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Implementation of the DW-MRI Calibrated with the Histological Data to Estimate the Tumor Cell Load and Heterogeneity

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Abstract
Diffusion-Weighted Imaging (DWI) or Diffusion-Weighted Magnetic Resonance Imaging (DW-MRI) is a key non-intrusive imaging procedure for the analysis of cancer and tumor treatment evaluation, based on Brownian movement of water molecules in tumor tissue. The formation of tumor makes the cells very tightly packed blocking the movement of cells in the molecule. Thus the tumor tissues compared to normal healthy tissues produce signals with less attenuation on isotropic maps. However, there is no possible evidence stating the relation between the Diffusion Weighted Data and the densities of cells.

To link the clinical cross-sectional data of low-resolution with the histological data of high-resolution, we have invented a digital image processing algithm, in order to assess the relation between the Diffusion Coefficient values (D value) and tumor cellularity obtained from Diffusion Weighted MRI Scan Images of Large cell carcinoma of the lung and the Endometrial papillary serous carcinoma (EPSC). Color de-convolution was performed followed by cell nuclei classification and division on the obtained histology pictures to examine the neighborhood and cell-type explicit densities.

In rundown, our outcomes show that tumor cell check i.e., number of cells influenced and non-influenced from disease and heterogeneity of the cells can be anticipated from DWI imaging information, which may open new gates to refine and manage the patient-explicit improvement of the treatment methods, customized analysis and treatment enhancement.

Keywords: DWI, Image Analysis, Image Processing, Brownian Movement, Histological Data, Histopathology

Introduction

Cancer now-a-days is one of the main sources of mortality around the globe. In the clinical treatment stages of the cancer cells growth and reduction, the histopathology of tumor models gives the premise for determination and treatment scheduling. Nonetheless, biopsies and pre clinical path reflect just the extremely restricted tumor zones, which might be of incapacitated significance concerning the vast majority of principal tumor load and respective individual treatment methodologies. The second significant crucial of tumor portrayal is non-invasive imaging, which yields a for the most part low-quality images of the nearby tissue, planning information through different segments, (for example, liquid proportion, radiation narrowing, cell thickness, extracellular grid thickness and so on....) on pixel magnitude.

This decrease of data hinders the basic understanding of the pictures. A model for non-intrusive (not involving the introduction of instruments into the body) imaging of ultra structural information available in pretty much every medical MRI is DW - MRI imaging. The capability of DWI on non-intrusive estimation of cell thickness has been the focal point of center for research. It is demonstrated to be viable in estimating the response for treatment reaction. The main property of DWI is it mirrors the movement of water particles in the tissue. For

instance, the dense packing of the cells is related to irregular mobility of water molecules also known as Brownian Movement, which is related by a less weakened signal.
Most often, cell thickness is higher in the solid portion of the tumor tissue than in typical one and accordingly the rate of diffusion is estimated to be limited because of the solid packing of the cells in tissues affected with tumor.
In this research we have used Matlab as the software platform with various image processing tool boxes. The main objectives include: segmentation of the cell nuclei; model for estimation of cell nuclei densities, calculating the cell count basing on correlation between D value estimated from the DWI. We initially take the serial histological images as input and apply the pre-processing in which we apply Gaussian de blurring techniques to remove the external noises occurred while digitizing the image. Then, we performed colour de-convolution which gives raise to Hematoxylin channel and KL1 Channel which are then again processed to give output to Grey-level image and Masked image respectively. From the output images, cell nuclei segmentation and classification is done which includes the spotting of candidate nuclei regions and segmenting the cell nuclei affected with cancer apart from the non- cancer affected cells.
Some investigators looked at cell thickness variations to respective ADC values, to assess tumour cell count. These investigations depended on the negative correlation values and the tumour development numerical models. For example, a researcher estimated the cancer growth rate in rat brain basing on a mathematical growth model along with the knowledge of ADC.
Most investigations address animal tumours or xenograft and considers about mean value for the various individual tumours without measurable evaluation of statistical quantification. The ADC is normally processed as the average estimation of various Regions of interest (ROIs).

