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# Leveraging Computer Vision for Quantitative Analysis of Stained Organoid Images to Evaluate Glioblastoma Drug Efficacy

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## Abstract:

Glioblastoma, also referred to as a grade IV astrocytoma, is a fast-growing and aggressive brain tumor. Despite years of research, the life expectancy after diagnosis with treatment remains at 14 to 16 months. When cancer recurs, which is not uncommon, no treatment options are available for patients. Organoid culture has slowly replaced traditional cell culture in chemotherapeutic drug testing for various cancers. Characterization of organoids is done with immunohistochemistry analysis, and viability in drug studies is corroborated with live/dead assays. These imaging studies are primarily qualitative.

This research study proposes enhancing this qualitative analysis with quantitative insights from the Computer Vision(CV) principles of Artificial Intelligence(AI). The proposed algorithm automates the processing of any set of organoid images to identify and numerically count stained cells, extracting objective viability percentages from visual data. This research showcases the potential of Computer Vision engineering to accelerate and improve Glioblastoma research by supplementing qualitative observations with rigorous computational metrics. Integrating these quantification methods with traditional visual evaluation could lead to more consistent, unbiased, and insightful drug testing, potentially expediting Glioblastoma therapy discovery.

*Keywords* —Artificial Intelligence, Computer Vision, Computer Science, Bio-engineering, Brain Tumor, Clinical Trials

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## I. INTRODUCTION

Glioblastoma (GBM) is a malignant tumor arising from astrocytes, supportive cells in the human brain. It invades the nearby brain tissue but generally does not spread to distant organs. Astrocytes are the most abundant cells in the brain that maintain critical physiological functions in the brain and play a supportive role to the neurons. GBM remains incurable despite years of research, and the tumor recurs invariably, and when it does, there are currently no treatment options. Treatment for GBM has remained unchanged in the last twenty years. The current standard care for Glioblastoma is maximal surgical resection followed by concurrent temozolomide (a type of chemotherapy) or in combination with radiotherapy. When there is a recurrence, no standard care is established as of now, with treatment alternatives including surgery, systemic therapies, and multimodal therapy with limited success. This limited success of therapy with an almost assured recurrence can be attributed to the aggressive infiltrative nature of GBM and the intratumor heterogeneity observed within a tumor.

  

Another reason that can be attributed to Glioblastoma remaining incurable is that there are no model systems that can faithfully recapitulate the tumor microenvironment with the heterogeneous cell population. Glioblastoma has tumor cells that are genetically and phenotypically different between patients and within a patient's tumor. A model that can faithfully recreate the tumor in vitro would provide invaluable research insights to improve the available treatment options.

## II. ORGANOID CULTURE

Organoid culture has exploded in the last decade, slowly replacing traditional 2D cultures and animal models. Traditional 2D culture has answered some of the basic biology questions in Glioblastoma but fails to provide accurate drug results. Traditional 2D cultures do not mimic the in vivo conditions of the disease in the human body. Animal models offer the 3D microenvironment to the tumor cells, but it is physiologically different from human physiology. Animal models have also been very expensive, time-consuming, and labor-intensive to fabricate. Organoid culture, where the tumor cells are cultured in natural or synthetic hydrogel, fills the gaps between the 2D culture and animal models.

Organoids are then employed in drug testing and the viability of the organoids is assessed using assays that can quantitate the Adenosine Triphosphate (ATP) of the cells in the organoids. Quantifying ATP cells helps assessrapidly dividing cells' metabolic health and vitality. ATP assays can be measured using different methods, including colorimetric, bioluminescent, and fluorescent assays.

### *A. RESEARCH APPROACH*

ATP bioluminescence is the most employed method of measuring ATP in the cells. The ATP bioluminescence assay exposes a sample of cells to the enzyme luciferase and the substrate luciferin. Only living cells in the sample will produce a bioluminescent signal. While ATP assay provides quantitative viability data, the live/dead assay generates descriptive images. Extracting quantifiable cell counts from these images through Computer Vision could complement the ATP assay with visual correlates.

Automated image analysis would add spatial understanding and rapid corroboration to the luminescence data. In summary, combining orthogonal quantitative ATP measures with computational quantification of live/dead images could provide a more complete and efficient assessment of glioblastoma drug effects.

Our research approach uses OpenCV algorithms to process confocal images of live/dead stained organoids. HSV masking segments-stained cells based on hue. Thresholding and contour mapping enable cell counting to determine viability ratios. This automated pipeline mimics and enhances human image assessment to quantify treatment effects objectively.

