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Delta projection imaging on contrast-enhanced ultrasound to quantify tumor microvasculature and perfusion

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Abstract

Rationale and Objectives—To assess the delta (Δ) projection image processing technique for visualizing tumor microvessels and for quantifying the area of tissue perfused by them on contrast-enhanced ultrasound images.

Materials and Methods—The Δ -projection algorithm was implemented to quantify perfusion by tracking the running maximum of the difference (Δ) between the contrast-enhanced ultrasound image sequence and a baseline image. Twenty-five mice with subcutaneous K1735 melanomas were imaged first with contrast-enhanced grayscale and then with contrast-enhanced power Doppler (minexCPD) ultrasound. Δ -projection images were reconstructed from the grayscale images, then used to evaluate the evolution of tumor vascularity during the course of contrast enhancement. The extent of vascularity (ratio of the perfused area to the tumor area) for each tumor was determined quantitatively from Δ -projection images and compared with the extent of vascularity determined from contrast-enhanced power Doppler images. Δ -projection and minexCPD measurements were compared using linear regression analysis.

Results— Δ -projection was successfully performed in all 25 cases. The technique allowed dynamic visualization of individual blood vessels as they filled in real-time. Individual tumor blood vessels were distinctly visible during early image enhancement. Later, as an increasing number of blood vessels were filled with the contrast agent, clusters of vessels appeared as regions of perfusion, and identification of individual vessels became difficult. Comparisons were made between the perfused area of tumors in Δ -projections and in minexCPD images. The Δ -projection perfusion measurements correlated linearly with minexCPD.

Conclusion— Δ -projection visualized tumor vessels and enabled quantitative assessment of the tumor area perfused by the contrast agent.

Keywords

Ultrasound contrast agents; power Doppler; angiogenesis; vascular disrupting agent; tumor perfusion

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INTRODUCTION

Vascular targeting has evolved into an attractive approach for cancer therapy. Disruption of the tumor vasculature deprives the neoplasm of oxygen and eventually leads to tumor cell death. Several promising vascular disrupting agents (VDAs), including pharmaceutical [1,2] and non-pharmaceutical [3-6] agents, are currently being investigated in different phases of clinical trials. Such VDA evaluation requires an accurate method for the direct assessment of tumor vascularity in response to treatment. Measurement of tissue microvessel density by tumor biopsy is one potential approach for monitoring therapy. However, repeated assessment is often not possible due to the invasive nature of the technique, and the procedure is prone to sampling error due to the small sample size. Destruction of blood vessels by the probing biopsy needle could further complicate the assessment. Direct visualization of tumor vasculature by imaging is an attractive alternative for assessing VDAs as it can be performed repeatedly and it provides a global view of the change in tumor vascularity either across an anatomic plane or over the entire tumor volume.

Diagnostic ultrasound is one of the most commonly used modalities for cancer imaging. The equipment is portable and is widely available in large and small imaging centers. It provides both high spatial and temporal resolution, and the Doppler mode enables direct visualization of tumor blood vessels. Currently, clinical scanners allow visualization of blood vessels that are 0.2 to 0.5 mm in diameter, however, to image smaller blood vessels similar to those targeted by VDAs, ultrasound contrast agents must be employed. Various ultrasound techniques have been proposed that use a contrast agent in combination with power Doppler [3,4,7-11] or grayscale imaging (fundamental [7,12], harmonic [13-15], sub-harmonic [16]). Each approach has its strengths and weaknesses.

While contrast-enhanced power Doppler imaging is robust and exhibits high sensitivity in detecting microvasculature, the technique is prone to motion and blooming artifacts. Harmonic and sub-harmonic modes of imaging detect the signal generated primarily from the nonlinear oscillation and collapse of microbubbles. These approaches visualize the contrast agent directly but suppress the background tissue. To assay tumor vascularity, it is important to image both tumor tissue and the vasculature. Fundamental grayscale imaging allows the visualization of both the background tissue and the contrast agent; however, the contrast enhancements are difficult to appreciate due to strong echoes from the background tissue. Techniques that emphasize vascular structures in the contrast-enhanced images have been introduced recently on commercial scanners, but the details of the algorithms and their validation are not available in the open literature.

In this study we propose an image processing technique, Δ -projection imaging, performed on grayscale images to visualize both the tumor vasculature and the surrounding tissue. In the same anatomic plane, the measurement of the vascular area of the tumor relative to the cross-sectional area of the tumor allows quantitative estimation of the percent area of the tumor receiving contrast agent. This area is intimately related to fractional blood volume or tumor perfusion and in this article we have used these terms interchangeably.