2. Existing Method

The existing concept was to analyze whether we could use the apparent diffusion coefficients (ADCs) to estimate the cell densities and necrotic tumour fractions. For this research the tumor tissues from many cancer xenograft lines are considered for DWMRI which are determined from histological sections.
Defining the technique briefly, the tumors were placed on the iso-centers of the magnets. The magnetic field was reduced before the start of the imaging procedure. Localisation of the tumor and tumor location determination was done using the segittal scan. The inclinations of the tumors were marked on the patients' skin after completion of imaging procedure. The Diffusion coefficient correlated with the cell density in all of the xenograft lines but the ADCs correlated with necrotic fractions in only a few xenograft lines. Their investigation suggested that ADCs could be used to measure cell densities of tumors, but might not be for all tumors, but basing on the dead cell regions of the tumor tissues.

3. Proposed Method

In our research we designed a model basedalgorithm for tumor cellularity and heterogeneity assessment using the variety of image processing techniques. The main steps in our work include estimating the cell thickness based on image analysis, Correlating the DWI-data and tumor cellularity by comparing the diffusion value coefficients D and the cell thickness of tumor samples, Estimation of cell count in the tumor. The tumor outlines are obtained by segmenting the tumor.
An algorithm is developed to detect the nuclei of the cells in given histological images. We assumed each cell to have a single nucleus and the cell thickness is calculated by mathematical division of number of nuclei found within the area of the tumor. Our research could be used in precise evaluation of the cancer. Finding the tumor cell load and cell count accurately. Aids in fast detection of the cancer and therapeutic planning for the cancer patients accordingly.

4. Literature Survey

The AuthorsTomohiro Hayakawa and V. B. Surya Prasath in their research paper described Pathology as a high needful field in the modern science and medical field. The nuclei sectionalization part is main step in cancer detection because cancer assessment, analysis and classification of different types of cancers' depend on the precise division results of cell nuclei. Previously during the conventional cancer assessment, the pathologists assess the biopsies and come to final conclusions based on the cell architecture. Due to recent development of technology, computerized approaches are widely used mainly in the area of digital pathology. Approaches are also made for cell nuclei estimation, classification and division are also on rise. These techniques help in minimizing the intervention of humans and come to precise and accurate conclusions in clinical information. Their review paper provides the information about various nuclei segmentation techniques and experimental methods.
Another author theorizes that quantitative estimation of information on tumor cell load and heterogeneity helps in developing very effective and unique treatment techniques. They concluded that relation can be inferred between Diffusion co-efficient D from the DW-MRI and cell density. However, such relation cannot be same

for all types of patients and tumors. To make it possible and unique the relation must be established without the tumor resection. They proposed biopsies contain useful information for this purpose if they are localized and evaluated systematically. They proposed the super pixel method for the automatic localization of the biopsies from the D coefficient value maps. Needle biopsies provide accurate and sufficient data to derive a quantitative relation between the Diffusion constant and the cell density, given that only if the data is collected from the low, intermediate and high D-value regions from MRI. Tumor heterogeneity and cell load can also be estimated from these data. This procedure assists in clinical workflow of cancer diagnosis and personalized cancer treatments.
Another author in his famous journal states that Diffusion-weighted MRI imaging (DWI) is a powerful MRI method since it evaluates the tissue structure abnormalities and detects the microscopic level change in mobility of water at the cellular level. Accordingly, DWI has capability to identify pathological data anatomical brain images. This important advantages of tissue characterization and detection makes this process suitable for gliomas and also other processes where standard anatomical processing is proved insufficient. The paper mainly focused on various applications of DW-MRI, the diffusion constant for assessing and treating gliomas.
The Author Carolin Reischauer in his paper prospectively evaluate the possibility of observing response to chemotherapy in patients with cell lung carcinoma by using the functional diffusion maps. Nine patients of different genders and ages are taken into consideration. Magnetic Resonance Imaging was performed at regular intervals after initiation of the chemotherapy. Treatment response is characterized by reduction in tumor length across on computed tomography (CT). On end their outcomes demonstrate the changes in ADCs basing on the patients and also the percentage change in the pixel levels with increased ADCs on FDMs is by all accounts a reliable and great biomarker for prior estimation of treatment response in lung cancer.

