

行政院國家科學委員會專題研究計畫 成果報告

藥物載體之超音波操作技術(III) 研究成果報告(精簡版)

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中 華 民 國 97 年 05 月 09 日

行政院國家科學委員會補助專題研究計畫 ☒ 成果報告
☐ 期中進度報告

藥物載體之超音波操作技術(三)

計畫類別：☒ 個別型計畫 ☐ 整合型計畫

計畫編號：NSC95-2221-E-002-442-

執行期間：95年8月1日至96年7月31日

計畫主持人：曹建和

共同主持人：

計畫參與人員：

成果報告類型(依經費核定清單規定繳交)：☒ 精簡報告 ☐ 完整報告

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☒ 出席國際學術會議心得報告及發表之論文各一份

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執行單位：國立台灣大學電信工程研究所

中 華 民 國 97 年 5 月 6 日

中文摘要

隨著醫療發展的演進，藥物之改良釋放劑型開始蓬勃發展，其目的無非是因為這些改良釋放劑型在控制藥物釋放時間以及決定最佳的釋放時間上，我們可以較容易的掌握。且改良釋放劑型(modified release dosage form)在降低毒性、增加療效以及減少投藥次數上有顯著的效果，所以可以預期的是藥物改良劑型(modified - release dosage form)仍然將是未來醫藥發展的趨勢。醫用超音波具有使用便利、安全性高、成本較低以及診斷可信度高等許多優點，在疾病的診斷與治療上，已經佔有舉足輕重的角色；我們在本計劃中將使用自製以及商用的微氣泡來做研究的材料，他們可以被用來當作藥物載體或是用來探究超音波藥物傳輸系統載體特性的探討，我們期望結合超音波兼具觀察與釋放的優點以及自製的藥物載體以及商用對比劑的特點，建立了一套超音波藥物傳輸系統，並期望它能適用於腫瘤治療或是其他的臨床醫療上。

在本計畫中，我們將整個研究分為四個部份來進行研究：一、對比劑的超音波信號特性分析。二、微氣泡對超音波訊號的響應模擬。三、載體破裂控制。四、載體殼層參數探討與掌握。

英文摘要

With the progress of biomedical engineering, the modified-release dosage mechanism has started to develop prosperously in recent years. It is easier to handle modified-release dosage for controlling and determining the optimal time of releasing medicine, and the modified-release dosage has obvious effects on diminishing toxicity, raising curative effect and decreasing the times of medication. Hence, we could expect that modified-release dosage form will still be the tendency of the medical development. Ultrasound has several advantages, such as convenience to use, safety of non-invasive, lower cost, and reliable diagnosis. Consequently it has become to play a significant role both on the diagnosis and therapy.

In this project, we will use self-made and commercial contrast agents for our research. It can be used for drug carrier and we will also survey their acoustic characterization. We attempted to set up an ultrasound drug delivery system(UDDS) by combining the excellent acoustic properties of self-made contrast agents and the advantages of ultrasound for monitoring and releasing the drug and expect to utilize it in cancer therapy or other clinical medicine. Therefore, this project will aims at the following issues:

- Analyzing the acoustic characteristics of contrast agents
- Stimulating the nonlinear microbubble response
- Controlling the destruction of drug carrier by ultrasound
- Research on the shell effect and mechanic parameter

前言

對於超音波反應於組織的特性而言，衰減係數是個重要的估測參數。一般估測組織的衰減係數大略可分為利用背散射訊號估測以及穿透訊號估測兩種。其中，背散射訊號的估測方式可適合於大部分的應用。但是卻有散射成份干擾的困難。應用穿透訊號的方式是較為適合於估測衰減係數，但是由於必須在組織兩端皆放上探頭，因此只適合體外實驗用。近年來由於超音波對比劑的應用增加，使得我們可以發展一個新方法，使用對比劑來估測體內組織的衰減係數。由於對比劑有高度非線性的特性，因此我們藉由使用多組頻率激發組織之後的對比劑，我們可應用其所產生的諧波訊號達到應用穿透訊號估測組織衰減係數的目的。

對於結合對比劑應用與組織衰減係數估測的方法開發，我們計算經由對比劑回波訊號中的諧波成分來估測理論中組織的衰減係數，其中必須利用周期譜的方式求得訊號的功率頻譜密度。

研究目的

醫用超音波開始應用於臨床醫學上，至今已經超過五十年之久。由於其診斷方式是以回波信號估測達到非侵入式的優點，因此，它具有使用便利、安全性高以及成本較低廉等優點而常被應用於肝臟病變的診斷。然而使用回波訊號所得到的訊息，常常因為組織散射干擾等因素而使得診斷結果不如使用於穿透訊號來診斷的系統（如：骨質檢測）精確。

已導致現今超音波對肝臟疾病診斷常被定位在用於輔助抽血檢驗的結果。

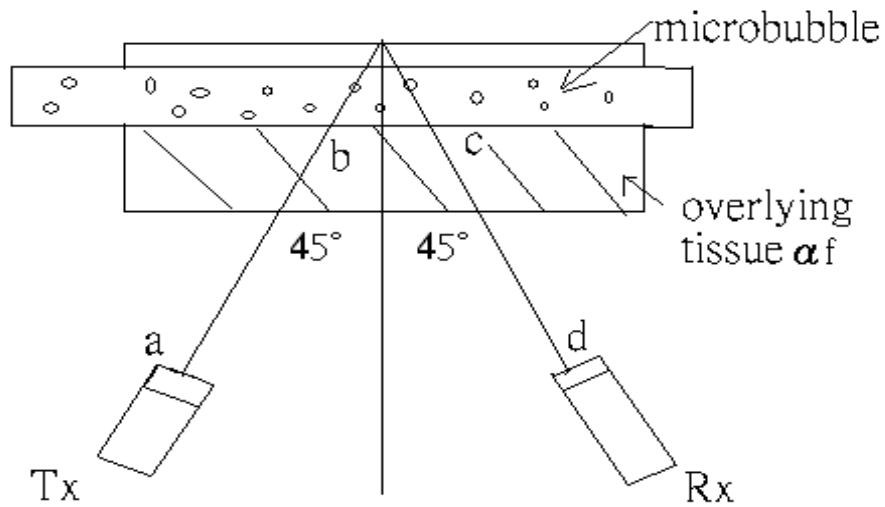
一般而言，我們利用超音波訊號經由物質所產生的信號衰減、背散射係數、信號漂移、聲速等參數以辨別肝臟的病變（如：纖維化、肝硬化、脂肪肝等）。而由於近年來超音波對比劑技術的蓬勃發展，應用其高度非線性的行為，使得利用高次諧波成分達到穿透式的硬化診斷成為一項可能的技術。雖然，衰減係數對於診斷大範圍的組織病變可以得到相當準確的估測。但是卻無法診斷隨機分布於肝臟內的小病變。因此，本論文的基礎為藉由使用多組頻率激發組織之後的對比劑，我們可應用其所產生的諧波訊號達到應用穿透訊號估測組織衰減係數。最終利用所得到的衰減係數以診斷局部病變的所在位置以及肝臟整體的健康狀況。

理論背景

應用對比劑的諧波信號估測肝組織衰減係數必須假設待測組織包覆一條大血管使其中存在著隨機散佈的微氣泡。在此，我們將利用功率放大器激發微氣泡，並將其所產生的諧波信號是為隨機信號原，用以估測肝組織所造成的衰減。

圖一為上述環境的簡化模型，我們可看到肝組織及其包覆的血管。微氣泡隨機分佈於血管當中。在此所定義的待測組織位於血管與探頭之

間。發射探頭傳送一大功率信號激發微血管內的微氣泡使其產生二次諧波，之後諧波信號穿過待測組織，並由接收探頭接收。當訊號從發射到接收，會與組織極為氣泡交互作用而產生四次改變，以下會描述其訊號改變藉以得到最後的訊號模型。



