

# Medical Image Fusion for Brain Tumor Diagnosis Using Effective Discrete Wavelet Transform Methods

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## Abstract

**Background:** The field of clinical or medical imaging is beginning to experience significant advancements in recent years. Various medical imaging methods such as computed tomography (CT), X-radiation (X-ray), and magnetic resonance imaging (MRI) produce images with distinct resolution differences, goals, and noise levels, making it challenging for medical experts to diagnose diseases.

**Objective:** The limitations of a single medical image modality have increased the necessity for medical image fusion. The proposed solution is to create a fusion method of merging two types of medical images, such as MRI and CT. Therefore, this study aimed to develop a software solution that swiftly identifies the precise region of a brain tumor, speeding up the diagnosis and treatment planning.

**Methods:** The proposed methodology combined clinical images by using discrete wavelet transform (DWT) and inverse discrete wavelet transform (IDWT). This strategy depended on a multi-goal decay of the image information using DWT, and high-frequency sub-bands of the disintegrated images were combined using a weighted averaging method. Meanwhile, the low-frequency sub-bands were straight-forwardly replicated in the resulting image. The combined high-quality image was recreated using the IDWT. This method can handle images with various modalities and resolutions without the need for previous data.

**Results:** The results showed that the outcomes of the proposed method were assessed by different metrics such as accuracy, recall, F1-score, and visual quality. The method showed a high accuracy of 98% over the familiar neural network techniques.

**Conclusion:** The proposed method was found to be computationally effective and produced high-quality medical images to assist professionals. Furthermore, the method can be stretched out to other image modalities and exercised by hybrid techniques of wavelet transform and neural networks and used for different clinical image analysis tasks.

**Keywords:** CT and MRI, Image fusion, brain tumor, wavelet transform methods, medical images, machine learning, CNN

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## I. INTRODUCTION

Medical imaging is essential for the diagnosis and management of many disorders in contemporary healthcare [1]. Modalities such as computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), and single-photon emission computed tomography (SPECT) provide detailed visualization of the tissues, bones, and other structural characteristics of the human body [2]. To improve the speediness of the diagnosis, several studies have proposed image fusion methods, transforming spatial domains into deep learning [3]. Although numerous image fusion methods have been discussed such as MRI/CT, MRI/PET, and MRI/SPECT [4], the study underscores that MRI/CT is the most favorable open-source single fusion method. CT and MRI scans are examples of medical images that offer valuable information on the inner workings of the human brain. Several problems with these pictures, including low contrast, noise, and abnormalities, can make it challenging for medical experts to make precise diagnoses. Image fusion, which merges many photographs of the same scene to produce a single, improved image, is one approach used to overcome these problems. Furthermore, simple averaging, maximum selection, and principal component analysis (PCA), which are three traditional methods for fusing medical images all have drawbacks, including information loss, picture artifacts, and decreased contrast [5].

Deep learning methods have proven inefficient in a single framework with small training data due to the manual labeling of the dataset by medical experts. Moreover, complex deep-learning frameworks are time-consuming and require significant hardware configurations [6]. To overcome these limitations, several sophisticated image fusion methods have been proposed. The overall quality of medical images can be enhanced by fusion, enabling simpler

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understanding and helping doctors diagnose patients more precisely. This study was conducted to investigate whether DWT and IDWT might be used to combine medical images. The IDWT combines frequency components to produce a new, improved image, while the DWT decomposes an image into many frequency components. DWT has the potential to split an image into several frequency sub-bands, and the fusion can provide a fused image with higher visual quality and features. The objective of this study is to develop an effective and dependable medical image fusion method combining DWT and IDWT to improve aesthetic appeal and diagnostic precision. The suggested method with CNN aims to remove redundant and extraneous information while maintaining important information from many images [7].

Improved medical images enable more accurate diagnoses, potentially leading to early diagnosis and treatment of diseases. Additionally, fusion methods can reduce radiation exposure of patients during medical image operations [8]. The article is organized into different sections namely (2) related works, (3) methods used in the proposed work, (4) results and discussion of all the comparisons centered on metrics and output screenshots of the proposed application, and (5) conclusion of the proposed work and further studies.

This study aimed to provide an easy-to-use software solution for image fusion that medical practitioners may employ. The GUI for the software utility will be integrated using the PyQt library and developed with the Python programming language. The main objective is to create an image fusion method that can improve the informational value of medical images and assess the performance compared to other cutting-edge methods currently used. In this context, DWT and IDWT were used to accomplish the proposed method and examine how the effectiveness is affected by various wavelet functions.