5. Data Analysis Methods

Depending on the tumor Imaging and histological information, we deduced an algorithm for tumor cellularity and heterogeneity assessment. The algorithm consists of following steps:

- Cell size evaluation based on image processing. A process is developed to find the nuclei of the cells from input histological images. We assumed a single cell to have a single nucleus and thickness is calculated by mathematical division of the cell count detected with the tumor area.
- Correlation of image -data and tumor cellularity by comparing the diffusion coefficients D. This data helps in evaluating the quantity of cancer cells in tumor regions of each voxel for analyzing the tumor tissue heterogeneity, including the evaluation of the regions which are affected and which are not affected by the cancer disease in the regions where proper data is not provided along with the evaluation of number of cells affected or cell countfor total tumor tissue.
- Estimation of the cell count in the all regions of tumor. The tumor outline is evaluated by sectionalization of the tumor for the given DWMRI Information. Tumor cell count is evaluated for each voxel and then the total cell count is obtained by adding the total number of cells in all voxels of the DWI information processed from the tumor.

5.1. Histological Image Analysis

We put forward acomputerized algorithm for the diagnosis, segmentation and categorization of tumor cells in cancer affected tumor tissue using Haemotoxylin and Eosin staining for cell nuclei, and KL1 (marker) staining for cells' cytoplasm. The methodology is shown in the Fig. 1 and tested on DWI histological images.

5.1.1. Pre – Processing

The primary input MRI image is a 40- fold magnified image of a very high file size. To make every detail visible, a 20-fold magnification has been chosen for to perform histological assessment. During tumor tissue slicing and the digitization process, the spatial correspondence is lost among the corresponding neighbor tumor slides and data was difficult to compare with other slices. So before analyzing the data the Gaussian blur removal technique is applied to reduce the noise.

5.1.2. Color Deconvolution

A computerized color deconvolution process is performed on pre-processed image to divide the input image into Hematoxylin stain channel and KL1 stain channel. Hematoxylin is a naturally derived dye which is used as histology stain/ Ink in histology. KL1 is the most sensitive marker used along with H&E stain. The Hematoxylin channel contains the background or the plane part of the tissue. While the KL1 Channel contains the tumor cells. Corresponding Mask Image is generated using the KL1 channel which separates the tumor cells from the background region.

5.1.3. Cell Nuclei Segmentation

Segmenting the cells from the histological data is a great challenge, since cells vary in shape and size. Also any minor mistake or noise at the time of tumor tissue slicing or digitizing may make it even more difficult. In this

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paper we have implemented an automated and precise cell segmentation algorithm in total of two steps. In the first step, the centers of each cell is accurately located using the local intensity minima and maxima and the gradient information. In step two, the cell centers determined in step one are used as initial seeds for cell nuclei segmentation and extraction. Grey level image and mask image are generated from the Hematoxylin channel and KL1 Channel respectively. The gradient for the image was computed using the Sobel operator.