MATERIALS AND METHODS

Animal and Imaging Studies

Female C3H/HeN mice (6 to 8 weeks old; $n = 25$) obtained from Charles River Laboratories (Wilmington, MA, USA) were used in this study. Tumors were implanted in each mouse as previously described [3]. This involved injecting 1 to 2 million K1735 melanoma cells subcutaneously into the right flank of the anesthetized animals (intraperitoneal administration of 140 mg/kg ketamine and 1.3 mg/kg xylazine). Following cell inoculation, the tumors were

imaged after growing to 10 to 20 mm in at least one dimension (approximately 3 to 8 weeks). All the animal procedures were conducted according to the protocol approved by the University's Institutional Animal Care and Use Committee.

For tumor imaging, general anesthesia was induced and maintained with isoflurane and oxygen in each mouse, and the hair coat overlying the tumor was removed by clipping and by application of a depilatory cream. The animals were imaged with a broadband high-frequency ultrasound transducer (7-15 MHz, Philips ATL 5000, Philips Ultrasound, Bothell, WA), which was mounted on a stand and fixed in a position aligned along the longest axis of the tumor. The imaging transducer focuses the ultrasound beam at 1 cm depth with a beam width of 0.6 mm in the focal region. The beam width broadens distal to focus but is within 1 mm at 2 cm depth. 0.2 mL of an ultrasound contrast agent (Definity, Bristol-Myers Squibb, North Billerica, MA) was injected via a 26 gauge tail vein catheter. The mean diameter of the microsphere bubbles as described by the manufacturer of the contrast agent ranges between 1.1 μm and 3.3 μm , and 98% of the microspheres are less than 10 μm in diameter. Following contrast agent injection, grayscale B-mode images were immediately acquired with a low mechanical index (MI) of 0.1. The depth and field of view for each animal study were adjusted such that the tumor occupied the maximum area in the image. The average ($\pm$ standard deviation) pixel size of all the images studied was 510 $\mu\text{m} \pm 66 \mu\text{m}$. Δ -projection images of each tumor were constructed from grayscale images (further details are given below) to demonstrate tumor perfusion.

A smaller dose of 0.02 mL of the contrast agent (compared to that used for Δ -projection) was injected and minimum exposure contrast-enhanced power Doppler (minexCPD) was performed to confirm tumor perfusion (further details are given below). Along with the low dose of contrast agent, the highest dynamic range available on the scanner was used to minimize potential blooming artifacts. To ensure high-quality images, minexCPD was performed at an MI of 0.8, and the ultrasound scanner was externally gated at 0.5 frames.s⁻¹ to minimize bubble destruction [3,8].

In previous studies, minexCPD acquired at a low frame rate yielded measurements of the percentage area of flow (PAF) for the slow flow conditions that occurred within the tumors [17]. PAF values determined from the Δ -projection and minexCPD studies were compared (further details are given below). All images were recorded on videotape and digitized for analysis, and at the completion of the observations each mouse was euthanized.

Description of Δ -projection Imaging Algorithm

Given a grayscale image sequence $\{\mathbf{a}_0, \mathbf{a}_1, \dots, \mathbf{a}_n\} \in F^{\mathbf{X}}$, with a pixel value set $F = \{0, 255\}$ on spatial domain $\mathbf{X}$, the delta projection sequence $\{\mathbf{d}_n\}$ of $\{\mathbf{a}_n\}$ is the *running* maximum of the difference (Δ) between the pixels of $\{\mathbf{a}_n\}$ and baseline image $\mathbf{a}_0$.

$$\{\mathbf{d}_n\} = \{\max(\mathbf{a}_0, \mathbf{a}_1, \dots, \mathbf{a}_k) - \mathbf{a}_0\}_{k=0}^n.$$

In contrast-enhanced imaging, the image sequence $\{\mathbf{a}_0, \mathbf{a}_1, \dots, \mathbf{a}_n\}$ represents images acquired as a function of time in a fixed plane. The '**max**' operator in the equation operates on individual pixels at the same coordinate through image sequence. For visualization of image enhancement, the projection is superimposed on the baseline image $\mathbf{a}_0$ in color, where color represents the magnitude of enhancement as indicated by the color bar in the images. The sequence of Δ -projection images is played back in real time to demonstrate the evolution of blood vessels as they are enhanced by the incoming contrast agent.