Experiments demonstrated consistent viability quantification between the algorithm and manual counting. The computer vision technique achieved accurate, reproducible cell segmentation and enumeration across treatment groups. Our automated analysis also matched ATP luminescence assays in predicting the most effective drug by indicating the lowest viable cell ratio. This validates the algorithm's capacity to evaluate treatment efficacy rapidly.

This research successfully integrates Artificial intelligence-based Computer Vision and Machine learning to extract quantitative insights from qualitative organoid images. Automating the laborious manual image analysis removes subjectivity and boosts throughput compared to current glioblastoma drug screening. This AI-based methodology could accelerate the discovery of novel glioblastoma therapies.

## III. OBJECTIVE

The main objective of this research is to create an algorithm that can automatically count and differentiate between dead and live cells in the maximum projection of confocal images of Organoids. Such an algorithm should analyse the

  

projected 2D images captured through confocal microscopy and accurately detect and quantify the stained dead cells and live cells based on their distinct colors.

Developing this algorithm will enable rapid, objective quantification of cell viability within organoid samples. In addition, automating such analysis with software programs will improve throughput for assessing treatment efficacy and drug screening compared to slower manual counting. The algorithm proposed in this paper aims to leverage AI computer vision techniques to reliably segment and enumerate dead and live cells within organoid confocal projections.

## IV. METHOD

Organoids are employed in drug testing, and the organoids' viability is assessed using assays that can quantify the adenosine triphosphate (ATP) of the cells in the organoids. ATP is a molecule in the cells that helps in vital biochemical functions of the cells and is an important energy source to the cells. When cells die, they stop producing ATP, and the remaining ATP is quickly degraded in dead cells. Thus, ATP is an indicator of biologically viable cells.

Temozolomide is the first choice of drug for glioblastoma patients. Temozolomide causes tumor cell death by alkylating deoxyribonucleic acid (DNA). P53 activator has not been approved yet for glioblastoma patients. Glioblastoma tumors widely have mutated P53, which is a tumor suppressor gene and this drug activates the tumor suppressor gene P53 and causes cancer cell death.

Quantifying ATP helps in assessing the metabolic health and vitality of rapidly dividing cells. ATP assays can be measured using different methods including colorimetric, bioluminescent, and fluorescent assays. ATP bioluminescence is the most employed method of measuring ATP in the cells. The ATP bioluminescence assay exposes a sample of cells to the enzyme luciferase and the substrate luciferin. Only living cells in the sample will produce a bioluminescent signal. The amount of light emitted is directly proportional to the amount of ATP. The cells in the organoid are provided with the enzyme luciferase and substrate luciferin. The luciferase reaction needs ATP in the cells to work and hence only live cells will produce luciferin which in turn produces a bioluminescent signal that can be measured with the luminometer.

The live/dead assay serves as a qualitative method for evaluating cell viability based onesterase activity and plasma membrane functionality. This assay comprises two components: ethidium dibromide, integrated with RFP protein to impart a red stain to cells, and calcein AM, combined with GFP protein to provide a green stain to cells. When these components are introduced to the organoids, ethidium dibromide can penetrate the nuclei of deceased cells due to their non-functional and deteriorated plasma membranes. Consequently, it results in staining the cell nuclei. On the other hand, Calcein AM relies on esterase present in the live cell's membrane to be enzymatically cleaved, releasing GFP protein that stains the cell membrane in green. The organoids are subsequently imaged using a confocal microscope, capturing Z-stacks of both fluorescent proteins in each stack, which are then superimposed to generate a maximum projection image. There is significant value in extracting quantitative data from these qualitative images and cross-referencing them with the ATP assay results for the same treatment groups.

## V. COMPUTER VISION

Computer Vision harnesses the capabilities of artificial intelligence to instruct computers in interpreting and comprehending visual data. Within the realm of computer vision, color detection holds paramount significance, finding extensive utility in domains such as image processing, robotics, and industrial automation. It entails the development of algorithms geared towards the recognition, analysis, and comprehension of colors present in digital images and videos.

This research paper introduces the concept of employing computer vision for the automated identification and quantification of stained glioblastoma cells as a means to assess the efficacy of drug treatments. The proposed methodology involves classifying live and dead cells based on the distinct stain colors they exhibit while also ensuring

  

an accurate tally of these cells to determine population ratios.

This approach facilitates a swift and quantitative evaluation of treatment impacts on cell viability, ultimately expediting the analysis of glioblastoma drug effectiveness. Such automation in glioblastoma drug testing holds promise for enhancing the development of more efficient therapeutic strategies. The images were obtained by staining the organoids with live/dead assay and imaging them in a Nikon AR1 confocal microscope.

