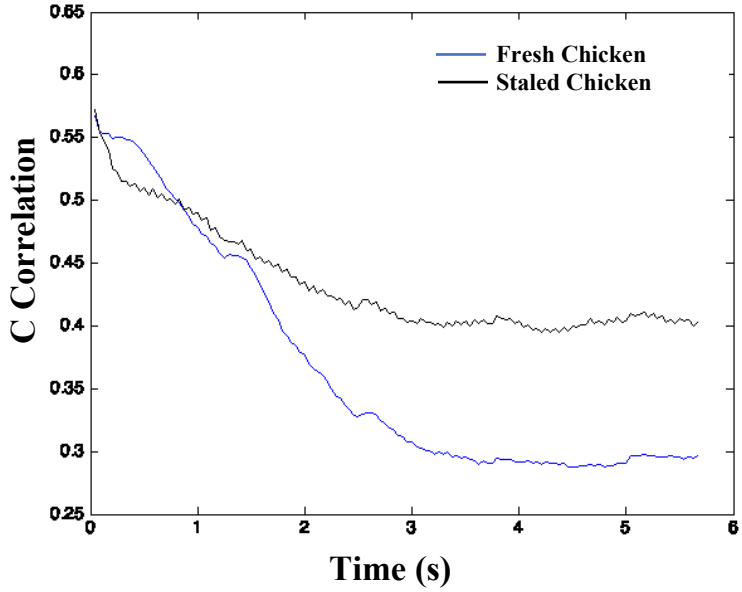



**Figure 10.** Time depending change of correlation function for fresh and staled chicken meat.

The correlation between the first pattern and subsequent patterns was examined. The main point to note here is the fluctuations in correlation, more important than the first pattern and correlation. For this reason, when the fluctuations in the correlation are examined, it is seen that there are more fluctuations in staled chicken than in fresh chicken. But when looking at

this fluctuation, it can be seen that it is less obvious than the contrast parameter. In order to obtain clearer results, it was decided to examine the correlation between the photo in each frame and the next photo. When we examine Figure 11, the correlation change in fresh chicken is maximum 0.002. In other words, the similarity rate of the two patterns is high. However, the variation between the two patterns in the staled chicken was measured as 0.07 maximum. The correlation seems to change in progress of time.

In staled meat, where living microorganisms reproduce, the correlation between speckle patterns decreases due to the random movements of microorganisms. Because fresh meat has a constant speckle pattern, it moves towards an almost constant correlation in a short time.

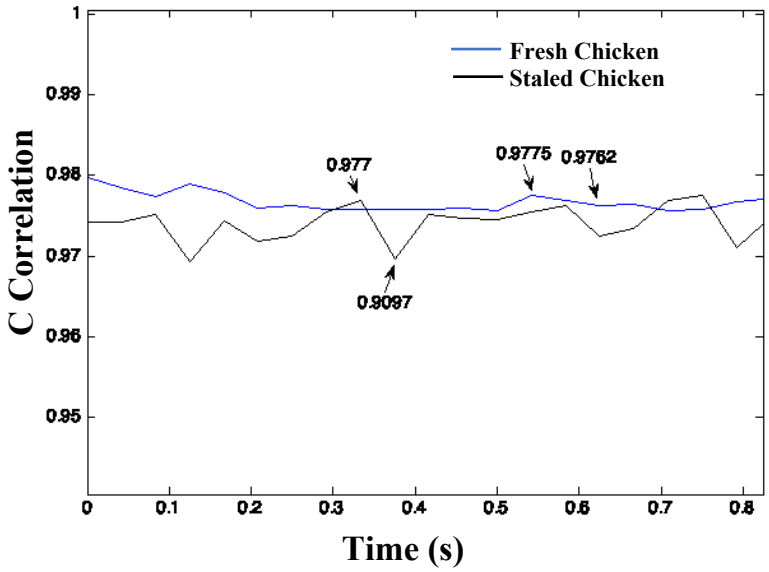



**Figure 11.** Time depending change of correlation between the first pattern and subsequent patterns for fresh and staled chicken meat.

### 3.2. Analysis Of Measurements Taken With Photodiode

In order to more precisely and simply determine the changes that occur in the staled chicken, the laser speckle pattern formed on the chicken meat was lowered to the photodiode. And the change in light intensity falling on the photodiode over time was analyzed using an oscilloscope. Changes in the laser speckle pattern of chicken meat caused by the movement of microorganisms were detected sensitively in the oscilloscope. The oscilloscope's speed is adjustable. Depending on the oscilloscope model, 100 million measurements can be taken per second. While taking a measurement, the oscilloscope was set up to record a measurement every 0.1 seconds.

