Chapter 10
Characterization of Bioengineered Tissues by Digital Holographic Vibrometry and 3D Shape Deep Learning

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Abstract One of the critical components of large-scale manufacturing of bioengineered tissues is the sensing of information for quality control and critical feedback of tissue growth. Modern sensors that measure mechanical qualities of tissues, however, are invasive and destructive. The goal of this project is to develop noninvasive methodologies to measure the mechanical properties of tissue engineering products. Our approach is to utilize acoustic waves to induce nanoscale level vibrations in the engineered tissues in which corresponding displacements are measured in full-field with quantitative optical techniques. A digital holographic system images the tissue's vibration at significant modes and provides the displacement patterns of the tissue. These data are used to train a supervised learning classifier with a goal of using the comparisons between the experimental vibrational modes and the ones obtained by finite element simulation to estimate the physical properties of the tissue. This methodology has the promise of mechanical properties that would allow technicians to noninvasively determine when samples are ready to be packaged, if their growth deviates from expected time frames, or if there are defects in the tissue. It is expected that this approach will streamline several components of the quality control and production process.

Keywords Digital holography · Tissue engineering · Vibration · Pattern recognition · 3D deep learning classification

10.1 Introduction

Mechanical properties are critical and often overlooked factors defining the quality of bioengineered tissues. Current tests used to measure the physical properties of engineered tissues, such as uniaxial tensile tests and nanoindentation, tend to be invasive and destructive and thus not applicable for in-process quality control. To measure a tissue destined for therapeutic applications, all devices must be aseptic and not damage the tissue.

The goal of this work is to develop a noncontact nondestructive method to measure the properties of bioengineered tissues during production. We use acoustic vibration and digital holography to measure the sample. The tissue this work examines is Apligraf, created by Organogenesis [1] and is a circular tissue consisting of an epidermal and dermal layer laying on a porous plastic membrane. Circular vibrations induced by noninvasive acoustic waves create natural modes as can be predicted with Bessel functions on a circular plate according to classical plate theory [2]. Holography operates by mathematically interpreting the interference between two beams of light, one of which reflects off the sample, in a digital sensor [3], thus operating noninvasively.

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10.2 Methods

Our methodology used three components to measure important information of the sample: optical coherence tomography (OCT), digital holographic vibrometry (DHV), and nanoindentation. All measurements and experiments were performed on samples of Apligraf generously donated by Organogenesis Inc.

First a sample was measured with OCT (Telesto SD-OCT, Thorlabs). A b-scan taken at multiple points was used to estimate the thickness of each layer of the Apligraf nondestructively.

DHV, the primary tool, uses a speaker and a holographic sensor (Fig. 10.1) to induce and measure vibrations. The sensor uses a 532 nm light to illuminate the sample while it is still in its packaging. A subwoofer placed above the sample outputs single-tone frequencies and sweeps between 70 Hz and 500 Hz. We identified vibrational modes of the 0th order and imaged the sample at 12 equidistant points within a single vibration curve by modifying the phase of camera strobing for image acquisition. This gave a full-field-of-view video with 12 frames that showed the nm-scale waveforms oscillating in the sample.

Samples were also measured with nanoindentation at 4 multiple random points with a 51 μm-radii probe on a 3.45 N/m stiffness cantilever (Chiaro, Optics 11). During indentation, the sample was covered by a lid with a small opening to minimize evaporation. The epidermis was measured and then peeled back with a scalpel and forceps, and the tissue's dermis was measured the same way. This was used to validate the modulus of the epidermal and dermal layers to compare to simulated results and to algorithm predictions.

To demonstrate the utility of the method, two experiments were performed. In the first experiment, a single sample was measured with nanoindentation and then with DHV. Four different speaker volumes were measured at each mode, measuring different pressures applied by the acoustic waves to the sample, to test for linearity of the tissue response.

For the second experiment, the sample was subjected to controlled drying to change the thickness and/or stiffness of the sample and measure the resulting change in frequency of vibrational modes over time. Due to the destructive nature of the drying, two different samples from the same production batch were used for nanoindentation and DHV. Each sample was dried for 90 minutes in a 23 °C oven (Isotemp, Fisher Scientific) for controlled humidity and temperature, and measured at several time points: 0, 3, 6, 10, 15, 30, 60, and 90 minutes. Each sample was put back into the oven with an open lid after measurement until the next time interval was reached.