Using OpenCV, a popular Computer Vision library, the confocal images were first converted to HSV color space to facilitate recognition of the different cell stain colors. Masks isolate live and dead cells based on their distinct hues in HSV space. Additional filtering further separated stained cells from the background.

The masked HSV images were then converted to grayscale, and contours were drawn around clustered cells. Contour counting quantified total live and dead cell groups. NumPy thresholding functions set intensity values corresponding to each stain color. Pixels above or below the thresholds were tallied to enumerate individual live and dead cells per clustered contour. Tuning the thresholds improved sensitivity to identify and count more cells despite noise accurately.

In summary, OpenCV enabled efficient HSV masking, grayscale conversion, and contour mapping and utilized NumPy-based thresholding to segment and count distinctly stained live and dead cells automatically.

Such a software-based automated computer vision approach applied on multiple image data sets achieved rapid quantification, consistent with results from assays.

## VI. FLOWCHART

Here are the key steps involved in the proposed algorithm using OpenCV for image processing:

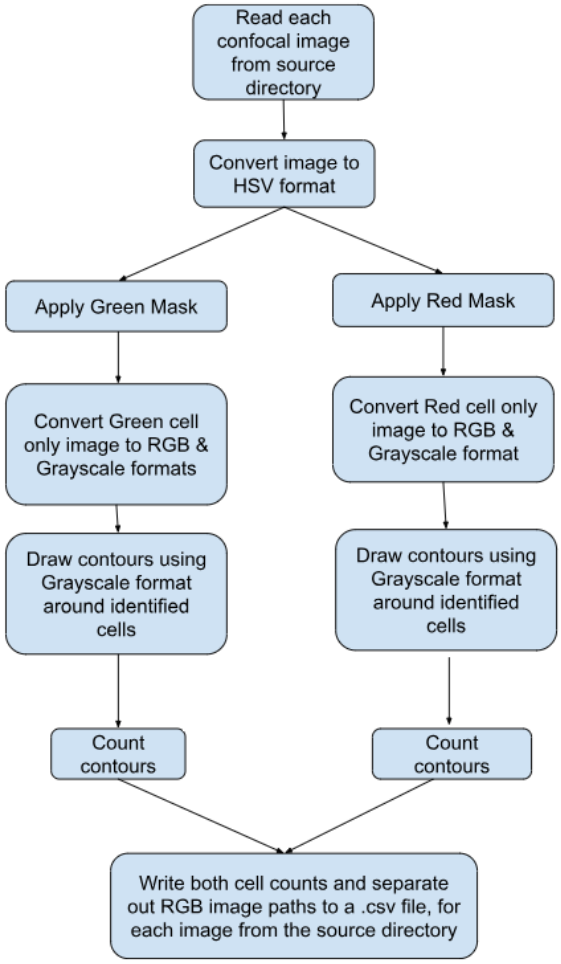


Figure 1: Flow chart describing the steps followed in the ML program

## VII. SOFTWARE USED

### *B. OpenCV*

OpenCV (Python-based Computer Vision Library) is a popular open-source Computer Vision and machine learning software library. It was built to provide a common infrastructure for computer vision applications and accelerate machine perception use. The library is cross-platform and free for use under the open-source BSD license.

OpenCV has a comprehensive set of both classic and state-of-the-art computer vision algorithms. These include facial recognition, object detection, motion tracking, and segmentation. The algorithms can process images and videos to identify objects, faces, and shapes. OpenCV also supports

  

computational photography, augmented reality, and stitching images together to produce a high-resolution image.

A key feature of OpenCV is its real-time performance. The library integrates with programming languages like Python, C++, and Java. It can take advantage of multi-core processing and GPU hardware acceleration. For this research purpose, we used the OpenCV Python library to detect color and draw contours to count the live and dead cells.

## VIII. RESULTS

Optimized hue thresholds combined with contour mapping and counting allow precise segmentation and quantification of the distinctly colored live and dead cells amidst complex image backgrounds. The quantification relates to the qualitative observation of the images in that temozolomide at 1mM causes very few viable cells to be present in the organoids. The 500µM temozolomide group organoids also had more green cells and red cells, which correlates with the huge error bar in the viability assay in that this specific drug concentration was only effective in certain organoids and not all organoids.

The live/dead quantification also correlates with the ATP assay in that it predicts the least number of green cells and the greatest number of dead cells in the P53 100µM treatment group, which was the least viable in the drug treatment group. The limitation of the study was that only n of 1 image was provided for the treatment groups. Increasing the number of organoid images analyzed per treatment group can improve the overall accuracy and robustness of the computer vision algorithm's viability quantification. Once the accuracy is improved, the algorithm's capabilities could also be extended to quantify protein expression levels in immunohistochemistry images of organoids.