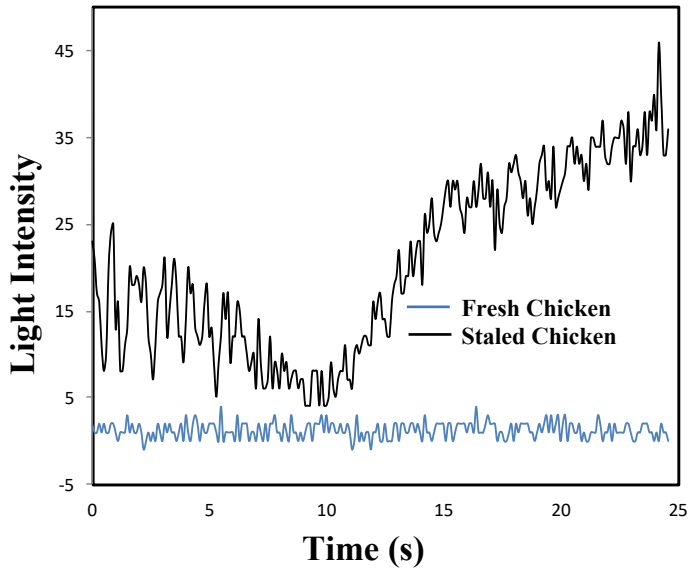



**Figure 12.** Time depending change of light intensity for fresh and staled chicken meat

As can be seen from Figure 12, the light intensity remains constant over time, since the speckle pattern falling on the photodiode in fresh chicken meat is constant. In staled chicken meat, the speckle pattern is mobile due to the movement of microorganisms. Because of this movement, the intensity of light falling on the photodiode changes in the course of time. The change in light intensity in the staled chicken over time demonstrates that the chicken begins to get contaminated and microorganisms reproduce on it. In this way, by analyzing laser speckle patterns, fresh chicken meat and staled meat can be distinguished from each other.

## 4.RESULTS AND DISCUSSION

In this project, a simple, non-destructive and fast optical method was proposed to detect living microorganisms that cause staled meat products, especially chicken meat, by using laser speckle imaging. By analyzing the static and dynamic speckle density patterns reflected from the samples and the change in correlation between the patterns over time, the following results were reached:

- ✓ A simple optic measurement system has been designed where we can transfer the laser speckle patterns on chicken meat to the camera and examine the changes in the patterns.
- ✓ A static speckle pattern formed on fresh chicken meat. A dynamic speckle pattern was observed on waiting chicken meat.
- ✓ It has been shown that the analysis of dynamic speckle patterns from staled chicken meat enables the detection of existing microorganisms.
- ✓ For the resulting speckle patterns, time depending change of the contrast parameter and correlation between the patterns were examined.
- ✓ An approximately 3-fold increase was observed in the contrast parameter for staled chicken meat compared to fresh chicken. This 3-fold increase enables the difference between staled chicken and fresh chicken to be understood.
- ✓ The change in the correlation between the patterns over time was examined in two different ways. First, the correlation between the first pattern and the next pattern was examined. Looking at the fluctuations in correlation, it was seen that there were more fluctuations in staled chicken than in fresh chicken. Second, the correlation between the photo in each frame and the next photo was examined. In fresh chicken, the similarity rate of the two patterns was observed to be high. In the staled chicken, the correlation was seen to change over time.

- ✓ In order to determine the changes occurring in the staled chicken more precisely and in a simple way, the measurement system was developed and the variation of the light intensity in the photodiode and speckle patterns over time was examined. In the measurements taken from fresh meat, it was observed that the light intensity remained constant by the time of progress, while it changed over time in the waiting chicken.
- ✓ The change of light intensity in the staled chicken over time proves that the chicken begins to contaminated and that microorganisms grow on it. Thus, fresh chicken meat and staled meat can be distinguished from each other by analyzing laser speckle patterns.

As summarized in Section 1.3, there are limited studies in the literature to examine foods by laser speckle method. In this project, unlike other studies, the system has been simplified by using photodiodes and examining the results, and it has been made much easier to apply in devices such as refrigerators at homes. The use of a photodiode instead of a camera increases applicability to many commercial devices. In addition, by using the laser speckle method in areas other than biomedical, it will contribute to the literature and is of great importance in the development of applications.

## 5.SUGGESTIONS

- ➢ Our improved system can be designed portable. Thus, it can be used in the home, food distribution industry and storage areas in markets.
- ➢ To make it portable, it can be made to be read directly from the computer instead of the oscilloscope. In this way, the system is planned to be developed.
- ➢ Once the system is developed, an academic study can be conducted on contaminated chicken meat by taking measurements on different types of chicken samples.

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An interesting and practical project. Time dependent change is a good idea. But there are questions in the data not explained. As an example, why the contrast measured by the staled meat is larger than fresh meat？ The fresh meat is supposed to be time independent, thus it should have a larger contrast value than the staled as discussed in page 4 of the report. Fig. 10 is also contrary to what is expected and the author noticed it and switched to analyze correlation in short time scale in Fig. 11 and then use oscilloscope to measure time dependence of intensity in Fig. 12. No reason or discussion for the reason of getting Fig. 10 and what is the short time scale from？ Also no explanation why light intensity is non monotonic function of time in Fig. 12 for staled chicken.