To relate the mode shapes and frequencies of the complex system, we trained a machine learning algorithm with a finite element (FE) simulation in Ansys APDL. We developed a composite linear-elastic FE model with thin shell elements (Fig. 10.2b). The model includes three layers: the epidermis, dermis, and porous plastic membrane on which they sit. To emulate the triangular pattern of the Apligraf packaging, we applied fixed supports to 30 degree increments evenly spaced

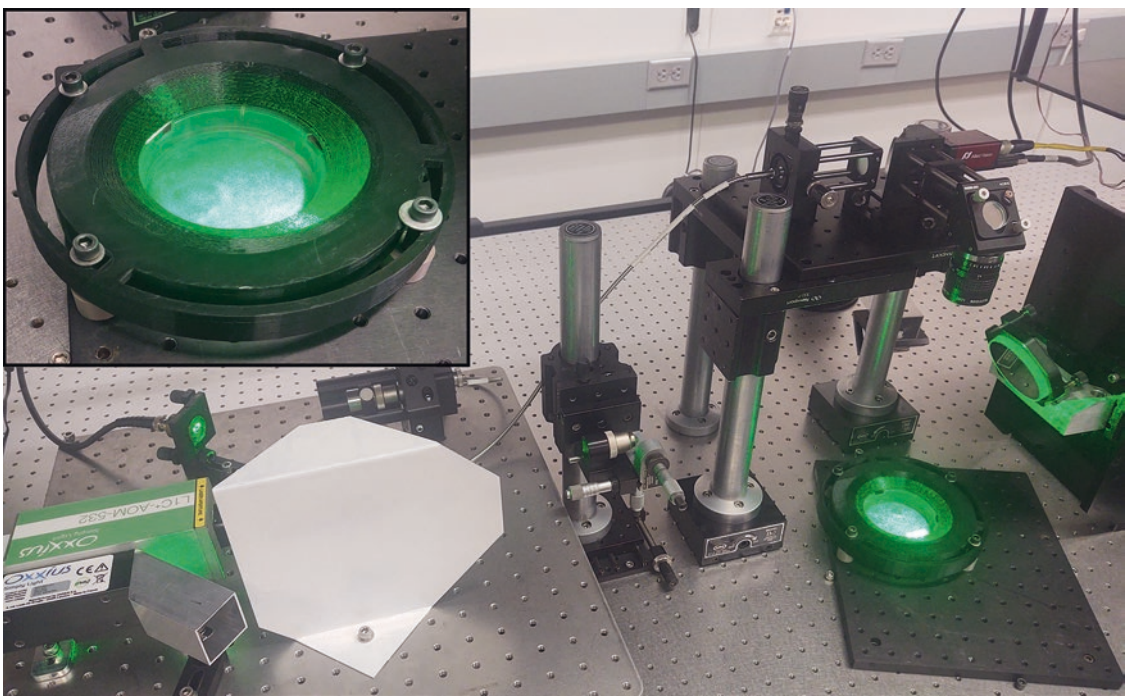

Fig. 10.1 DHV sensor with inset of tissue during measurement [4]