圖一 估測衰減的理論模型

假設訊號 a 為由發射端所傳送的單頻訊號，其中令振幅為 A_1 且頻率為 f_1 如下：

$$a = A \exp(j2\pi f_1 t)$$

首先，訊號 a 必須先穿過待測組織才會到達血管以激發微氣泡。在此，假設 (1) 對組織而言，穿透方向的散射成份相較於背散射時的狀況

是可以忽略的。(2) 由於微氣泡為極端非線性的物理特性，因此，與其相比較軟組織所產生的諧波成份會遠小於微氣泡所產生的部份。因此，我們在此可假設軟組織為隨頻率有 α 倍的指數衰減。我們可得訊號 b 為訊號 a 通過組織而得到血管前訊號：

$$b = A \exp(-\alpha f_1) \exp(j2\pi f_1 t)$$

在液體當中的微氣泡，經歷外壓時所產生的震盪行為可以 (RPNNP) 方程式所描述：

其中 R 為瞬時微氣泡半徑、 R_0 為微氣泡平衡時半徑、 ρ 和 μ 為液體的密度及黏滯度、 σ 為表面張力、 Γ 為 polytropic exponent、 P_0 為液體靜壓。由於我們必須得到微氣泡振盪函數。因此我們假設其隨時間所產生振盪半徑 R 為與平衡半徑 R_0 上一個線性組合的振盪成分 $R_0 x$ ：

$$R = R_0 (1 + x)$$

在此，我們有興趣的成份為信號的基本頻率 f_1 以及其二次諧振項 $2f_1$ 。因此，我們可將 R 代入 (7) 以及將 RPNNP 方程式簡化至二次向並加入輸入信號 b 而得：

$$\rho R_0^2 (1+x) x'' + \frac{3}{2} \rho R_0^2 x'^2 = \left(P_0 + \frac{2\sigma}{R_0} \right) \left[1 - 3\gamma x + \frac{3\gamma(3\gamma+1)}{2} x^2 \right] - \left[P_0 + \frac{2\sigma}{R_0} (1-x+x^2) \right] - 4\mu (x' - x'x) + A \exp(-\alpha f_1) \exp(j2\pi f_1 t)$$

因此，我們所得到的解 x 為：

$$x = A_0 + A_1 \exp(j2\pi f_1 t + \phi_1) + A_2 \exp(j2\pi 2 f_1 t + \phi_2)$$

其中 (1) A_0 為經由信號激發而改變的平衡半徑 (2) A_1 為經由信號激發之後所產生的微氣泡基頻強度 (3) A_2 則為微氣泡產生的二次諧波成份。而除了 A_0 之外， A_1 與 A_2 皆為信號頻率相關的振幅，Miller [19] 指出微氣泡可視為一個頻率選擇震盪器，其震盪頻率與微氣泡高度相關。我們可令當微氣泡振盪很微小以至於可將其是為線性振盪時所產生的振盪頻率為微氣泡的振盪頻率如下：

$$\omega_0^2 = \frac{1}{\rho R_0^2} \left[3\gamma \left(P_0 + \frac{2\sigma}{R_0} \right) - \frac{2\sigma}{R_0} \right]$$

可看到其與平衡半徑 R_0 高度相關。

在本實驗中所量測到經由激發微氣泡所得到的散射信號為探頭取樣

空間當中，微氣泡所產生振盪信號的總和。在此我們假設（1）使用一般微氣泡（free gas）時，氣泡半徑對於系統所發射的頻率範圍內都是均勻分布的函數（2）在探頭取樣空間中，氣泡本身是不會交互影響的。因此，在模型上可是為互不相關（3）在取樣空間中，因為微氣泡隨機位置所產生的隨機相位。假設為均勻分布在 $[0, 2\pi]$ 範圍當中。由於以上假設，我們可認定接收信號的功率頻譜密度可視為一個以發射訊號為振盪頻率的解

$$E[x^2] = A_0^2 + A_1^2 \big|_{f_0=f_1} \exp(j2\pi f_1 t) + A_2^2 \big|_{f_0=f_1} \exp(j2\pi 2 f_1 t)$$

其中：

$$A_0 = \frac{1}{2} A_1^2 \left[3\gamma - \frac{3\gamma(3\gamma+1)}{2} \right] / \left[-3\gamma + \frac{2\sigma}{R_0} \left(P_0 + \frac{2\sigma}{R_0} \right) \right]$$

$$A_1 = \frac{1}{4\mu} A \exp(-\alpha f_1)$$

$$A_2 = \frac{1}{2} A_1^2 Y \chi_2$$

以及：

$$\chi_2 = (9 + 4\delta^2)^{-\frac{1}{2}}$$

$$Y = \left[\left(\frac{5}{2} + \frac{3\gamma \left(P_0 + \frac{2\sigma}{R_0} \right) (3\gamma + 1) - \frac{4\sigma}{R_0}}{2\rho\omega_0^2 R_0^2} \right)^2 + \delta^2 \right]^{\frac{1}{2}}$$

因此，我們可得經由激發微氣泡所得到的背散射訊號 c 為：

$$c = A_0^2 + \left(\frac{A}{4\mu}\right)^2 \exp(-2\alpha f_1) \exp(j2\pi f_1 t) + \left(\frac{1}{2}Y\chi_2\right)^2 \left(\frac{A}{4\mu}\right)^4 \exp(-4\alpha f_1) \exp(j2\pi 2 f_1 t)$$

最後，由微氣泡所產生的訊號 c ，將藉由通過待測軟組織而計算其衰減係數 α ，信號 c 包含了基本頻率與二次諧振項。因此，二次諧振成份相當比基頻多經歷一次衰減。信號 d 則為接收探頭所得到最終信號：

$$d = A_0^2 + \left(\frac{A}{4\mu}\right)^2 \exp(-4\alpha f_1) \exp(j2\pi f_1 t) + \eta(f_1) \exp(-4\alpha f_1) \exp(j2\pi f_1 t) + \left(\frac{1}{2}Y\chi_2\right)^2 \left(\frac{A}{4\mu}\right)^4 \exp(-8\alpha f_1) \exp(j2\pi 2 f_1 t)$$

其中，第二項為組織所產生的背散射係數。因此，我們可分析訊號 d 為基頻成份除了精穿透所得的衰減情形之外，還包括組織所產生的背散射成份，而二次諧振項僅存在穿透訊號。

實驗及分析方法

以下將介紹超音波 RF 訊號的實驗方法、實驗器材配置、以及所使用的衰減估測技巧。

● 衰減係數的估測方法

基於以上理論分析的部分我們可以了解接收信號 d 包含組織背散射成份、基頻訊號、以及二次諧振訊號。我們發覺利用二次諧振訊號以估測衰減係數可迴避組織所帶來的干擾。因此，以下將解說使用窄頻訊號所產生的二次諧振估測組織衰減係數的實驗方法。

假設發射一頻率為 f_1 的窄頻訊號。並經由衰減係數為 α 的軟組織衰減過後，並將接收訊號的二次諧振成份利用帶通濾波器取出。我們可得如下的訊號成份，令其為 S_{overlay}

$$s_{\text{overlay}}(f_1) = \left(\frac{1}{2} Y \chi_2 \right)^2 \left(\frac{A}{4\mu} \right)^4 \exp(-8\alpha f_1) \exp(j2\pi 2 f_1 t)$$

倘若我們利用相同的發射訊號，但發射到另一個系統為無待測軟組織，但仍然存在相同環境的微氣泡。則應用同樣的接收方式並利用相同的帶通濾波器時，我們應該可得到類似無衰減成份的二次諧振訊號：

$$s(f_1) = \left(\frac{1}{2} Y \chi_2 \right)^2 \left(\frac{A}{4\mu} \right)^4 \exp(j2\pi 2 f_1 t)$$