## II. LITERATURE REVIEW

Fusion is an essential technological advancement in a variety of fields, including robotics, medicine, and remote sensing. In terms of medical image methods, MRI focuses on the soft tissues, while CT offers a clearer image of the bony structures. Image fusion is primarily used to reduce the quantity of data and also create images that are more precise and intelligible from both a human and machine perspective. The initial stage in the wavelet-based fusion approach is the breakdown of the two input images using DWT [9]. A multimodal image fusion adds structural and functional information, providing a storage solution. A previous study [10] integrated multimodal images using DWT and empirical mode decomposition. The suggested method combines spatial and functional data from the original image [11]. In this context, the combination of brain CT and MRI scans is recommended, with hybrid fusion response being dominant in the method.

The adaptive approach, Empirical Wavelet Transform (EWT), modifies the basic functions following the information in the source image. Selecting an optimal fusion mechanism is critical, with three main categories of fusion rules, namely decision, feature, and pixel level. As the name implies, pixel-level fusion fuses the source images by pixel, while the selection of characteristics including edges and lines form the basis of feature-level fusion. The data points in the images are used to run the decision-level fusion, while an adaptable wavelet-based fusion technique that combines local energy maxima (LEM) with EWT has been developed [12]. The fused image is distortion-free and has superior outcomes when integrated with brain MRI and CT scans.

Curvelet Wavelet Transform (CWT) was used in a study [13] for treating edges as basic components, offering maturity and good image processing adaptability, anisotropy, and suitability for image properties. Over the years, multi-modular image combination calculations and tools have developed as excellent assets in the clinical usage of therapeutic imaging structures [14].

Current methods often a long time and require numerous samples, while the proposed method uses Dual Tree Complex Wavelet Transform (DTCWT) to extract complex and related information from each image [15]. A three-layer rule-based image decomposition method is suggested [16], comprising spatial low-pass filters and local extrema to decompose input images. Fusion rules are applied to maintain tumor illumination, considering the contrasting characteristics of MRI and CT images. MRI images depict characteristics of tissue with increased moisture content, while CT images largely focus on high-density tissue. Conventional medical image fusion methods may be divided into spatial, transform, and hybrid approaches. Some methods such as the Laplacian pyramid, are inaccurate in their descriptions of image contour and contrast. Furthermore, DWT, contourlet transform, and ST, among others, fall short of capturing salient features effectively, resulting in Gibbs's effects and artifacts in the merged images.

Methods such as Structure Tensor Optimization and Structural Similarity are used to divide low frequencies. HID produces fused low-frequency images by using base layer texture information. Using 50 CT and MRI scans, the suggested methods are contrasted with 12 state-of-the-art approaches. Fusion rules take advantage of base layer texture information [17]. The synchronized anisotropic diffusion equation [18] was used to suggest unique multi-

modality medical image fusion methods (S-ADE). The decomposition of two source images into a single image is achieved using the modified S-ADE model, which is more suited for CT and MRI images. The modified anisotropic Laplacian method is used to construct the fusion decision map on texture layers.

Information fusion architecture, inspired by the human mind enhances efficiency through computerized evaluation. Various filters such as softening filters-based multiplication, Brovey high filter (HPF), intensity hue saturation (IHS), and principle component analysis (PCA) were used to provide excellent data on the image combination used in satellites. The choice of filters, including HPF with smoothing filter-based modulation, significantly impacts the outcomes as exemplified in the study by Zhang and Wand [19] in characterizing, dividing, and combining images to retrieve important data.

Neural network-based methods play a crucial role in combining medical images, with the pulse-coupled neural network (PCNN) drawing inspiration from cat vision. PCNN-based algorithms operating in the global domain, enhance image fusion by aiding in the retention of specific details. Huang et al. augment the effectiveness of PCNN by adding the Laplacian energy of the image block as input excitation for every neuron. In the past, parameter-adaptive PCNN algorithms were used in the high-frequency band of the NSST area to enhance performance and raise the quality of the fused image. Multilevel time fractional frequency domain elements of the image may be obtained using the discrete fractional wavelet transform (DRWT)-based method. A dictionary-learning-based technique for fusing multimodal medical images was presented [6] based on coefficient fusion and sparse representation.

A conditional generative adversarial network with multiple generators and discriminators is known as MGMDcGAN. In the first cGAN, the generator aims to produce a fused image that closely resembles reality using a specially crafted content loss designed to deceive two discriminators. The objective of the discriminators is to identify structural differences between the fused and the source image. Based on this knowledge, a previous study [20] applied the second cGAN with a mask to improve the dense structural information in the final fused image while preventing the loss of functional information. MRI PET, MRI SPECT, and CT SPECT are three different types of medical image fusion that may be implemented using MGMDcorgan as a unified method. This can be achieved using a guided image filter (GIF) [20] and an NSST with integrated phase congruency-based fusion rules [12]. Using the aforementioned transform process, the GIF provides the tiny features that are then merged, while MRI and CT scans are used to validate this procedure. In medical fusion [21], the non-subsampled contourlet transform (NSCT) is efficient and the highest image resolution is necessary for MRI and CT fusion. Several multimodalities in clinical domain such as registered tomography and appealing reverberation images, are combined to produce an integrated image [22] with 96.5% accuracy. When CT and MRI images are combined, a mark image known as the shearlet transform is produced (NSST). In terms of high robustness and invisibility, the suggested method performs better than several of the latest methods [23].