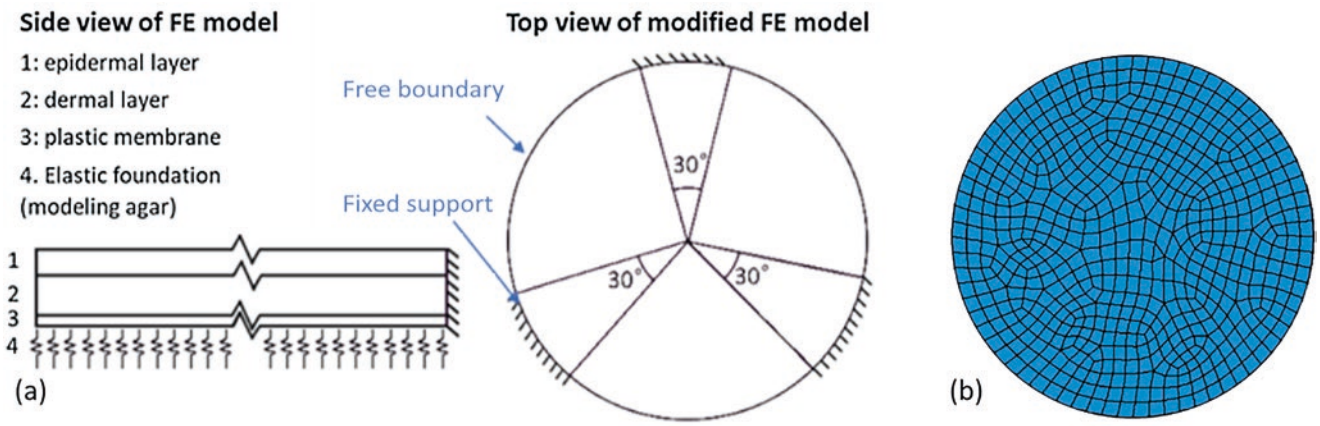

Fig. 10.2 (**a**) A schematic of the model used in the FE simulation (**b**) the mesh pattern of the FE model [4]

around the edge (Fig. 10.2a). We then added an elastic support underneath the sample to emulate the agar upon which the system sat.

We performed a parametric modal analysis, the stiffness of dermis and epidermis were modified in the range of 10–60 and 3–20, and the thickness was in the range of 50–200 for both layers. For this model, Poisson's ratio and density were assumed to hold constant at 0.48 and 997 kg/m^3 respectively for both layers of tissue. This model outputs the mode shapes and frequencies of several modes for each combination of stiffness and thickness values.

A 3D shape classification supervised learning model was built by leveraging Pointnet deep learning architecture [5]. Specifically, the model was trained on the point cloud dataset from the FE simulation to classify the stiffness and thickness of the epidermis and dermis, where each data point was organized as a separate simulation file. Only 0th order modes, such as shown in Fig. 10.4, were selected by finding all modes with triangular symmetry defined by three partial cross sections (Fig. 10.3b). In total, a dataset that included 1440 simulation files was selected, and randomly split by the ratio of 3:1 into the training and testing subsets. We treated the 1763 nodes in the mesh files as point cloud images, with x,y, and z coordinate values of each point as the representative features. The frequencies of FE stimulation were extracted for the corresponding 0th mode. Each property was encoded as either 0 or 1 if the stiffness or thickness value was less or no less than the medium value of all simulation files, respectively.

In our supervised learning model, the Pointnet architecture was modified, by adjusting the hidden layer nodes to better fit the FE simulation data (Fig. 10.4). The input transformer network (T-net) linked the input layer with the first primary multilayer perceptron (MLP), and the feature transformer network linked two primary MLPs together. The global feature was generated by maxpooling the second MLP output, and we concatenated the input frequencies as an additional feature. The overall 257 features were processed by the final MLP to generate the output class.

10.3 Results

For the first time point of the drying experiment, the fundamental frequency was identified when the speaker played a 149 Hz tone. Inputting this into classical plate theory equations for a thin round plate, we calculated a stiffness of 1.1 GPa. Meanwhile, the nanoindentation measurements indicate a modulus around 36.5 ± 26.2 kPa for the epidermis of the tissue.

In the variable pressure experiment, the maximum positive displacement measured at the first four vibration modes for four different acoustic forces applied by the speaker shows roughly linear relationships (Fig. 10.5), indicating that the tissue is within the linear region at these low displacements. For these analyses, the largest displacement is taken from the point in the 12-image set of measurements that reflects the top of the sinusoidal curve. Taking the steepest and flattest slope indicated in Fig. 10.2 and converting to strain using a thickness of 400 μm, we calculate a stiffness of 1.3 kPa or 570 Pa.

In the second experiment, OCT measurements of thickness for both epidermal and dermal layers of the Apligraf tissue decreased with drying time (Fig. 10.6), thus establishing a change to the tissue and physical properties for the DHV sensor to measure.