若我們可分別得到以上兩種訊號，則我們可以利用相除之後取對數的方式得到在頻率 f_1 之下的局部衰減係數：

$$8\alpha f_1 = \ln \left(\frac{s(f_1)}{s_{\text{overlay}}(f_1)} \right)$$

當我們想要估測大範圍的頻帶時，我們仍可應用窄頻訊號做估測。我們可藉由求出各個不同頻率所對應的衰減功率所得到的斜率。因此，假設傳輸另一個窄頻訊號 f_2 其中 ($f_2 > f_1$) 時，應用之前方式，我們亦可得到一相對於頻率 f_2 之上的局部衰減係數

$$8\alpha f_2 = \ln \left(\frac{s(f_2)}{s_{\text{overly}}(f_2)} \right)$$

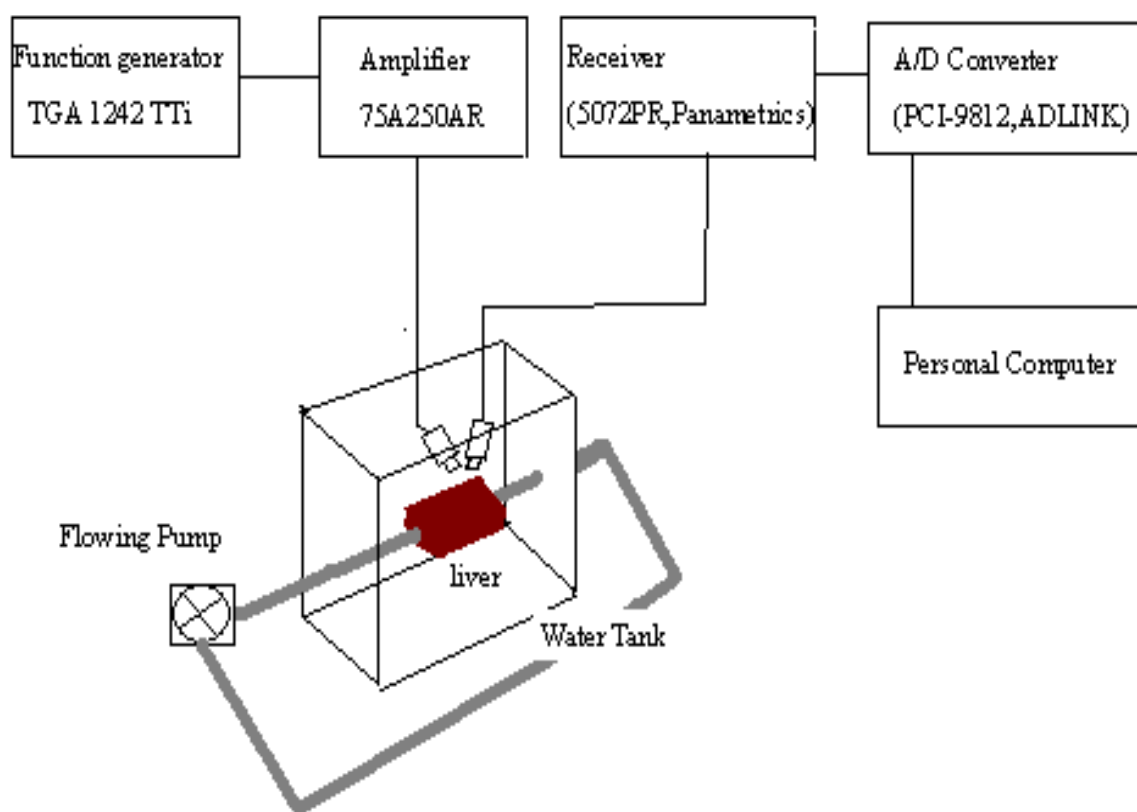
則我們可利用在 f_2 以及 f_1 的局部衰減係數得出在頻率範圍(f_1, f_2) 上的衰減係數 α 為：

$$\alpha = \frac{1}{8(f_2 - f_1)} \left[\ln \left(\frac{s(f_2)}{s_{\text{overly}}(f_2)} \right) - \ln \left(\frac{s(f_1)}{s_{\text{overly}}(f_1)} \right) \right]$$

● 超音波 RF 訊號分析與實驗環境架設

我們設計一個窄頻長脈衝，以高斯視窗函數(Gaussian window) 濾波器濾出主要頻帶，避免旁辦和儀器所產生的非線性干擾接收回來的訊號。

訊號的波形由電腦設計，然後將其輸入至訊號產生器產生 (Functional generator) 之後，經由功率放大器放大並由 2.25MHz 中心頻率的探頭髮射超音波訊號。訊號會因此通過仿體(Phantom)，為了接收由微氣泡所產生的二次諧振成份，我們使用另一支 5MHz 中心頻率的探頭接至接收器(receiver)(5073PR, Panametrics)。再傳至數位濾波器(oscilloscope, HP54645D)。示波器再經由 GPIB 介面傳送資料至電腦、存至記憶體及硬碟。訊號處理平台為 Matlab6.5 應用程式。探頭聚焦深度為兩公分。