A previous study [24] shows how to strengthen visual contrast through fusion using discrete wavelet transform (DWT). Initially, three different methods were applied to three photographs and the two improved images were combined, while the third and the linear fusion image were both improved by using DWT. The fusion of three photographs on two levels and the comparison effectively increased proficiency. Furthermore, the lack of local image features in SR-based image fusion leads to low detail and registration misalignment sensitivity. To address these issues, a study [25] introduced gradient regularization convolution SR multi-source image fusion. The high- and low-frequency image components were separated. The optimal high-frequency image component was specified by the sparse coefficient produced by CSR gradient regularization. For use with either extreme or normal fusing, low-frequency components are preferred, enhancing both registration sensitivity and image preservation.

Compared to existing strategies, the suggested method is anticipated to deliver improved fusion performance. Machine learning-based methods for image fusion have also drawn more interest recently [26] but pose computational challenges and require substantial training data, which might be a drawback in applications for medical imaging. Many methods for DWT-based medical image fusion have been reported in the available literature. The DWT, among others, has found widespread application due to its ability to extract both spatial and frequency information from images.

### III. METHODS

This study aimed to design a method for fusing medical images that incorporated data from each of the sources into the final images using DWT and IDWT. The objective of this procedure is to enhance the depiction of the target region, providing a more comprehensive and precise representation to improve diagnosis and treatment planning. The limitations inherent in a single medical image modality underscored the need for medical image fusion. Each modality has advantages and disadvantages, and the combination of images from various modalities can effectively

mitigate specific drawbacks associated with each. For instance, an MRI scan has poor spatial resolution but great contrast compared to a CT scan with high contrast but low spatial resolution [27].

A single image with high spatial resolution and contrast was generated by merging images from the two modalities, namely DWT and IDWT. DWT is a widely embraced method for breaking down images into many sub-bands, each of which represents a different degree of information. The DWT coefficient matrix was then used to reconstruct the fused image using IDWT. Fig. 1 depicts the schematic illustration of the proposed classification of brain tumor images. The MRI and CT images were used as input in this model to create the grayscale picture, for the precondition source. The image was separated after DWT processing to identify the coefficients of the CT and MRI, then the estimated values were reported once the coefficients had been combined.

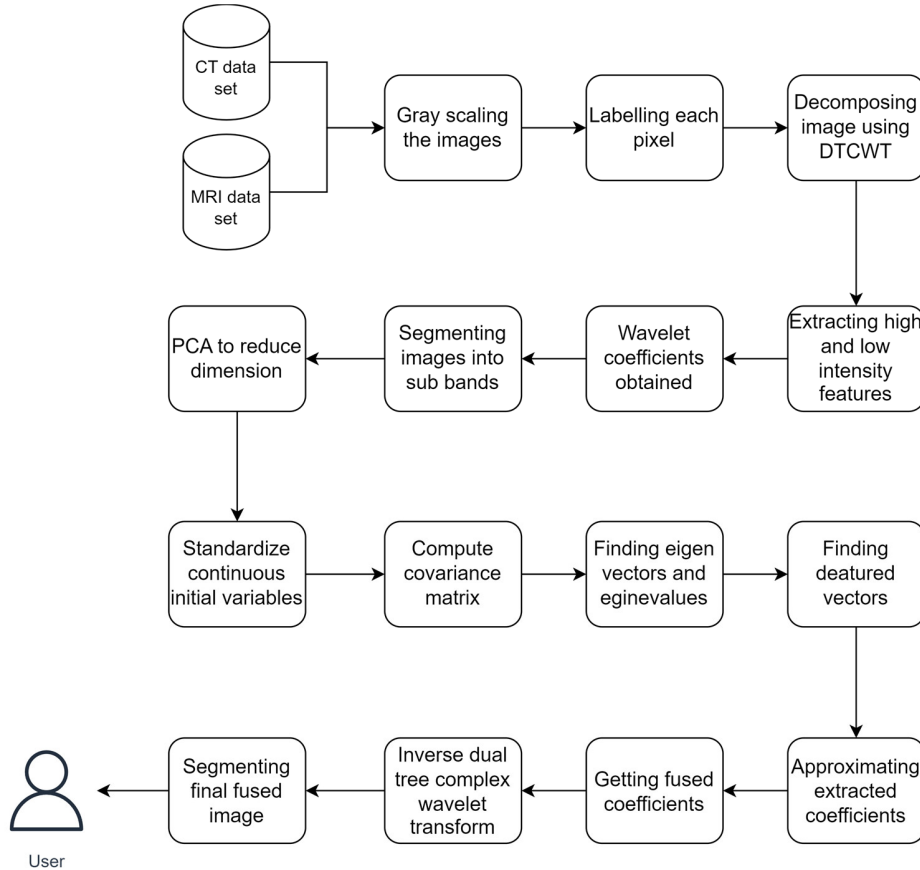


Fig. 1 Schematic illustration of the proposed classification of brain tumor images

#### A. Module Description

The dataset was collected from Kaggle. The total number of images in the dataset was 5,600, containing training and testing datasets as well as CT and MRI images of brain tumor patients. The training dataset consisted of 4000 images, while the testing dataset was composed of 1600 images. The sample datasets for MRI and CT are shown in Fig. 2.