The algorithm is illustrated in Figure 1. The serial dataset shown in panel (a) of Figure 1 represents a time series of cross-sectional images consisting of pre-contrast (baseline) and post-contrast (target) images. Parallel ray tracing between the baseline and the target image at index i ($\mathbf{a}_i$) is performed through the tumor pixels, and the maximum difference (Δ) between the target image and the baseline image ($\mathbf{a}_0$) is projected back onto the i^{th} image plane. The projection data are superimposed on the baseline image in color to form image $\mathbf{d}_i$ (Fig. 1b). The sequential process of ray casting, Δ -projection, and superimposition is repeated iteratively until the entire dataset is analyzed. For each image in the time series, the percent area of flow is determined by measuring the area of the tumor enhanced as described below.

Image Analysis for Δ -projection and minexCPD

Δ -projection imaging—The dataset consisted of images acquired before and after contrast injections. A region representing the tumor was outlined in the first pre-contrast B-mode image of the dataset. Sequential projection images of the tumor region in the images were reconstructed from the serial contrast-enhanced images by ray tracing the running maximum change in image enhancement as described above. A threshold gray level difference was applied to segment blood vessels enhanced by the inflow of contrast agent from the surrounding tissue. The threshold value was determined by studying the baseline pre-contrast images of five randomly selected mice. In each animal, the threshold was increased in a stepwise fashion in 50 sequential pre-contrast images. For each threshold value, projection images were constructed and examined quantitatively. Quantitative assessments were based on measuring the highest percent area of flow [PAF(Δ)] in the 50 images as described below. The threshold that showed no enhancement (PAF(Δ) $\leq$ 2.5 %) was identified and used for constructing projection images of all the animals. In summary, the procedure consisted of increasing the gray level difference stepwise until the Δ -projection images showed the expected absence of enhancement in the pre-contrast images.

The projection image data were superimposed in color on the grayscale images. In principle, this approach mapped the pixels of the blood vessels that were enhanced by the passage of the highly echogenic contrast agent. For each image in the time series of the projection images, the percent area of flow [PAF(Δ)] was determined by taking the percent ratio of pixels (n_Δ) enhanced by contrast agent to the total number of pixels (N) in the tumor cross-section:

$$\text{PAF}(\Delta) = 100 * (n_\Delta / N).$$

With the inflow of contrast agent, an increasing number of blood vessels are filled and the measured percent area of tumor flow PAF(Δ) increases with time. Following peak enhancement, there is no further increase in PAF(Δ) and the PAF(Δ) versus time curve plateaus. An initial increase followed by a plateau in PAF(Δ) was observed experimentally as illustrated in Figure 3 of Results. The plateau value of PAF(Δ), representing the total area of tumor perfused, was measured and compared with the data obtained from minexCPD imaging.

minexCPD imaging—A region representing the tumor was outlined in the pre-contrast image and superimposed on the post-contrast enhanced images. Using the color palette on the Doppler scans, a lookup table for the color levels was constructed [18]. The color lookup table was used to identify colored pixels within the tumor. As for Δ -projection imaging, the percent area of flow PAF(D) for each minexCPD image was determined by the percent ratio of pixels (n_D) enhanced by contrast agent to the total number of pixels (N) in the tumor cross-section:

$$\text{PAF(D)} = 100 * (n_p / N).$$

Comparison Between Δ -projection and minexCPD

The images recorded at the peak values of PAF(Δ) and PAF(D) were visually compared to determine whether their depictions of tumor perfusion were similar. The peak values of PAF(Δ) and PAF(D) were also fitted to a linear regression model ($y = mx$), and the slope (m) and regression coefficient (R) were used to assess correlation between the two measurements.

RESULTS

In the fundamental B-mode images before contrast injection, the melanoma typically had a uniform echogenic texture and its boundaries were distinct (Fig. 2a). Δ -projection imaging provided a clear dynamic display of the neovasculature of each tumor (Figs. 2b to 2e). Since the microbubbles of the contrast agent were intravascular, the colored pixels showed the microvessels within the tumor to be on the order of 250 μm in diameter (as measured directly from the images). Initially, the larger blood vessels ($>250 \mu\text{m}$ in diameter) with a faster flow rate were revealed and then the smaller diameter vessels (estimated from the images to be on the order of $\leq 250 \mu\text{m}$ in diameter), presumably with slower flow rate, became visible. These later vessels appeared as either branches of the larger vessels or as new blood vessels surrounding a main vessel. As the contrast flowed into the smaller vessels, their branches appeared (Fig. 2c) and the complex network of tumor blood vessels, formed as a result of angiogenesis, became increasingly visible (Fig. 2d). With the further inflow of the contrast agent to the capillaries and venules, the visible vascular network became increasingly filled with microbubbles, so that eventually individual vessels could not be identified (Fig. 2e); these changes can be better appreciated in real-time video displays than in static individual two-dimensional images. As the contrast filled more blood vessels, the density of the vessel network in the images increased, and the aggregate of blood vessels appeared as regions of perfusion. The area of the tumor perfused by the contrast agent increased with time and then leveled off after maximum perfusion was achieved. The plateau of the area vs. time curve corresponded to the total area of tumor perfusion (Fig. 3).