超音波實驗系統架構圖

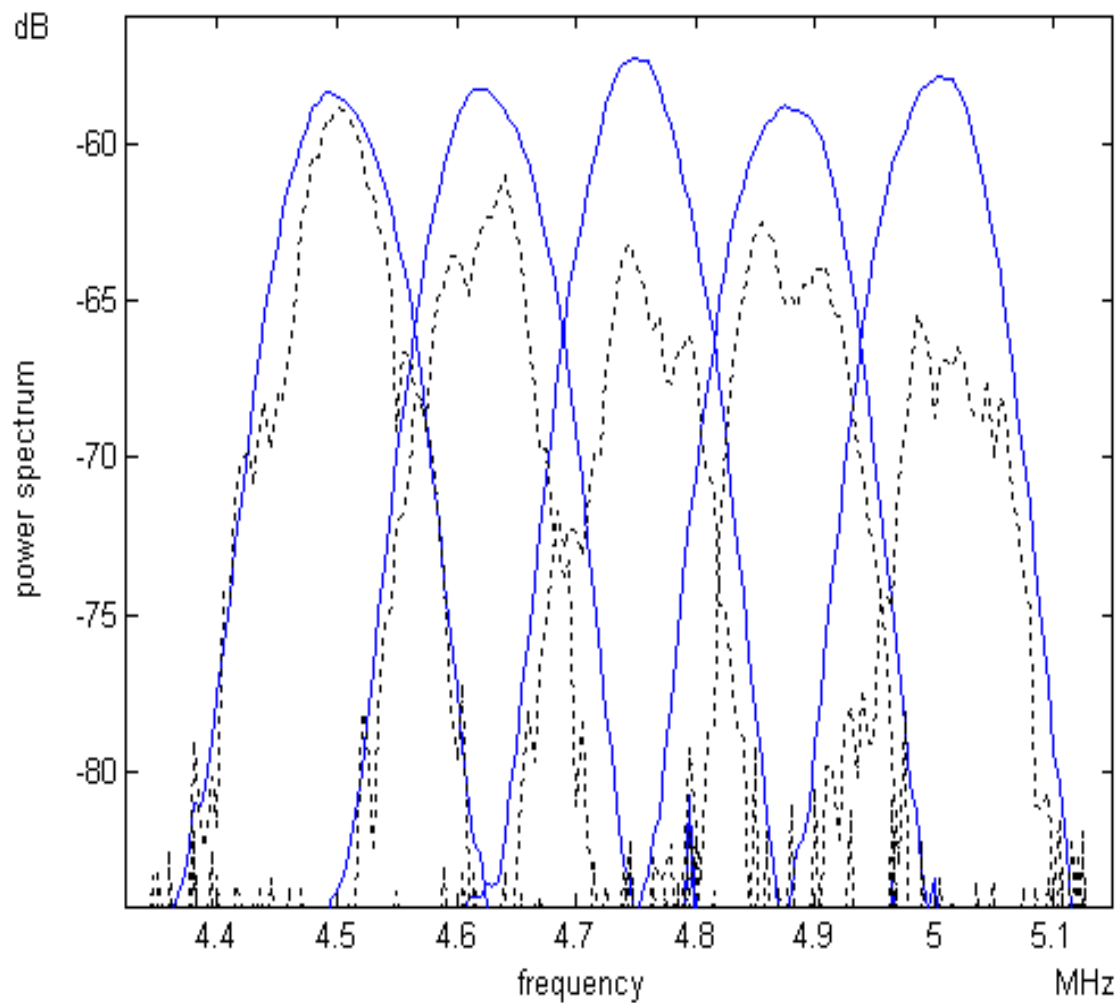
● 超音波實驗操作步驟如下

1. 實驗仿體與探頭架設方式如上圖，利用一為發射探頭做為超音波發射源，收取其 RF(radio frequency) 訊號做分析。探頭架設為發射與接收探頭成四十五度角以方便反射。
2. 使用一沉水馬達、水管、與仿體所架構的循環系統以模擬血流。其中，循環系統使用蒸餾水作為循環的媒介，以防止水中產生額外的微氣泡
3. 在每 100ml 的蒸餾水當中，加入 15ml 的空氣(free gas) 到循環系統當中。其中，循環的水流必須足夠快速以使得在每次訊號發射時，確保氣泡大小為均勻分佈。並且每次發射時都能打到不同的氣泡。
4. 在循環系統當中，使用方型仿體做為方便肝組織覆蓋的平台。
5. 發射多組窄頻訊號以量測頻率範圍為 0.5MHz 頻寬的衰減係數，其中 PRF 為 5KHz。
6. 接收訊號使用取樣頻率為 20MHz、12bit 的類比數位轉換器(analog to digital converter)將訊號輸入到電腦，並儲存於硬碟。
7. 分析訊號。

研究結果與討論

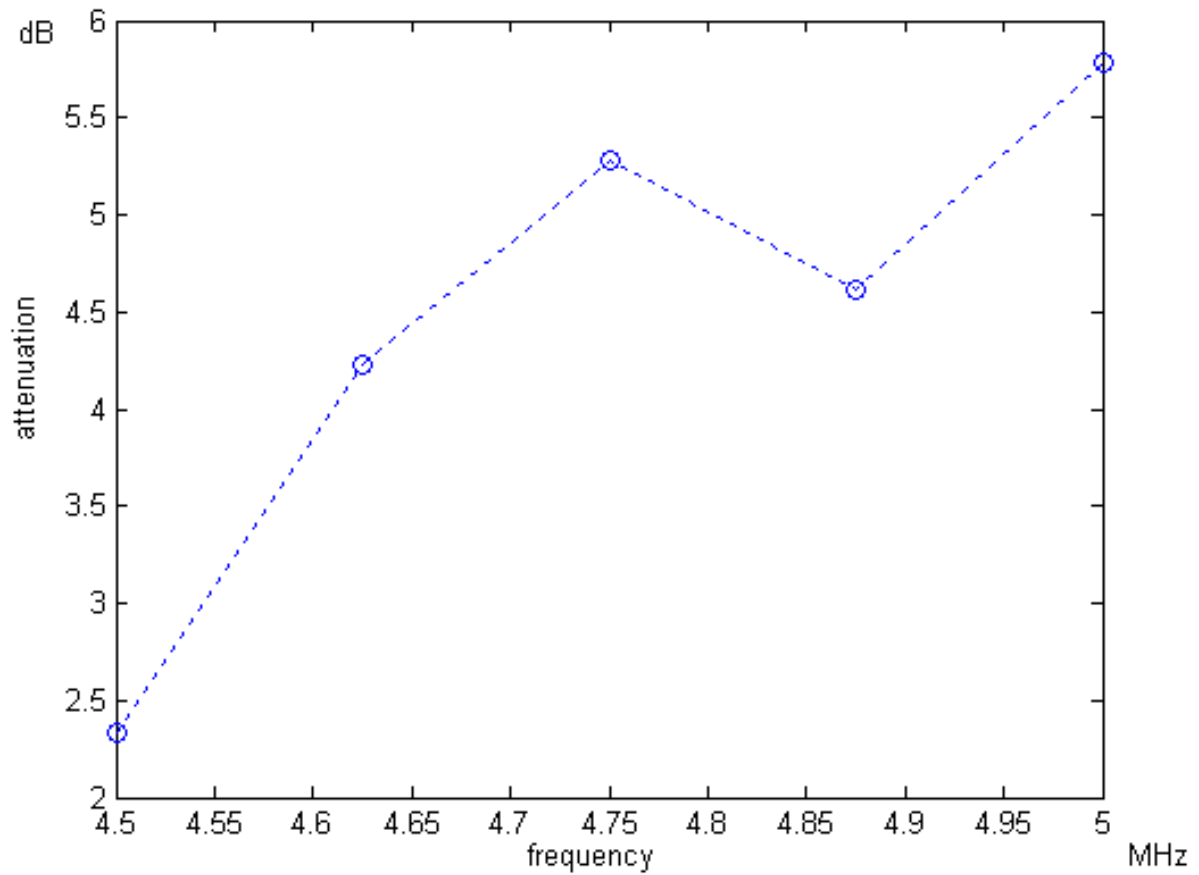
我們使用五個不同中心頻率，在頻帶範圍[2.25MHz 2.5MHz]之間發射訊號，以激發在循環系統當中的微氣泡。並接收二次諧振成份。其頻率範圍在[4.5MHz 5MHz]當中的二次諧振回波。

實驗訊號的量測分成兩個部份，第一部分為在放入組織之前，先行量測在循環系統內微氣泡二次諧振的統計特性。由於微氣泡的散射訊號本身為一個隨機程序，因此，我們必須求得在二次諧振頻率上的功率頻譜密度。利用 150 筆接收樣本對其作傅利葉轉換後取絕對值、對數、程已 20 之後作算術平均得到週期譜。第二部份則是重複第一次的量測方式，但此時在循環系統與探頭之間加入了覆蓋在管線上的肝組織樣本，加上了肝組織的影響後，我們發覺取樣所得的功率頻譜密度相較於覆蓋之前較為隨機。而我們可以圖示的方式比較利用五個頻率所得到包含肝臟及不包含肝臟時所求得的二次諧振項功率頻譜密度比。其中，藍色的頻譜為不包含肝臟組織的二次諧振項，而黑色成分則為包含肝組織的二次諧振項