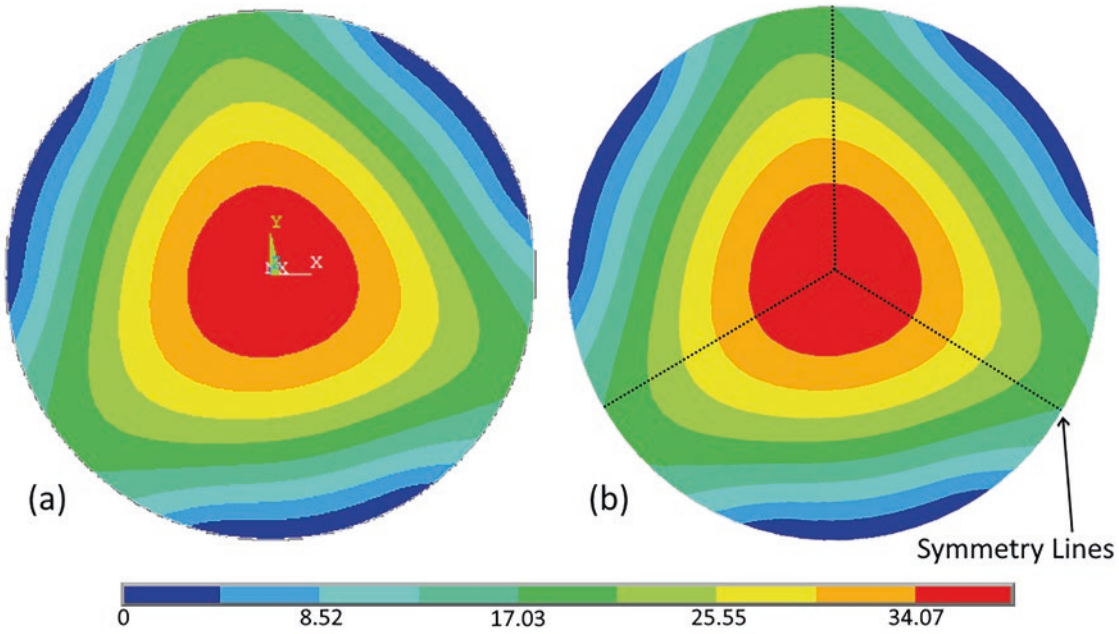

Fig. 10.3 Fundamental mode shapes calculated by the FE simulation of Apligraf tissue (**a**) an example of a fundamental mode output with relative z-displacements (**b**) symmetry lines overlaid onto a fundamental mode image

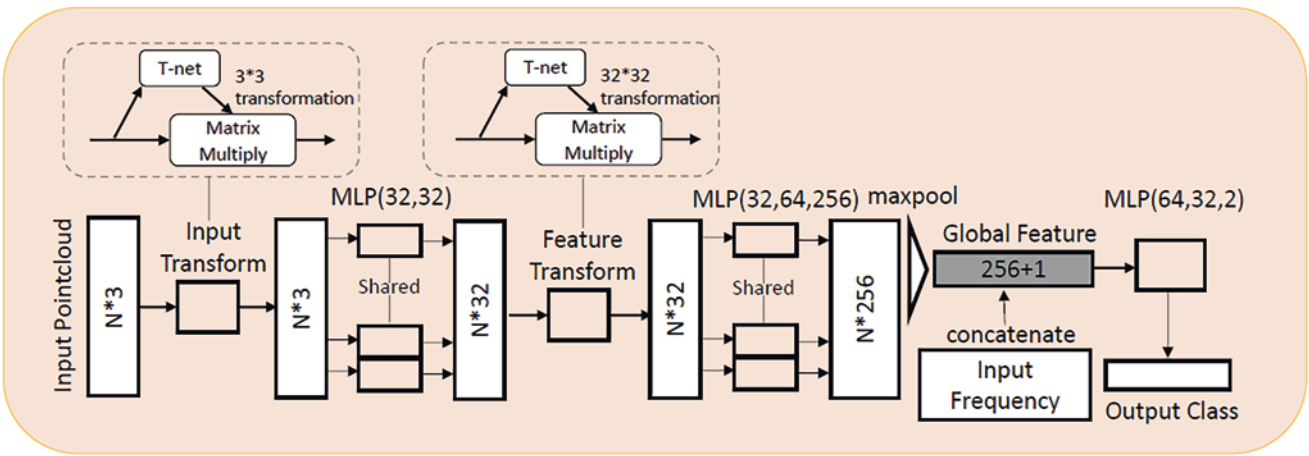

Fig. 10.4 Architecture of the Pointnet-based 3D classification model combined with frequency input. The Pointnet based classification network for FE simulation pointcloud takes 1763 nodes as input N, and x,y,z coordinate values as the representative features. Input and feature transformations are linked between two primary MLPs. Input frequencies were concatenated as an additional feature to the maxpool global features for the last mlp. The output was densed by softmax as binary classification

The frequencies at which each of the first four modes of vibration occurs decrease monotonically with drying (Fig. 10.7a). The modal FEA in which the layer thickness was decreased to match the measured data shows the opposite trend in frequency of the fundamental mode (Fig. 10.7b). This result indicates that it is more likely that the increase on modulus with drying dominates the vibratory behavior.