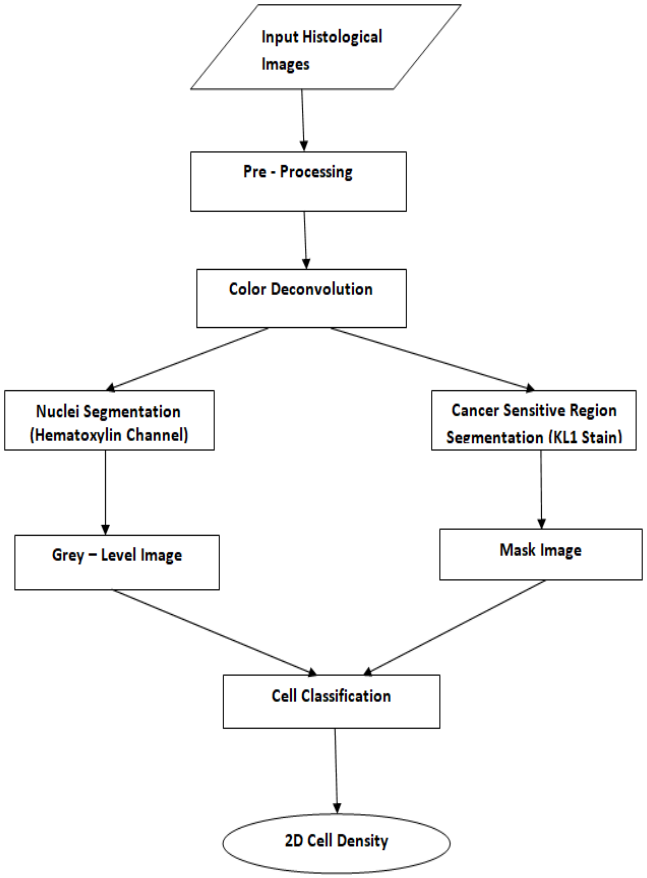

Figure 1: Flowchart of the Digital Image Processing Algorithm for Computerized Histological Image Analysis, from Input Histology Slides to Cell Count and Thickness Estimation.

6. Results

Matlab is used to execute mathematical estimations for a wide extent of usages. The basic level language used is on a very basic level equivalent to standard direct variable based math documentation.MATLAB is a numerical figuring condition and new age programming language. MATLAB permits plotting of various tables and graphs in two dimensional and three dimensional's, lattice controls, production of UIs and interfacing with codes or programs of different programming languages like C, C++, Java etc…,

Results after executing Matlab Code for Color deconvolution, Nuclei segmentation (Hematoxylin stain) and Cancer growth region division (KL1 stain) and Cell Nuclei categorization and Segmentation are as follows :

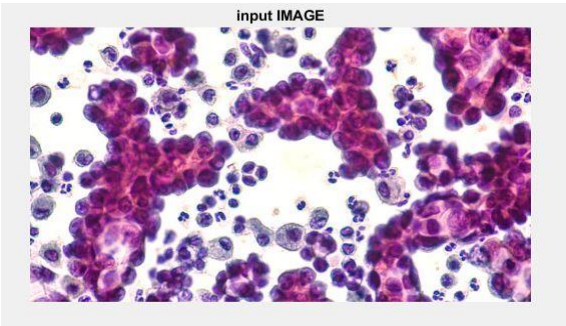

Figure 2: Input Histological Image.

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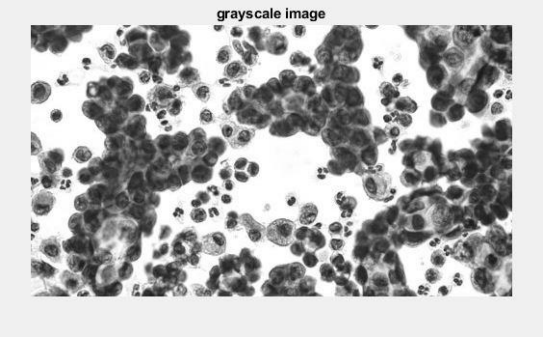

Figure 3: Grey Level Image.

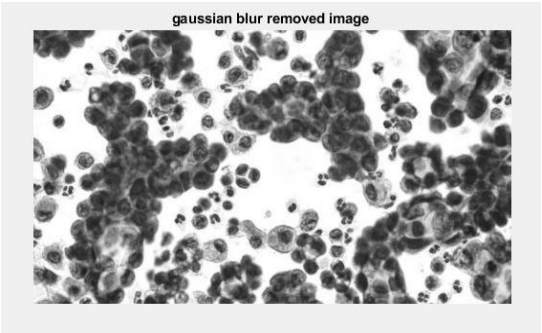

Figure 4: Gaussian Blur Removed Image.

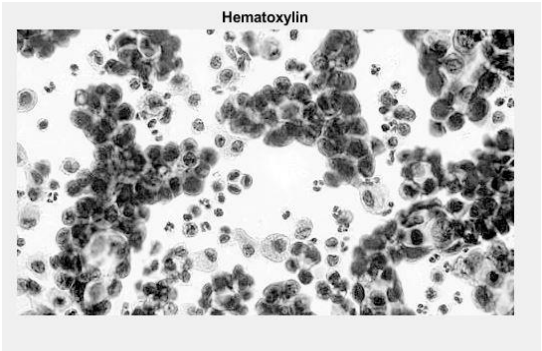

Figure 5: Hematoxylin Channel.