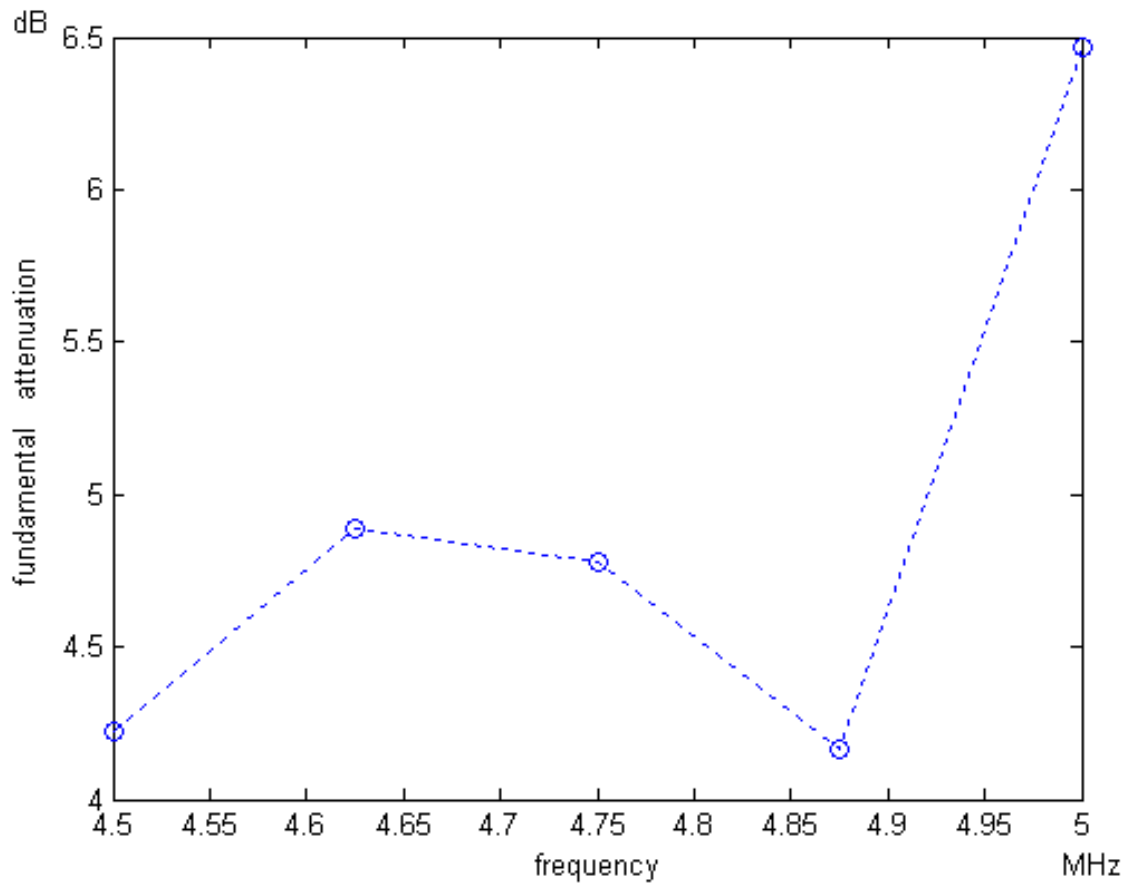
組織衰減係數的估測

在取得二次諧振成份的功率頻譜密度之後，接下來我們必須利用這五個取樣的頻率成分估測這 0.5MHz 頻寬範圍當中肝組織的衰減係數。首先，我們應用之前所提到的估測方式將上圖當中兩組頻率成份相減之後做圖並確定所得到的圖形結果是否為一個隨著頻率改變而呈線性變化的趨勢。如下圖所示



因此，經由上圖當中所呈現的結果得到，在此頻率範圍之間的衰減確實呈現近似於線性的變化方式。因此，我們可以利用所得到的資料估測大頻率範圍的衰減係數，所得到的結果為 0.0994dB/cmMHz 。

為了驗證在基頻訊號時，肝組織的背散射成分確實會影響微氣泡的回波訊號，進而使得利用基頻成分估測衰減係數變的困難。在此將上圖中基頻訊號的衰減估測圖型求出，如下圖



從上圖當中，我們便無法明顯的看出衰減係數與頻率的變化呈現線性關係。因此，我們可經由實驗證明，利用二次諧振以估測組織的衰減係數優於使用基頻訊號的估測方式。

由先前的研究得知，微氣泡的二次諧振成份相關於氣泡的大小以及在樣本空間當中氣泡的濃度分佈。在我們的理論當中，我們假設在每次取樣時，微氣泡大小與濃度的分佈均為均勻分佈，因此，我們可以得到在沒有組織衰減的情形之下，每個二次諧振頻率的回波強度都可以保持相同，在此實驗當中，我們也提出了在組織與管壁之間的介面反射成分可以忽略的假設。

生物體的應用

相較於傳統使用背散射訊號成分以估測衰減係數的方法，應用於二次諧振的方法可以視為穿透訊號的估測的方式。因此，應用二次諧振迴避不同組織所帶來的干擾成為一個可能的作法。在生物體當中，藉由注入商用對比劑而達到更準確的組織衰減估測，則是未來必須努力的目標。

解析度的提高

對於組織病變的診斷，除了估測的精準度以外，估測的解析度也同時是個重要的課題。當發射的訊號為窄頻訊號時，相對的，其發射脈波的長度就相較於寬頻訊號為長。因此，這使得對於組織估測的解析度因此受到了限制，並不利於在生物體當中的應用。如何在提高解析度並保持二次諧振成份的穩定間得到平衡亦為未來重要的課題。

發表論文

S.J. Lu, C.Y. Chuang and J. Tsao, "Dosage Prediction via Estimation of Shell Thickness and Concentration of Drug Carrier with Microbubbles," 29th IEEE EMBS Annual Int.Conf. (EMBC07), Aug. 23-26, 2007, Lyon, France, pp. 1090-1093. (NSC 95-2221-E002-442)

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S.J. Lu, C.Y. Chuang and Jenho Tsao

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Abstract- For drug delivery applications, dosage prediction before release and estimation after release are required functions. In this study, we attempted to establish a method to evaluate liposome concentrations and liposome shell thickness for dosage prediction. We use the Trilling model with parameter of phospholipids bilayers to simulate the frequency responses under the different acoustic pressure and establish an experimental protocol to evaluate the liposome concentrations and the liposome shell thickness.

Our results illustrate the changes on the signal strength for different concentrations and show that it is relatively stable to estimate the concentrations when the cycles are lower (15cycles). Besides, it is verified that the second harmonic signal is more sensitive in analyzing different concentrations. On the other hand, it is proved that the liposome shell thickness affect signal strength and thinner thickness will increase the second harmonic response.

Therefore, in accordance with the theoretical and experimental results, we would be able to estimate the concentration and the shell thickness of the liposomes. By numerical analysis methods, dosage prediction would be built.

I. INTRODUCTION

In medical ultrasound applications, microbubbles are closely tied to the diagnostic and therapeutic uses. In diagnostic applications, their sound scattering properties yield improved imaging, when the microbubbles are used as contrast agents [1]. The harmonics, subharmonics, and second harmonic responses from the bubbles assist in distinguishing the acoustic scattering of blood flow from that of the surrounding tissue[2]. The therapeutic use of microbubbles has recently become a subject of much interest, one of which is to serve as modified-release dosage forms.

The modified-release dosage forms start to develop prosperously recently. It is the purpose that we can properly handle modified-release dosage forms for controlling the time of medicine release and determining the perfect timing to release. However, the modified-release dosage forms have obvious effect on diminishing toxicity, raising curative effect and decreasing the times of medication. Hence, we could expect that modified-release dosage forms will still be a prospect of the medical development .

For drug delivery system , the drug must be preserved for

a period of time in order to utilize bubble characteristics for targeting and control the release at the best time. Dosage prediction before release and estimation after release is considerably significant.

The difference of harmonic responses generated from multi-lamellar vesicles with different numbers of layers and the number of bubbles can be used to estimate the shell thickness related to numbers of layers and predict the concentration of vesicles. However, there is no appropriate mathematical model and experimental protocol to determine the shell thickness of multi-lamellar vesicles and the concentration of vesicles.

In this study, we tried to adopt the Trilling model with parameter of phospholipids bilayers to simulate the frequency responses and establish an experimental protocol to evaluate the effect of shell thickness of liposomes to the estimation of its concentration. And we also attempted to get the optimal condition for measuring the concentration of liposomes. Further, we might use the study to predict the dosage via concentration.

II. MATERIALS AND METHODS

In this section, we will describe our liposomes as drug carrier first and then the simulations and experiments on the liposome concentration. Besides, the liposome shell thickness has a deep connection with signal strength, so we do simulation and experiments to evaluate the effect of shell thickness.