The 3D deep learning classification method presented the ability to predict the modulus of the epidermis based off of the spatial patterns in the FE simulation. Prediction accuracy of the thickness was 0.7101 for the epidermis and 0.7731 for the dermis, while prediction accuracy of the stiffness was 0.7668 for the epidermis and 0.4937 for the dermis.

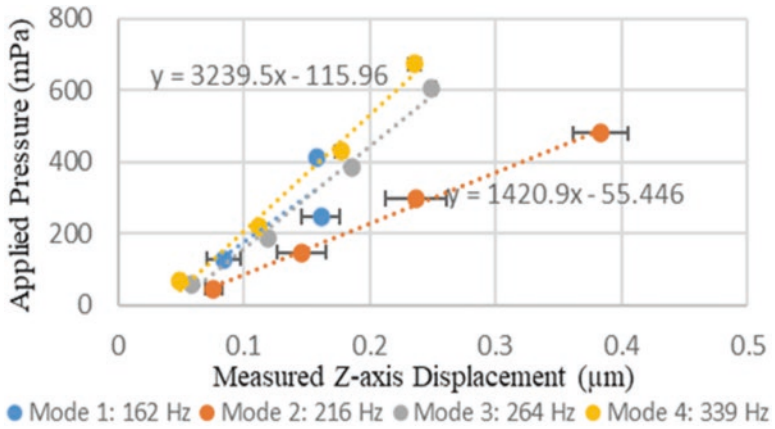

Fig. 10.5 Amplitude of tissue displacement for multiple applied pressures during vibration [4]