A web page was created for the image decomposition module asking visitors to contribute MRI and CT scans of patients. This web page has a two-file upload option to upload CT and MRI images of the brain with a maximum size of 512\*512 in the RGB format. This RGB image was transformed into a grey-scale format, which contained information about the intensity.

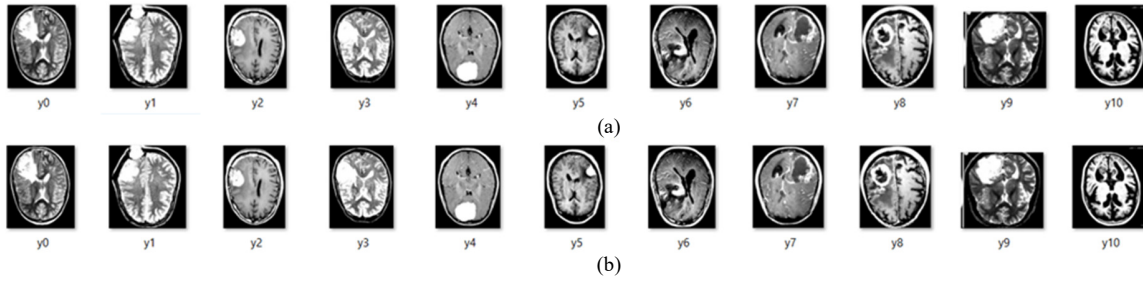


Fig. 2 The sample images of brain tumor patients (a) MRI images; (b) CT images

The process of extracting features and image fusion using DTCWT entails obtaining high- and low-intensity features as wavelet coefficients after decomposition, see in Fig. 3. This method generated visuals at different degrees of decomposition. To extract the characteristics of image, each level was split into sub-bands at six different angles, namely  $-15^\circ$ ,  $-45^\circ$ ,  $-75^\circ$ ,  $75^\circ$ ,  $45^\circ$ , and  $15^\circ$ . When the data was larger, PCA was used to extract the quiet and complimentary characteristics to reduce the dimension. The various steps in PCA include standardization, which aims to normalize the range of continuous variables to ensure equal contribution to the analysis. This step was accomplished mathematically by calculating the variation between each result and the mean, then dividing by the standard deviation. Following this, the covariance matrix was constructed since certain variables have duplicate information due to the strong correlation with one another. The eigenvalues and eigenvectors of the covariance matrix were also calculated to identify the primary components. The highlighted vector was selected from the calculated primary vectors, then the data were reconstructed using the primary axes. The estimated wavelet coefficients obtained were considered approximations. Subsequently, the estimated coefficients from the image fusion were fused, and the fused image was restored by the inverse dual-tree complex wavelet transform (IDTCWT).

The resultant fused image was used for the segmentation, a crucial process in computer vision, medical imaging, and pattern recognition. The process entails breaking the image into various areas or segments, each of which represents a distinct item or portion. The purpose is to isolate and separate the object or important elements of an image from its surroundings and one another. This process finds application, specifically in thresholding, clustering, region-based, and machine-learning-based methods.