A. Materials

In this study, we have manufactured our own microbubble with lipid-shell (a kind of liposomes) for the ultrasound drug carrier, whose diameter is about 1.4 μ m (figure.1). The lipid-shelled microbubble is composed of a multi-lamellar lipid shells, a little nitrogen and some normal saline. The shell thickness can be expressed in terms of number of layers also, which is known to range from 1 to 5 layers [3]. The lipid shell comprises DSPC, Cholesterol and DSPE-PEG-Ome. The shell thickness of liposome is controlled by sonicator which controls the numbers of layers. In the size analysis, the optical measurement of N4-PLUS COULTER was used to analyze size distribution of the lipid-shell microbubble. (as shown in figure 1.)

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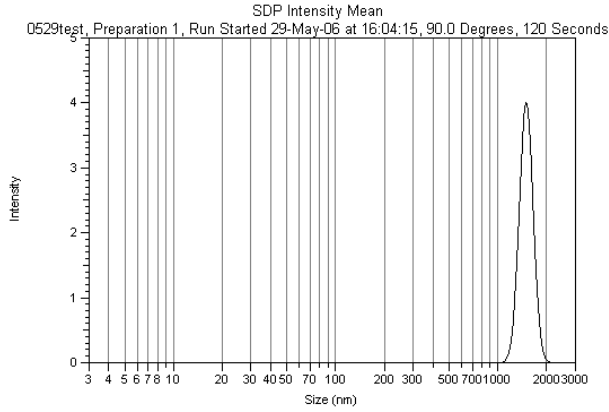


Figure1. The size distribution of original liposomes in N4PLUS Coulter

B. Simulation

Although the behavior of a bubble in an acoustic field has been studied for a long time, few theoretical describe the simulation of a real population of variably sized microbubbles in a finite beam. As the first step in our simulation, we will select the trilling model to simulate our microbubbles (liposomes) and summation all the radiation signals from large number of such bubbles. The *bubblesim* [4] is the main software to simulate the single bubble response, and we will modify the software to fit this study. The simulations include both the simulation of the concentration of liposomes and the simulation of the layers of liposome shell.

The concentration simulation

We started the process of simulation by deciding on a sample volume with N microbubbles whose size distribution is Gaussian distribution after the shell parameters of liposomes; Moreover, in order to fit the probe response, we use randomly different focal pressures in the simulation. The cycles of pulse are given 15, 20, and 25 cycles. The selection of concentrations will fit the experimental concentration.

The shell thickness simulation

We started the process of simulation by deciding on a sample volume with N microbubbles whose size distribution is Gaussian distribution after the shell parameters of liposomes; we adopted transmitted narrow-band pulse ($f_c = 2\text{MHz}$, cycles = 15, $\text{PRF} = 1\text{KHz}$) to simulate 3 different thicknesses of liposomes. We simulated the drug carrier (liposomes) consisting of gas, with mean diameter being 1.4 micron meter and shell thickness being 1, 3, and 5 layers (3.5, 10.5, and 17.5 nm). Using several numerical analysis methods, we can get the frequency response of all bubbles.

The equations and parameters used in our simulations are summarized below.

Equation of motion: Rayleigh-Plesset with radiation damping

$$a\ddot{a} + \frac{3}{2}\dot{a}^2 - \frac{p_L - p_o - p_i}{\rho} - \frac{a}{\rho c} \dot{p}_L = 0$$

Boundary condition: Pressure p_L at the bubble wall, using the exponential shell model

$$p_L = -4\eta_L \frac{\dot{a}}{a} + 12 \frac{d_{se}}{a_e} (G_s x_0 (1 - e^{-x/x_0}) + \eta_s e^{-x/x_0} \dot{x}) + p_0 \left(\frac{a_e}{a}\right)^{3\kappa}$$

where $x = \frac{a - a_e}{a_e}$, $\dot{x} = \frac{\dot{a}}{a_e}$, $x_0 = \frac{1}{8}$ and $x_1 = \frac{1}{4}$,

Shell parameters

$$d_{se} = 4.0\text{nm}, G_s = 50\text{MPa}, \eta_s = 0.8\text{Ns/m}^2$$

The Trilling model

The Trilling model [4] is derived from the acoustic approximation, the speed of sound is modeled as constant. It is only meaningful to the first order in the acoustic Mach-number $M = \dot{a}/c$. The order term of $1/c^2$ are removed from the ODE for the bubble surface, reducing it to

$$\ddot{a}a(1 - 2\frac{\dot{a}}{c}) + \frac{3}{2}\dot{a}^2(1 - \frac{4}{3}\frac{\dot{a}}{c}) - \frac{a}{\rho c} \dot{p}_L + \frac{p_o - p_L}{\rho} = 0$$

This equation of motion for a gas bubble was published by Trilling in 1952.

With a driving pressure $p_i(t)$, the Trilling equation is modified to

$$\ddot{a}a(1 - 2\frac{\dot{a}}{c}) + \frac{3}{2}\dot{a}^2(1 - \frac{4}{3}\frac{\dot{a}}{c}) - \frac{a}{\rho c} \dot{p}_L + \frac{a}{\rho c} \dot{p}_i(t) + \frac{p_o + p_i(t) - p_L}{\rho} = 0$$

The parameters used in simulations are Density of water = 998 kg/m^3 , Shear Modulus = 50MPa , Polytropic exponent of gas = 1.4, External pressure Range from 0.5 to 1.5Mpa, hydrostatic pressure = $1.01 \times 10^5\text{ N/m}^2$, Distance = 2cm, Acoustic speed = 1540 m/s and Shear viscosity of liposome = $0.8\text{Pa}\cdot\text{s}$.

C. Experiments of concentration estimation

In the experimental setting (as shown in figure2), we use the ultrasound RF signals to measure the acoustic reflection. In this study, pulse trains ($\text{PRF} = 1\text{KHz}$, cycles = 15, 20, and 25 cycles) are transmitted with the 2.25MHz center frequencies, then backscattered signals are received and digitized for processing. We record the second-harmonic signal strength in several concentrations to find the relation between concentrations and strength. In addition, we like to find the optimal method for the prediction of concentrations.

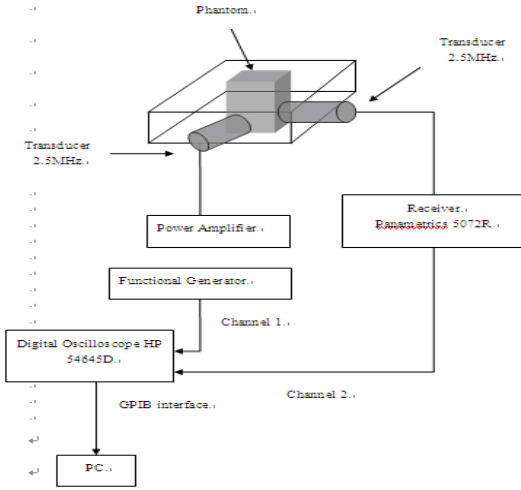


Figure2. The setup of the ultrasound

D. Experiments for shell thickness effect

In the experimental setting (as shown in figure2), we use the ultrasound RF signals to measure the acoustic reflection. In this study, pulse trains (PRF=1KHz, BW=5%) are transmitted with the 2MHz center frequencies, then backscattered signals are received and digitized for processing. We attempted to get whether different layers have difference in the frequency domain to develop the forecast of layers of liposomes.