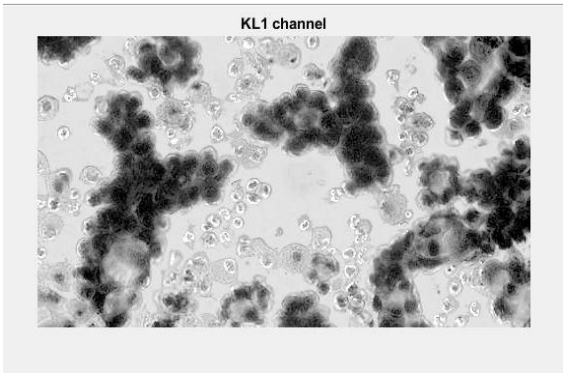

Figure 6: KL1 Channel.

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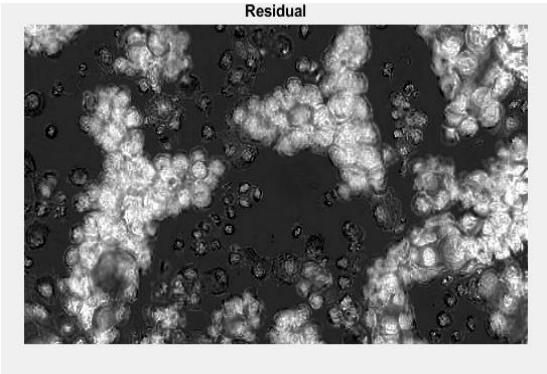

Figure 7: Grey- Level Residual Image.

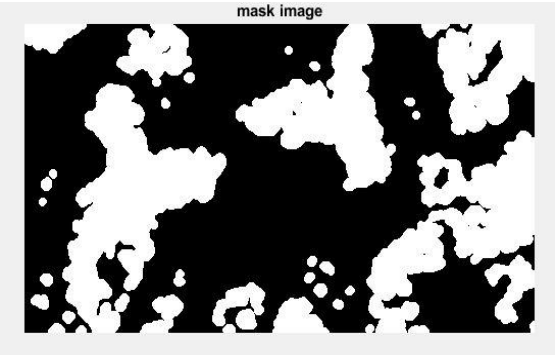

Figure 8: Masked Image.

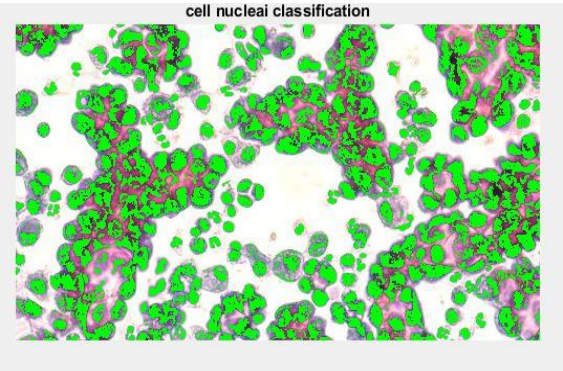

Figure 9: Candidate Nuclei Regions.

FINAL RESULTS AFTER THE CELL NUCLEI SEGMENTATION AND CLASSIFICATION ARE :

THE TOTAL NUCLEA FOUND : 637

THE TOTAL NUCLEA AFFECTED : 427

THE TOTAL NUCLEA NOT AFFECTED : 210

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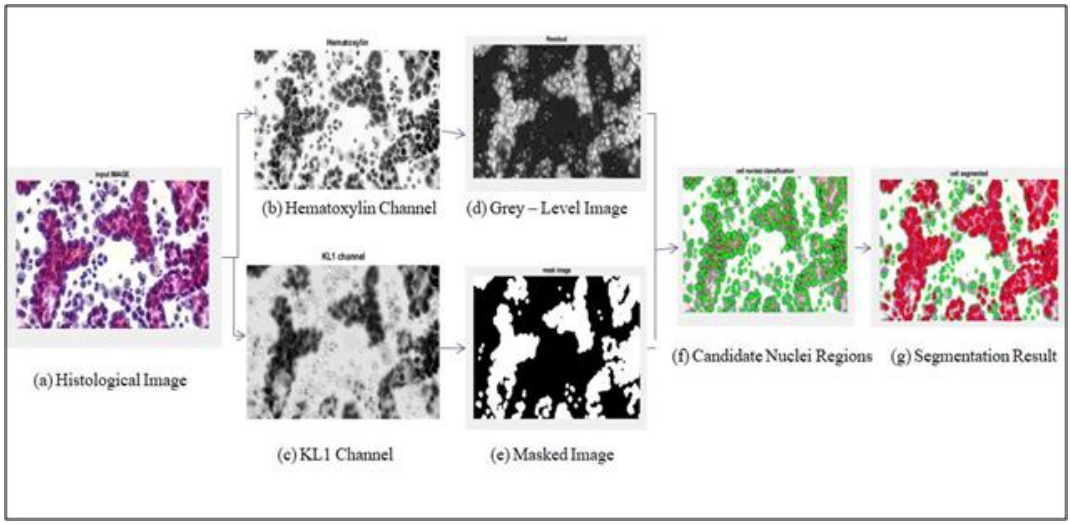