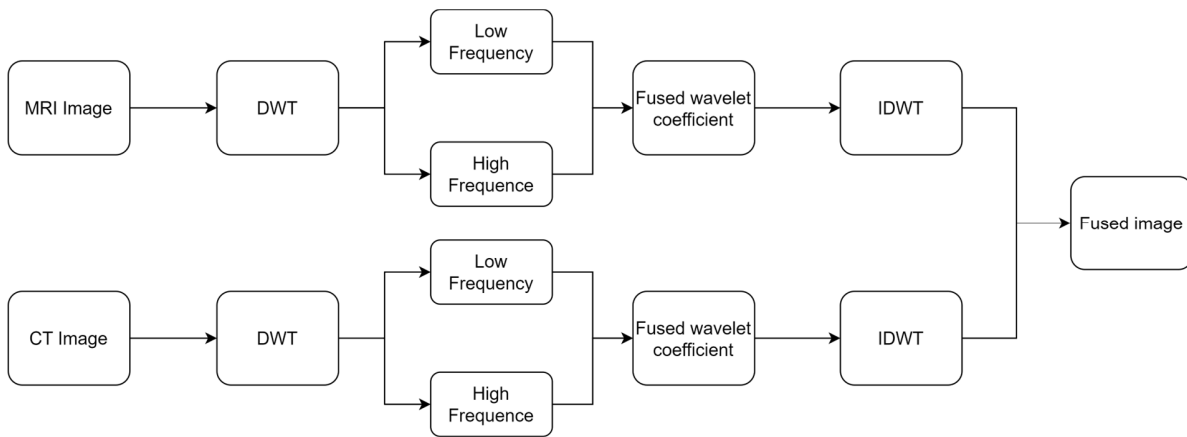


Fig. 3 Extracting features using DTCWT

### B. Evaluation

In the field of medicine, accuracy is crucial for reliable diagnosis, treatments, and prognoses, for instance, the effectiveness of a diagnosis is impacted by the accuracy of blood or genetic tests. Moreover, the safety and effectiveness of treatment may be impacted by the accuracy of medical devices such as a pacemaker, ventilators, or artificial joints.

$$\text{Accuracy} = \left( \frac{\text{Number of correctly classified images}}{\text{Total number of images}} \right) * 100 \quad (1)$$

Accuracy measures the averaged squared discrepancies between the highest strength of a signal and the power of the noise that degrades the accuracy of its illustration. A lower accuracy number denotes a greater quality of the fused image. The accuracy for the different strategies of the proposed model is depicted in Fig. 4-6, showing better performance compared to the existing model.

Peak signal-to-noise ratio (PSNR) is the relationship between the maximum possible power of an image and the power of corrupting noise that affects the quality of its representation. An improved quality of the fused image is shown by a higher PSNR value, which compares the compression quality between the original and reconstructed. In general, PSNR is used for image compression and filter techniques.

$$\text{PSNR} = 10 \log_{10} \left( \frac{(IL-1)^2}{\text{MSE}} \right) \quad (2)$$

IL refers to the quantity of maximum possible intensity levels in an image, starting from 0. The lower mean squared error (MSE) improves the PSNR value. Furthermore, the structural index similarity metrics (SSIM) evaluate brightness, contrast, and structure to determine the structural similarity between the original and fused images. The range of the SSIM value was between -1 to 1, denoting perfect similarity. An improved quality of the fused image was shown by a higher SSIM value. The degree of information retention was measured using the FF metric, and a greater FF value denoted a higher level of image quality.

Precision defines the percentages of confirmed identifications as truly accurate and when there are no null false positives, the model earns a precision of 1.0. The recall confirms how many positive inputs were accurately identified, a larger value of recall signifies the optimal ability to recognize positive inputs accurately.

$$\text{Precision} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Positives}} \quad (3)$$

$$\text{Recall} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}} \quad (4)$$

#### IV. RESULTS

Compared to existing strategies, the suggested method was anticipated to deliver improved fusion performance. The objective of this experiment was to provide an easy-to-use software solution for image fusion suitable for medical practitioners. The GUI for the software utility was integrated using the PyQt library and developed through the Python programming language. Users would be able to enter numerous medical photos, select the preferred method, and view the merged image. Moreover, the experiment included options for fusion, parameter adjustment, and image quality analysis.

##### A. Model Creation

The existing CNN model splits the dataset into 30% for testing and 70% for training, after importing the model, it was applied to both test and train the dataset. The images were labeled with YES or NO, representing brain tumors and no brain tumors, respectively. Subsequently, the dataset was loaded, the image was reshaped, and greyscale representations were shown. The existing model CNN produced lesser accuracy on CT, MRI and fusion images, see in Fig. 6.

The proposed method started with loading the dataset, followed by reshaping, training, testing, and validating the image. Subsequently, the image was shown at segmentation by labels 1 and 0, representing brain tumor, and no brain tumor respectively. Due to the varying image sizes, normalization was applied to ensure uniform pixel values between 0 and 1 for easier training and testing. To train the model, the proposed method was exercised with and without augmentation of the dataset at reshaping of images. Furthermore, performance evaluation entailed assessing loss and accuracy at different stages of training and validation. The proposed image fusion process has been tested using a variety of performance criteria to measure the quality of fused images, including accuracy, precision, recall, and F1-score. Also, the model identification was generated based on the number of positives and negatives in the confusion matrix.

Fig. 4 shows the accuracy of the proposed model with and without augmentation. Based on the results, the proposed model with augmentation produced greater accuracy and reduced losses at different epochs than without