Each Δ -projection image displays the cumulative sum of tumor vessels as the neovasculature was progressively enhanced by the inflow of contrast agent. The point of maximum enhancement represents the total percentage area of the tumor perfused by contrast agent, which in the Δ -projection images made in this melanoma model ranged from 14% to 100% (fully perfused). In visual comparisons between Δ -projection and minexCPD images, identical regions of the tumor were appeared either vascular or avascular, so there was a high visual correlation between the two imaging techniques (Fig. 4).

The PAF(Δ) and PAF(D) data fit closely to the linear model with a slope (m) of 0.95 and a regression coefficient (R) of 0.94 ($R^2 = 0.88$; Fig. 5). The slope of $0.95 < 1$ indicates that PAF(Δ) was 5% lower than that estimated by PAF(D).

DISCUSSION

It is now well recognized that angiogenesis and microvascular flow play important roles in tumor growth and metastasis [19,20], and there have been intense research efforts to develop antiangiogenic and antivascular therapies to impede tumor perfusion [1-6]. Thus there is a great need for methods that accurately assess tumor perfusion in pre-clinical and clinical studies. This study introduced the Δ -projection technique as a means of imaging tumor blood vessels and providing a quantitative assay of the area of the tumor receiving contrast agent; a

measurement closely related to fractional blood volume or tumor perfusion. The difference between Δ -projection images and simple maximum projection using the whole image sequence is that, whereas maximum projection produces a single image representing the cumulative sum of all tumor vessels, Δ -projection shows the time course of individual vessel evolution as the tumor is filled-in by the contrast agent. In all tumors, Δ -projection highlighted small individual blood vessels ($\leq 250 \mu\text{m}$ in diameter) that were usually not visible in the unprocessed contrast-enhanced B-mode images. The tumor microvessels that form as a result of angiogenesis are estimated to have a diameter of $< 100 \mu\text{m}$ [21] and were thus evident in the Δ -projection images. Further studies involving correlation with histology or other imaging methods like CT or MR will be needed for additional evidence. The Δ -projection technique appears to have some similarity to the vessel tracking features available on certain commercial scanners. Since the details of the commercial algorithms are unavailable in the open literature, a comparison is not possible.

In qualitative observations of the Δ -projection images, it was found that larger blood vessels, apparently with a faster flow rate, were initially filled with the contrast agent, and then the smaller diameter vessels, presumably with slower flow rate, became visible. Huber et al observed that the enhancement of tumor vessels was faster in carcinomas than in benign lesions [22]. The relationship between the size of vessels and the time of enhancement exhibited in this study could have important clinical implications. In the future, Δ -projection imaging could be used to estimate the proportions of different sized blood vessels in a tumor, and such data could help differentiate malignant and benign lesions.

Several previous studies have documented the role of contrast-enhanced power Doppler in assessing tumor vasculature [3,8,10,11,21,22]. To determine its feasibility for evaluating tumor perfusion, Δ -projection imaging was compared to minexCPD. Although a high MI of 0.8 was used for obtaining high-quality Doppler images, minexCPD was performed at a low frame rate to minimize ultrasound exposure and the associated microbubble destruction. Δ -projection and minexCPD demonstrated a high correlation. In both groups of images, the aggregate of large and small blood vessels appeared as colored regions. The unperfused areas of the tumor appeared as regions without detectable color in the images. Although the individual blood vessels in the areas of maximum enhancement were difficult to resolve in both the Δ -projection and minexCPD images (due to a high density of the blood vessels), the Δ -projection images better resolved the progressive filling of the neovasculature with the contrast agent. The difference may in part be due to the larger sample volume of the ultrasound pulses used in power Doppler images.

The percent areas of flow measured from Δ -projection and minexCPD imaging were linearly correlated. Given the complexity of the two techniques (thresholding in Δ -projection imaging and possible blooming artifact in minexCPD imaging), the observed high correlation should be interpreted conservatively as it does not represent equivalence, but an association. Δ -projection imaging did, however, provide an assessment of tumor perfusion consistent with existing approaches. While many have used different imaging methods to estimate perfusion [23], to our knowledge there is no universally accepted gold standard for the noninvasive measurement of tumor perfusion. MinexCPD imaging, although a sensitive method for visualizing regions of blood flow, has limitations, such as blooming artifacts that overemphasize tumor vasculature. These artifacts were minimized in this study by using a low volume of an ultrasound contrast agent and the highest dynamic range available on the scanner. The comparison between Δ -projection and minexCPD was made primarily because each used the same contrast agent and the same anatomic plane was imaged. The agreement between the two distinct approaches (Δ -projection and minexCPD) is evidence that the two methods are assessing the same tissue property: how well a tumor is perfused by the contrast agent.