Figure 10: Results for Computerized Cell Nuclei Detection: (a) Input Coloured Histological Image was Separated into (b) Hematoxylin Channel and (c) KL1 (Cancer Growth Region) Channels (d) Grey - Level Image Evaluated from the Hematoxylin Channel and (e) Masked Image Evaluated from the KL1 Channel, (f) Candidate Nuclei Regions (Green) were Obtained from (e) and (d) Images; The Final Segmentation Result (g) with Cancer Affected Regions (red) and Non-Cancer Affected Regions (Green), the Small Window on the Right Top of (g) Shows the Outline of Nuclei Regions and their Center Points Inside the Region.

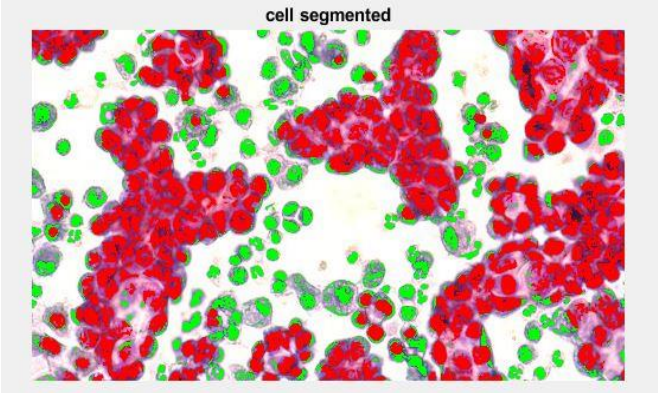

Figure 11: Segmented Image.

7. Discussion and Conclusions

After performing the diagnosis on the tumor, the proposed digital image processing method efficiently differentiated cancer-affected cells from non-cancer affected cells, and their respective countin the output image and also revealed the tumor heterogeneity. Based on the DWI data of the tumor and histology information, we deduced an algorithm for evaluation oftumor cell count and heterogeneity assessment.

Our investigation may assist with refining and changing the patient-specific unique advancement of medical tratment techniques. It remains constant for customary cytostatic drugs (for example allopurinol, aprepitant, azathioprine) also for profoundly specific catalyst inhibitors (for example novel purine diamine analogue used as a potential ATP-competitive catalytic inhibitor of topoisomerase II used in anti-cancer therapy), which currently are used in the therapy of cellular breakdown in lungs. Moreover, our research might be veryvaluable for the customized change of novel therapeutic methodologies targeting angiogenesis and associated growth factors like vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF). Here the information on cell count of cancer and non- cancer affected regionsand vessel thickness (which is key for pharmacokinetics) may permit the exact fitting of medication drugs doses and their targeted clinical application methods to decrease negative results and side effects and expand medications hostile to tumor proficiency. The generation of samples for the Histology study is the time consuming part. It has to be done with utmost care otherwise it may effect the outputs of the experiment.

Another big question remaining is how these findings might be applied in the clinics. This method or algorithm can be applied to various tumour tissues. But, the D value is the composition of the sum of interactions with many other biological particles apart from cell membranes like blood vessel thickness and condition, micro and

macro molecules, vascularization etc., Different results could be expected for different tissues.
To analyze and investigate further the proposed concept has be tested on the different types of lung cancers such as Large cell, Squamous cell, Adenocarcinomacarcinoma (10% , 25%, 40% of the lung cancers), sarcomatoid carcinomas etc., and also analyze how far the results varies.

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