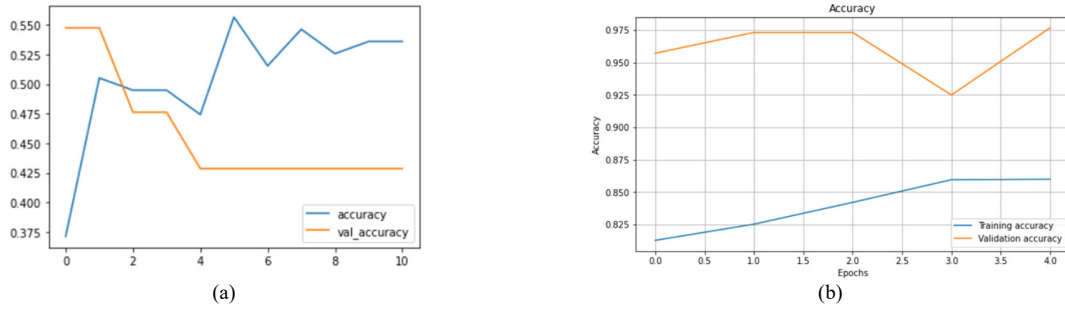


Fig. 4 Proposed model accuracy (a) without augmented; (b) with augmented

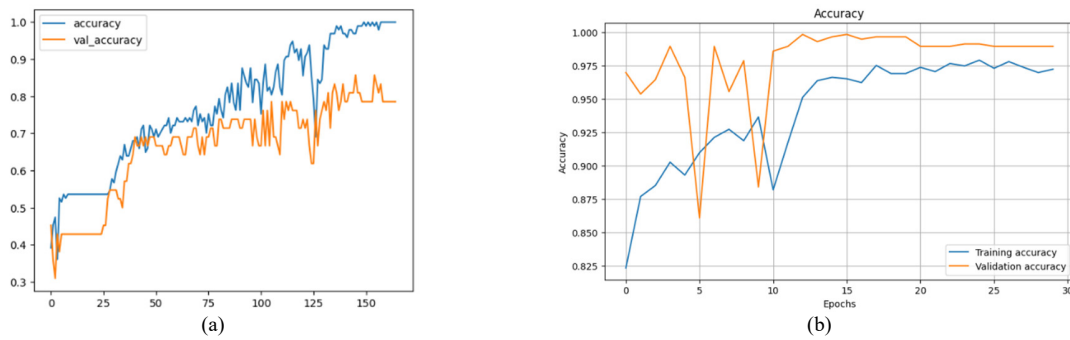


Fig. 5 Accuracy and Validation accuracy (a) the proposed system; (b) pre-trained model VGG16

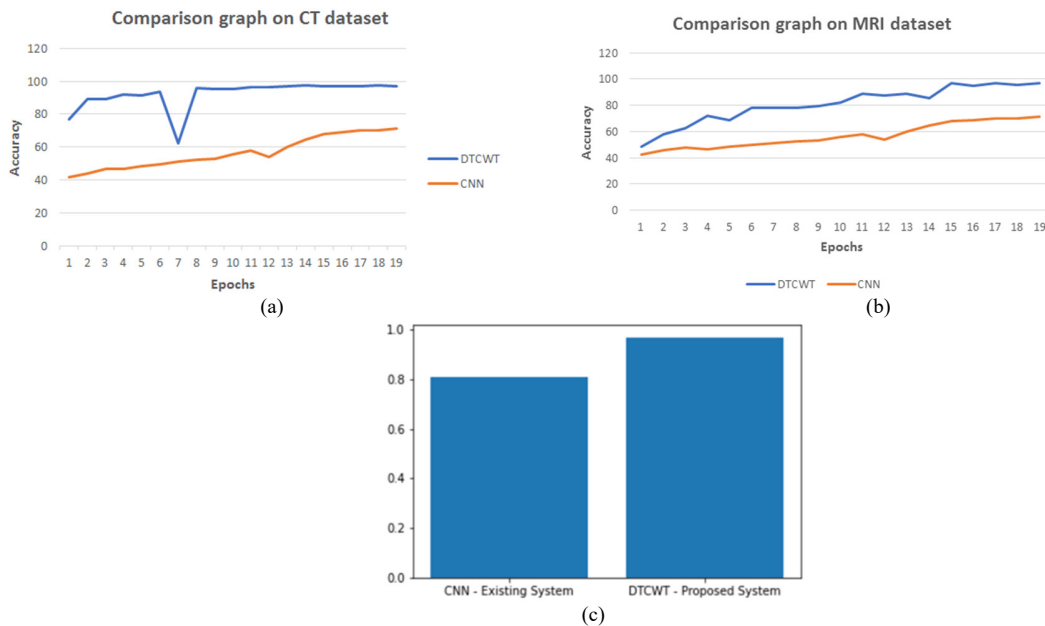


Fig. 6 Comparison between existing vs proposed on (a) CT dataset; (b) MRI dataset; (c) Fusion images

augmentation. Fig. 5(a) shows the accuracy and validation accuracy of the proposed system at 150 epochs. The percentage of accuracy was 98.92 and 85.75 at training and validation, respectively, on the CT dataset. To enhance performance, the pre-trained model VGG16 model recognized for its CNN architecture with 16 layers and weights, was incorporated and visualized in Fig. 5(b). The comparison between the existing and proposed system is depicted in Fig. 6, grounded on CT, MRI, and fused images, and based on different metrics. Here, the proposed approach recorded the performance metrics on the CT dataset as 0.9090 of precision, 0.8333 of recall, 0.86956 of F1-score,

and 0.9688 of accuracy. For illustration, Table 1 tabulates the performance metrics of the existing and proposed models on the MRI dataset. According to the assessment results, the proposed method produced a high-quality fused image with low MSE, it improves the PSNR and SSIM metrics which are tabulated in Table 2. In general, the assessment metrics showed that the proposed methodology was better than existing fusion methods in terms of quality and information retention, generating high-quality fused images.