High-quality Δ -projection imaging requires a lack of tissue motion between consecutive image frames. Movement of tissue between image frames could blur the image, and if the motion is significant and the consecutive images are decorrelated, there is potential for a flash artifact. None of the Δ -projection images in this study exhibited a flash artifact, since the transducer was fixed in a holder and also the tumors were distant from the thorax and likely unaffected by motion related to ventilation. Future studies could investigate the use of computer algorithms to factor out motion to produce Δ -projection images free from flash artifacts. In its current form, however, we anticipate that the Δ -projection technique will allow imaging of solid tumors where motion effects due to ventilation or contractions of the gastro-intestinal tract are likely to be minimal; these could be cancers of the skin, thyroid, prostate, uterus, ovaries, and musculoskeletal system. In principle, Δ -projection imaging could be used with any ultrasound contrast-specific technique, including fundamental and harmonic modes of imaging. However, there would be advantages in combining Δ -projection with fundamental grayscale imaging, which is universally available for a wide range of imaging frequencies (2-20 MHz) on low- to high-cost ultrasound scanners.

In summary, this study introduces the Δ -projection algorithm for imaging networks of tumor microvasculature. The results demonstrate that Δ -projection visualizes individual microvessels for qualitative and quantitative assessment of tumor perfusion. These results are encouraging, and we anticipate that further development and testing will expand the technique's application to cancer diagnosis and to monitoring antiangiogenic and antivascular therapies.

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Figure 1.

Diagram of Δ -projection imaging. (a) The running maximum grayscale difference between the i th ($\mathbf{a}_i$) and baseline image ($\mathbf{a}_0$) of the time series of contrast-enhanced images is projected onto the baseline image as pseudocolor to construct the Δ -projection image sequence $\mathbf{d}_n$. (b) The Δ -projection calculation is repeated on each image to form a time sequence of projection images.

Figure 2.

Time course of Δ -projection images showing increasing tumor vascularity. Colored regions represent vascular regions enhanced by the inflow of contrast agents. Images (a) through (e) are the images at 0, 5, 6, 7 and 18 seconds after a bolus injection of contrast agent. The bars on the right side of each panel represent 1 mm. There is a mild increase in echogenicity from the center of the tumor to the periphery (Figure 2a). The bright echo at the bottom of the panels is the reflection from the tumor-air interface distal to the imaging transducer. Blood vessels of 250 μm diameter (open arrows) or smaller become visible in the image (Figures 2b and 2c). The branching network of tumor blood vessels becomes increasingly complex with the inflow of more contrast agent (Figures 2d and 2e). The total area of perfusion in Figure 2e is 90%. Visually, although most of the area is covered by colored pixels, the interior has regions dotted with non-colored pixels showing lack of perfusion.

Figure 3.

The time evolution of the tumor area enhanced by the inflow of contrast agent. Time points marked by the arrows (a) through (e) correspond to images shown in Figure 2 at 0, 5, 6, 7, and 18 seconds after a bolus injection of contrast. The tumor area perfused by the contrast agent increases with time then levels off after a maximum perfusion is achieved. The plateau of the curve corresponds to the total area of perfusion.

Figure 4.

Visual comparison of tumor perfusion imaged by Δ -projection and by minexCPD. (a) Δ -projection image of a mouse tumor; (b) minexCPD image of the mouse tumor shown in (a); (c) and (d) number the correlated regions of vascularity from the the Δ -projection and minexCPD images in (a) and (b). Eight different regions designated by numbers 1 to 8 in the Δ -projection image (Figure 4c) correspond to regions 1' to 8' in the minexCPD image (Figure 4d). Although there are some differences, each tumor perfusion region (for example regions 1 and 1') is highly correlated in the two images. The total percent area of perfusion (PAF) was 63.9% by Δ -projection and 59.3% by minexCPD.

Figure 5.

Graph of the correlation between percent area of flow (PAF) determined from Δ -projection and from minexCPD imaging. The solid line is the linear regression fit of the data. The dotted line is the line of identity with unit slope. The PAF(Δ) and PAF(D) data fit closely to the linear model with slope (m) of 0.95 and regression coefficient (R) of 0.94 ($R^2 = 0.88$). The slope of $0.95 < 1$ indicates that the percent flow area estimated by Δ -projection was 5% lower than that estimated by minexCPD.