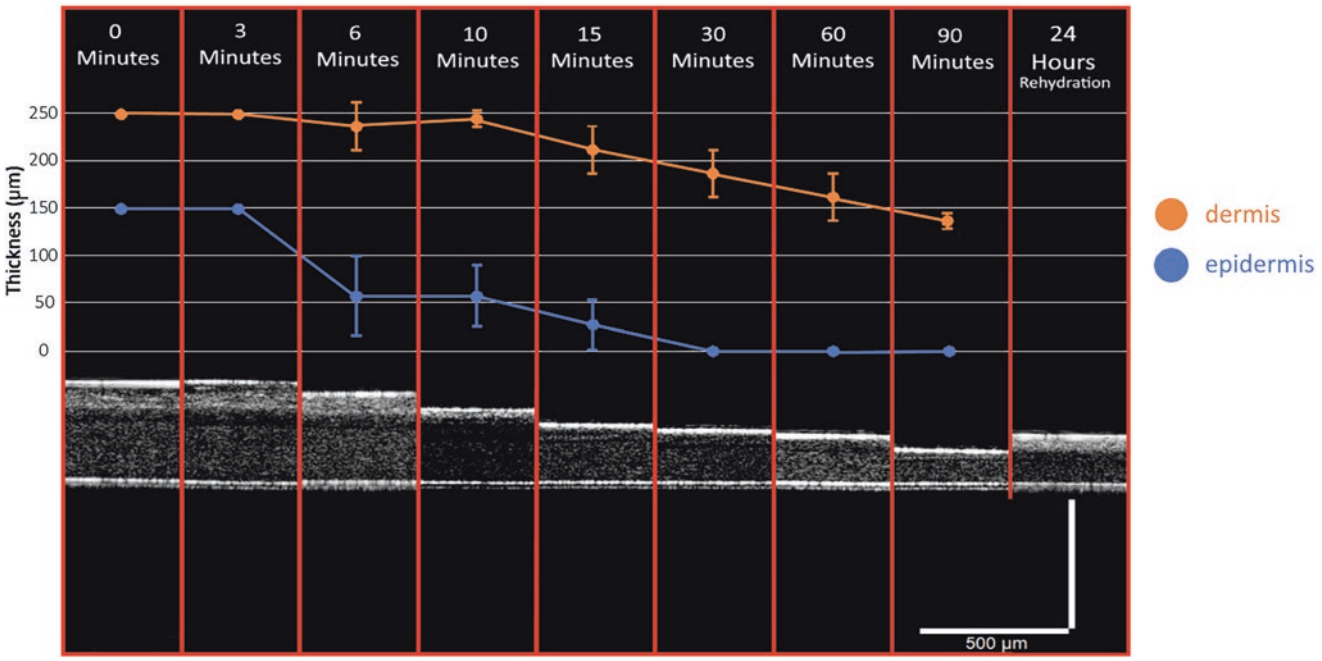

Fig. 10.6 Decrease of tissue thickness during drying. OCT images show the thickness, while a numerical of the epidermis (blue) and dermis (orange) thickness are overlaid [4]

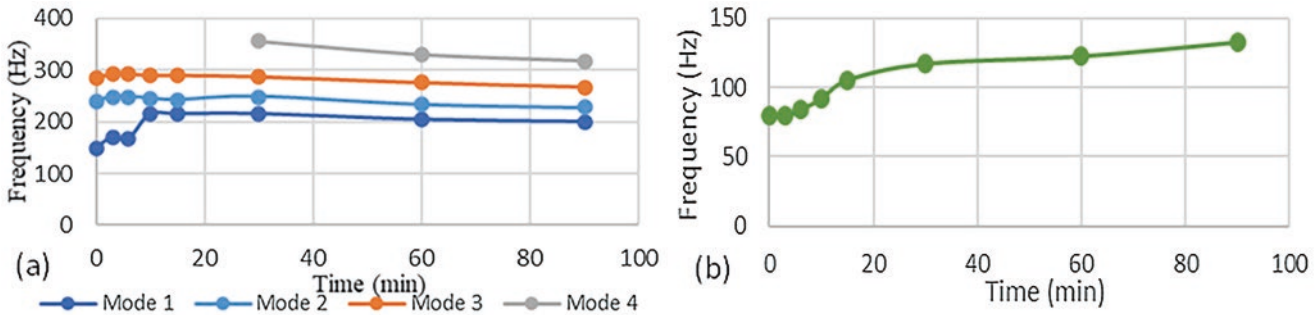

Fig. 10.7 (**a**) Frequency location for each mode during drying (**b**) Expected frequency of vibration from FEM [4]

10.4 Discussion

This study demonstrates the ability to measure the full-field surface displacements of engineered skin tissue nondestructively and aseptically within its packaging using stroboscopic holographic vibrometry. By utilizing multiple acoustic pressures, the linearity of the tissue behavior at such low applied pressures was confirmed. Drying the tissue demonstrated the ability of the system to detect changes in the physical properties. After an initial increase in the measured frequencies, a consistent decrease in the modes' frequencies was found as the tissue thinned. This contrasted expected frequency change from the FE simulation. We believe this is due to the number of factors that change. The FE simulation accounted for only thickness change, while the real tissue thinned by losing water, thus altering its stiffness, density, and possibly even Poisson's ratio.

Due to the complexity of the layered tissue, analytical methods and vibrometry measurements alone cannot currently extract precise mechanical properties of the Apligraf layers. Multiple attempts to calculate the stiffness of the system using different methods resulted in estimates of 1.1 GPa or 1.3 kPa, which were both orders of magnitude of the 36 kPa nanoindentation measurement.

The application of the Pointnet-based deep neural network can identify certain mechanical properties when fed an image from the FE simulation. Concatenating the frequency with the mode shape helps increase the accuracy slightly than using the shape alone. It shows less accuracy on dermis stiffness compared with other properties, which can be explained that the 3D shape of FE simulation is less sensitive toward the change of dermis stiffness. The next step after this is to fine-tune the model with experimental data. For several tissue samples, we have collected stiffness with nanoindentation, thickness with OCT, and modes with DHV. We will re-evaluate the fine-tuned model and use it to identify which features can be accurately predicted, and what pieces of information, such as what nodes will not affect the prediction accuracy, to determine whether they need to be collected.

Once completed, this noninvasive measurement technique and associated analysis method would likely take around 5 minutes allowing for in-line measurement during production. This QC assay has the potential to enable identification of faulty batches before the full growth time is reached, increasing efficiency of manufacturing.

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