TABLE 1  
 PERFORMANCE METRICS ON THE MRI DATASET

	Existing System				Proposed System			
	Precision	Recall	F1-Score	Support	Precision	Recall	F1-Score	Support
0	0.00	0.00	0.00	0	0.79	0.79	0.79	24
1	1.00	0.59	0.74	76	0.72	0.72	0.72	18
Accuracy			0.59	76			0.76	42
Macro avg	0.50	0.30	0.37	76	0.76	0.76	0.76	42
Weighted avg	1.00	0.59	0.74	76	0.76	0.76	0.76	42

### B. Model Use

A web application was developed for the image decomposition module asking visitors to contribute MRI and CT scans of patients. As shown in Fig. 7, the web application has a two-file upload option for CT and MRI images of the brain with a maximum input image size of 512\*512 in the RGB format. Subsequently, the RGB image is transformed into a greyscale, which contains information about the intensity. Using DTCWT, the original grey-scale MRI and CT images are deconstructed. In the next step, the website user has to select the coordinates for the CT and MRI during the image registration process. After the image registration process, the fusion method is performed to obtain the fused image. The final fused, as seen in Fig. 7(e), segmented image can be very helpful for the neuropathologist and neurologist to identify the brain tumor levels and provide treatment to cure patients.

TABLE 2  
 COMPARISON BASED ON EVALUATION INDICATORS PSNR AND SSIM

	PSNR	SSIM
Proposed	71.66	0.98
CNN	70.98	0.89
NSCT+RPNN [28]	31.68	0.50
NSST+PAPCN [29]	32.92	0.49
NSCT+LE [30]	31.61	0.48

Users of the web application can enter numerous medical photos, select the preferred method of fusion, and view the merged image. Moreover, the application has options for fusion, parameter adjustment, and image quality analysis. The overall objective is to advance medical imaging by creating a user-friendly software tool for medical practitioners.

## V. DISCUSSION

The image fusion process in the proposed framework entailed the application of DTCWT, which addressed the drawbacks of DWT by combining medical images. The proposed method was assessed using two separate medical image datasets, comprising MRI and CT scans. DTCWT offers better directionality and shift variance, making it easier to analyze the edges and contours of the original image. The results showed that the proposed method outperformed other cutting-edge methods in terms of visual quality, information preservation, and accuracy. One of the key conclusions from this study is the efficiency of DWT in breaking down the source image into several frequency sub-bands, which can aid in identifying and preserving pertinent data. The IDWT effectively combines sub-band coefficients from two source images, producing a fused image with minimal artifacts and includes pertinent data from both sources. It was also found that the quality of the fused image may be significantly impacted by the wavelet function and decomposition level used. The proposed model, applied to both MRI and CT datasets, showed that the symlet wavelet with a decomposition level of 4 produced the best results.