III. RESULTS

A. simulation and experimental evaluation of concentrations

Results for the second harmonic signals of simulation and experiments for several concentration are plotted in the figure 3, 4, and 5. These results illustrate the changes on the signal strength for different concentrations (circle: the experimental data, line: the simulation data). We compare the results of simulation with the results of experiments under three kinds of cycles.(15, 20, and 25 cycles). When the concentration of liposomes is below around 40% of the concentration (PS: when 100% of the concentration, DSPC is 10^{-4} mole, cholesterol is 5×10^{-5} mole, and DSPE-PEG-OMe is 2.5×10^{-6} mole in 30 ml.), the ultrasound strength is sensitive for the concentration of liposomes. As shown in [5], the linear scattering assumed in the simulation may no longer be valid, multiple scattering would happen and causes apparent discrepancy. In three kinds of conditions, we could observe that in the thinner concentrations there are precise relations between the simulations and the experimental results. In the condition

of 15cycles, the relation is better than others in the all concentrations. And what is more, it is obvious that when the cycles are higher, it excites the nonlinear response of microbubbles more easily, but it is relatively unstable to detect the concentrations. On the contrary, when the cycles are lower, it is relatively stable to detect the concentrations. It is verified that the second harmonic signal is sensitive in different concentrations; Therefore, it is sufficient to prove the possibility of prediction of concentrations by second harmonic signals.

B. The simulation and experimental evaluation of thickness of shell

In the study, we adopted transmitted narrow-band pulse ($f_c = 2\text{MHz}$, cycles = 15, PRF=1KHz) to simulate 3 different thicknesses of shell. In this study, the thickness of a layer of liposomes is about 3.5 nm under room temperature, the original liposomes generally have 3-5 layer (the thicknesses of shells are 10.5 - 17.5nm), and the handled liposomes generally have 1 layer (the thickness of shell is 3.5nm), so we simulated three kinds of shells (3.5, 10.5, and 17.5nm by thickness).

As a result of the simulation, we could clearly see that the thicker the shells of liposomes, the lower the strength of non-linear response. Therefore, in the figure 6, it is shown that the difference of strength of second-harmonic response between three and five layers of liposomes was 12dB and the difference of strength of fundamental response between three and five layers of liposomes was about 4dB in our experiments.

In the experiments, we reduce the layers of liposomes with the help of a sonicator. Utilizing microscope, it is verified that the layers of shells of liposomes could be reduced to about 1 layer from 3-5 layers.

According to the experiments in the figure 7, we would discover that the second-harmonic response had larger difference than fundamental response whether the center frequency is 2MHz. The difference of strength of second-harmonic response was about 6dB. Similarly, in the actual experiments, it was obviously observed that the thicker the shells of liposomes, the lower the strength of non-linear response. In Figure 7, we could find stronger nonlinear response.

IV. CONCLUSION

To sum up, it is demonstrated that we could get the concentration of liposomes by second harmonic signals under different focal pressures. In the meanwhile, it is obvious that the shell thickness would affect the signal

strength making the concentration prediction confused. It is useful for us to understand the liposome shell affecting drug packaged.

Therefore, we could realize the drug carrier structure and predict the drug dosage by simple volume calculation and drug binding estimation. In the future work, we would build a model to build a set of dosage prediction system.

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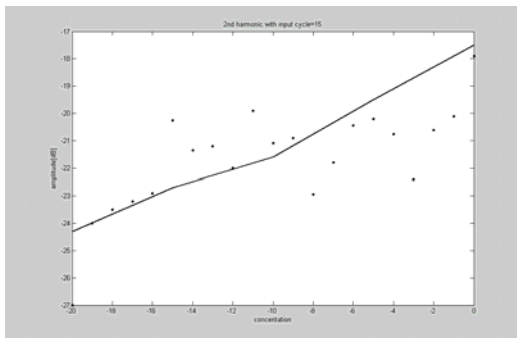


Figure3. The results of simulation and experiments in 15 cycles pulse. circle: the experimental data, line: the simulation data.

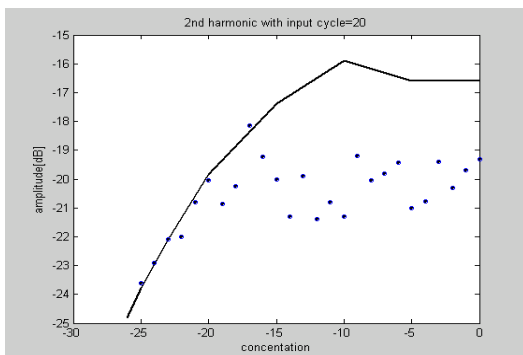


Figure4. The results of simulation and experiments in 20 cycles pulse. circle: experimental data. line: simulation data.

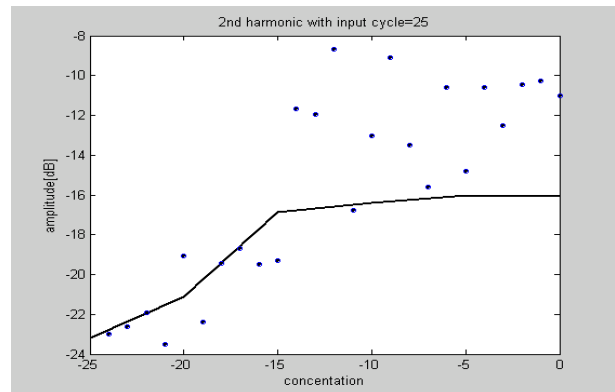


Figure5. The results of simulation and experiments in 25 cycles pulse. circle: the experimental data, line: the simulation data.

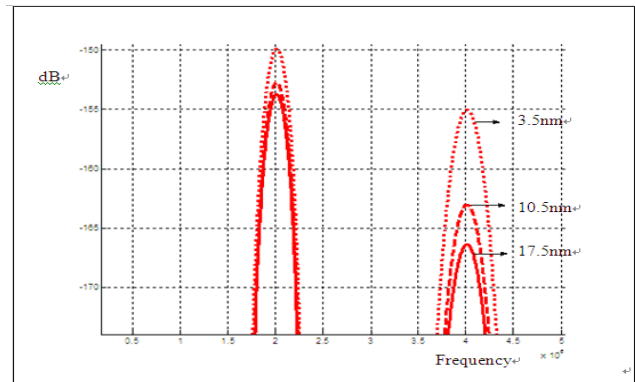


Figure6 .The spectrum of simulation of thickness

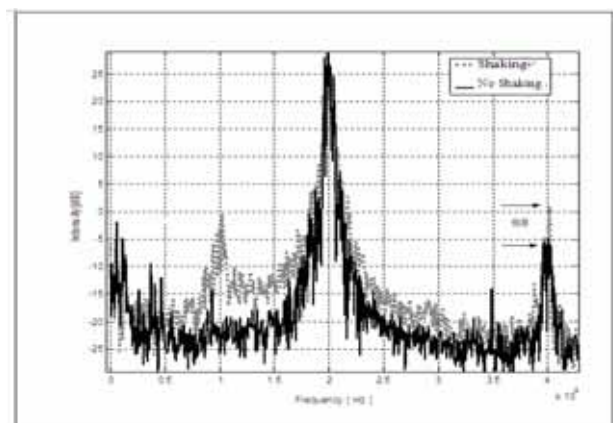


Figure7 .The spectrum of simulation of thickness

計畫名稱：藥物載體之超音波操作技術(三)

計畫主持人：曹建和

本計畫的研究成果，已部份發表於 IEEE 第 29 屆 世界醫學工程大會 [IEEE 29th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC07)], 本次大會於 2007/8/23~26 在 法國 里昂 舉行。
此次本人共發表兩篇與醫用超音波有關論文，其相關資料節錄於後：

S.J. Lu, C.Y. Chuang and J. Tsao, “Dosage Prediction via Estimation of Shell Thickness and Concentration of Drug Carrier with Microbubbles,” 29th IEEE EMBS Annual Int.Conf. (EMBC07), Aug. 23-26, 2007, Lyon, France, pp. 1090-1093. (NSC 95-2221-E002-442)

Tsao and J. H. He, “Ultrasonic Renal-Stone Tracking with Mesh Regularization,” 29th IEEE EMBS Annual Int.Conf. (EMBC07), Aug. 23-26, 2007, Lyon, France, pp. 2187-2190. (NSC 95-2221-E002-442)

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In this section, we will describe our liposomes as drug carrier first and then the simulations and experiments on the liposome concentration. Besides, the liposome shell thickness has a deep connection with signal strength, so we do simulation and experiments to evaluate the effect of shell thickness.