In Figure 2, the second panel is the mask of the green cells, and the third panel is the mask of the red cells.

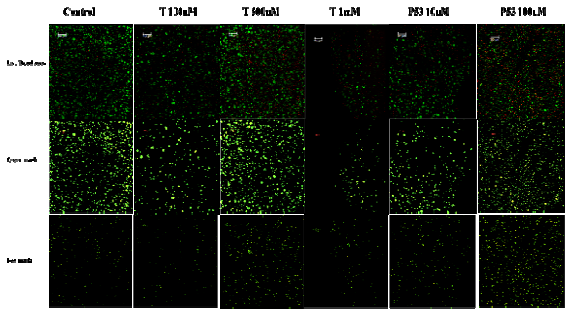

Figure 2: Represents the live/dead image of the organoids imaged in a confocal microscope

Figure 3, representsthe TP quantification of the organoids in different treatment groups:

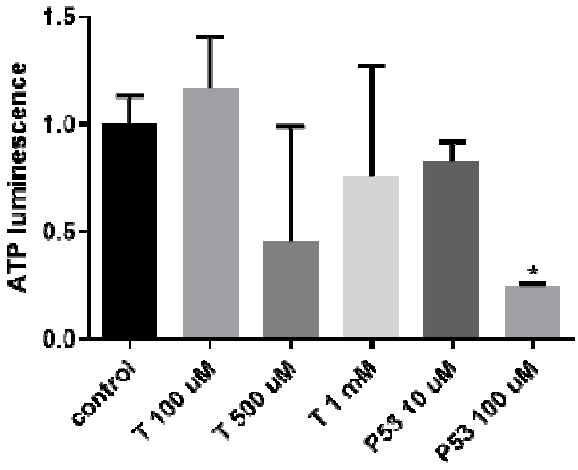


Figure 3: ATP quantification of the organoids in different drug groups

In Figure 4, the chart represents the result with respect to live-dead cellsacross different experiments.

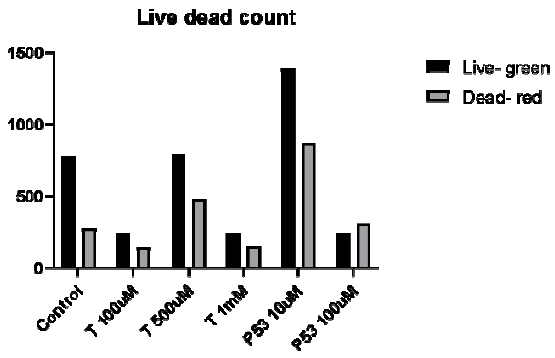


Figure 4: Live/dead cell quantification of the same organoids

  

## IX. FUTURE ENHANCEMENTS

The limitation of this research study was - only n of 1 image was provided for the treatment groups. Increasing the number of organoid images analysed per treatment group can improve the overall accuracy and robustness of the computer vision algorithm's viability quantification.

Future researchers can apply Convoluted Neural Networks (CNN) for Cell Segmentation: The results from this research show the promise of using Convolutional neural network-based advanced machine learning techniques, such as the U-Net CNN architecture, to achieve precise cell segmentation. U-Net employs an encoder-decoder structure with skip connections, enabling pixel-wise segmentation.

In addition, researchers can apply additional Noise Reduction with Filters: To reduce image noise, the paper recommends using median and Gaussian filters. The median filter effectively removes salt-and-pepper noise while preserving edges, while the Gaussian filter smoothens images by suppressing high-frequency noise. These filters can be combined with OpenCV denoising methods for further noise reduction.

## X. CONCLUSION

The automated quantification of live and dead cells using computer vision closely aligns with the outcomes of viability assays when predicting the treatment group with the least number of viable cells. In both the computer vision method and conventional assays, the same drug or combination consistently identifies as exerting the most potent cytotoxic impact on the glioblastoma organoids, as evidenced by the minimal live cell ratios.

This substantiates that the proposed automated image analysis methodology can swiftly and accurately gauge cell viability, demonstrating a level of accuracy akin to well-established quantification techniques. The consistent predictions affirm the efficiency and reliability of the computer vision approach as a viable alternative for assessing the efficacy of glioblastoma treatments.

In summary, by enhancing the precision of color detection, refining cell segmentation masks, and reducing image noise, the computer vision algorithm for automated glioblastoma cell analysis can be significantly improved in terms of accuracy and robustness.

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