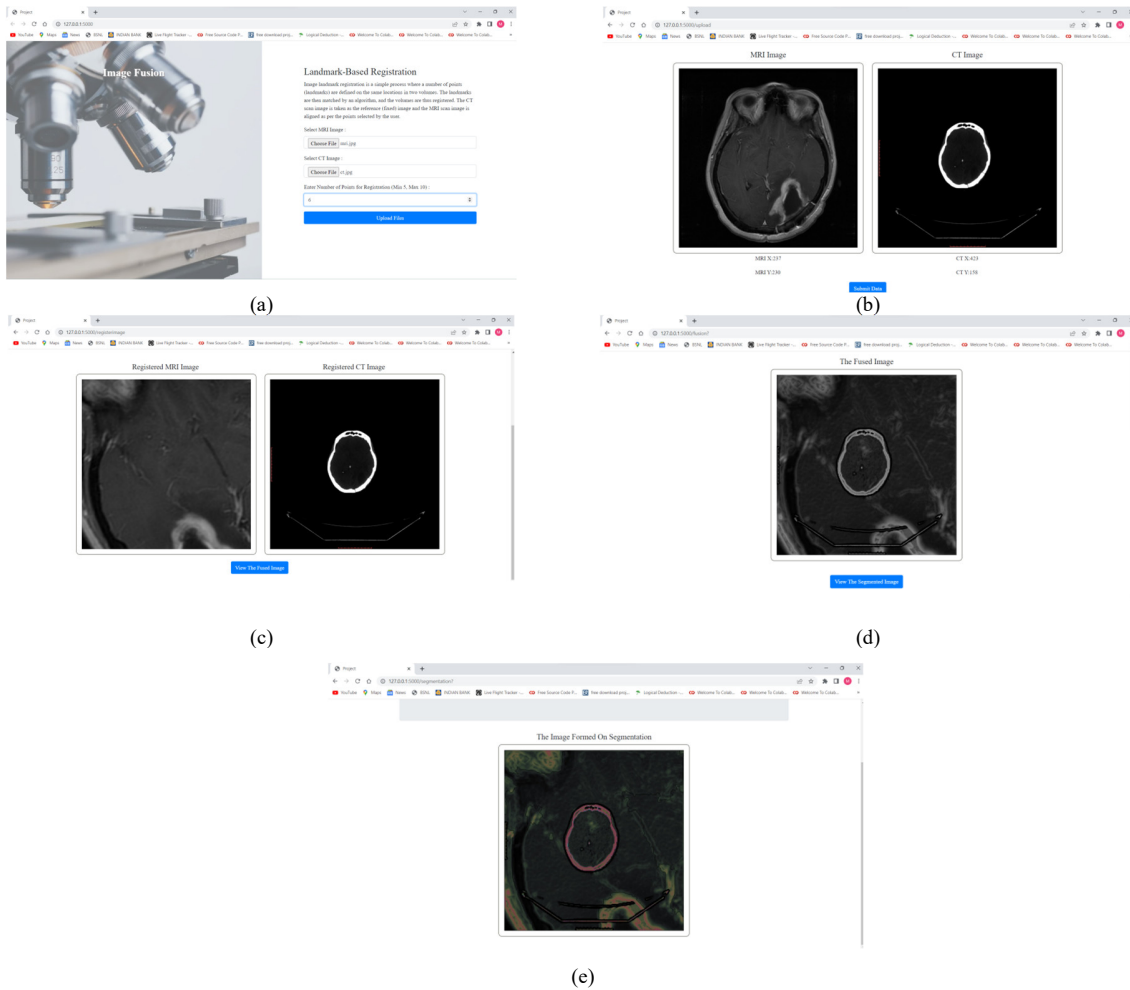


Fig. 7 Steps to use the proposed model in an application (a) User interface for uploading an MRI and CT image with registration process number; (b) User interface for uploading an MRI and CT image with registration process number and selecting the coordinates for registration process of MRI and CT image; (c) Registered MRI and CT image; (d) Fused image after applying fusion method on CT and MRI image; (e) Final fused segmentation image to diagnose a brain tumor.

The method also incorporated CNN, CNN-VGG16, and IDWT models based on CT, MRI, and fused images with different metrics. According to the assessment results, the suggested method produced a high-quality fused image with low MSE and high PSNR, SSIM, and FF values. A comparison was carried out with existing methods such as NSCT+LE, NSCT+RPCNN, and NSST+PAPCNN, see in Table 2. In NSCT+LE, a phase congruency and local laplacian energy-based rule in the NSCT domain was used. Congruency was applied in high-pass sub-bands, and energy was used in low-pass sub-bands to reduce the computational cost and improve fused image quality. NSCT+RPCNN used a fuzzy-adaptive reduced pulse-coupled neural network in the NSST domain, underscoring a simple structure, and fewer parameters to increase computational efficiency. Furthermore, the parameter-adaptive pulse-coupled neural network (PA-PCNN) was applied in the NSCT domain to preserve the energy and extract the image details, referred to as NSCT+PAPCNN. Compared with the existing methods in different transform domains, the proposed model had superior performance based on the PSNR and SSIM evaluation metrics. The deep learning-based algorithm CNN works better in edge detection. Therefore, in the future, the proposed model can be used with CNN to improve the accuracy of the fused image detection.

The assessment metrics showed that the proposed methodology surpasses existing fusion methods in terms of quality and information retention, producing high-quality fused images. In general, the results underscore the potential effectiveness of the proposed method for fusing medical images to assist medical diagnosis and treatment planning. Systems for medical imaging of CT and MRI are useful in identifying different bodily flaws and problems.

## VI. CONCLUSION

In conclusion, the proposed image fusion method showed promising outcomes in enhancing the quality of medical images using DWT and IDWT. The evaluation criteria, such as PSNR and SSIM, offer impartial measurements for assessing image quality. The results showed that the suggested method outperformed existing methods in terms of PSNR and SSIM values, suggesting improved image quality and greater accuracy with 98%. It was also found that the visual contrast and detail were improved, while the noise level was effectively lowered. A visual examination of the combined images validated the enhancement in image quality and diagnostic precision.

Future studies are needed to explore how the method can be applied to various medical image modalities, such as CT and MRI scans. There is also a need to investigate the efficacy of different image fusion methods, including multi-scale and deep learning, to improve algorithm compatibility and computational efficiency. A user-friendly tool for medical practitioners to execute image fusion in clinical settings might be developed as another field of study, which could speed up diagnosis and treatment planning.

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**Informed Consent:** There were no human subjects

**Animal Subjects:** There were no animal subjects

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