A. Materials

In this study, we have manufactured our own microbubble with lipid-shell (a kind of liposomes) for the ultrasound drug carrier, whose diameter is about 1.4 μ m (figure.1). The lipid-shelled microbubble is composed of a multi-lamellar lipid shells, a little nitrogen and some normal saline. The shell thickness can be expressed in terms of number of layers also, which is known to range from 1 to 5 layers [3]. The lipid shell comprises DSPC, Cholesterol and DSPE-PEG-Ome. The shell thickness of liposome is controlled by sonicator which controls the numbers of layers. In the size analysis, the optical measurement of N4-PLUS COULTER was used to analyze size distribution of the lipid-shell microbubble. (as shown in figure 1.)

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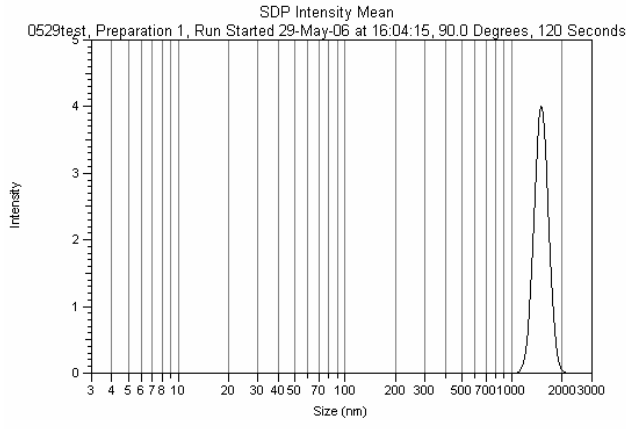


Figure1. The size disturbance of original liposomes in N4PLUS Coulter

B. Simulation

Although the behavior of a bubble in an acoustic field has been studied for a long time, few theoretical describe the simulation of a real population of variably sized microbubbles in a finite beam. As the first step in our simulation, we will select the trilling model to simulate our microbubbles (liposomes) and summation all the radiation signals from large number of such bubbles. The *bubblesim* [4] is the main software to simulate the single bubble response, and we will modify the software to fit this study. The simulations include both the simulation of the concentration of liposomes and the simulation of the layers of liposome shell.

The concentration simulation

We started the process of simulation by deciding on a sample volume with N microbubbles whose size distribution is Gaussian distribution after the shell parameters of liposomes; Moreover, in order to fit the probe response, we use randomly different focal pressures in the simulation. The cycles of pulse are given 15, 20, and 25 cycles. The selection of concentrations will fit the experimental concentration.

The shell thickness simulation

We started the process of simulation by deciding on a sample volume with N microbubbles whose size distribution is Gaussian distribution after the shell parameters of liposomes; we adopted transmitted narrow-band pulse ($f_c = 2\text{MHz}$, cycles = 15, PRF=1KHz) to simulate 3 different thicknesses of liposomes. We simulated the drug carrier (liposomes) consisting of gas, with mean diameter being 1.4 micron meter and shell thickness being 1, 3, and 5 layers (3.5, 10.5, and 17.5 nm). Using several numerical analysis methods, we can get the frequency response of all bubbles.

The equations and parameters used in our simulations are summarized below.

Equation of motion: Rayleigh-Plesset with radiation damping

$$a\ddot{a} + \frac{3}{2}\dot{a}^2 - \frac{p_L - p_o - p_L}{\rho} - \frac{a}{\rho c} \dot{p}_L = 0$$

Boundary condition: Pressure p_L at the bubble wall, using the exponential shell model

$$p_L = -4\eta_L \frac{\dot{a}}{a} + 12 \frac{d_{se}}{a_e} (G_s x_0 (1 - e^{-x/x_0}) + \eta_s e^{-x/x_1} \dot{x}) + p_0 \left(\frac{a_e}{a}\right)^{3\kappa}$$

where $x = \frac{a - a_e}{a_e}$, $\dot{x} = \frac{\dot{a}}{a_e}$, $x_0 = \frac{1}{8}$ and $x_1 = \frac{1}{4}$,

Shell parameters

$$d_{se} = 4.0\text{nm}, G_s = 50\text{MPa}, \eta_s = 0.8\text{Ns/m}^2$$

The Trilling model

The Trilling model [4] is derived from the acoustic approximation, the speed of sound is modeled as constant. It is only meaningful to the first order in the acoustic Mach-number $M = \dot{a}/c$. The order term of $1/c^2$ are removed from the ODE for the bubble surface, reducing it to

$$\ddot{a}a(1 - 2\frac{\dot{a}}{c}) + \frac{3}{2}\dot{a}^2(1 - \frac{4}{3}\frac{\dot{a}}{c}) - \frac{a}{\rho c} \dot{p}_L + \frac{p_\infty - p_L}{\rho} = 0$$

This equation of motion for a gas bubble was published by Trilling in 1952.

With a driving pressure $p_i(t)$, the Trilling equation is modified to

$$\ddot{a}a(1 - 2\frac{\dot{a}}{c}) + \frac{3}{2}\dot{a}^2(1 - \frac{4}{3}\frac{\dot{a}}{c}) - \frac{a}{\rho c} \dot{p}_L + \frac{a}{\rho c} \dot{p}_i(t) + \frac{p_\infty + p_i(t) - p_L}{\rho} = 0$$

The parameters used in simulations are Density of water = 998 kg/m^3 , Shear Modulus = 50MPa , Polytropic exponent of gas = 1.4, External pressure Range from 0.5 to 1.5MPa , hydrostatic pressure = $1.01 \times 10^5\text{ N/m}^2$, Distance = 2cm , Acoustic speed = 1540 m/s and Shear viscosity of liposome = $0.8\text{Pa}\cdot\text{s}$.

C. Experiments of concentration estimation

In the experimental setting (as shown in figure2), we use the ultrasound RF signals to measure the acoustic reflection. In this study, pulse trains (PRF=1KHz, cycles=15, 20, and 25 cycles) are transmitted with the 2.25MHz center frequencies, then backscattered signals are received and digitized for processing. We record the second-harmonic signal strength in several concentrations to find the relation between concentrations and strength. In addition, we like to find the optimal method for the prediction of concentrations.

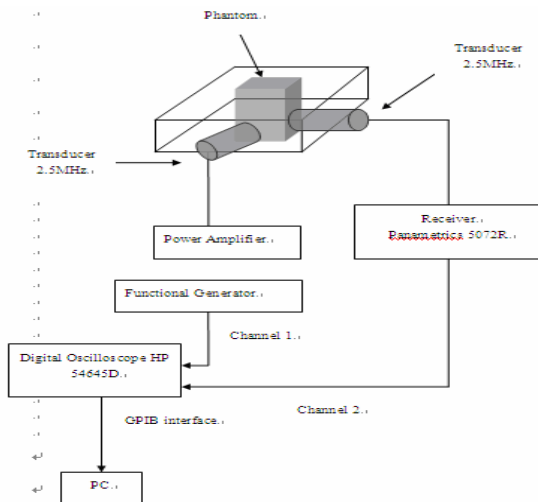


Figure2. The setup of the ultrasound

D. Experiments for shell thickness effect

In the experimental setting (as shown in figure2), we use the ultrasound RF signals to measure the acoustic reflection. In this study, pulse trains (PRF=1KHz, BW=5%) are transmitted with the 2MHz center frequencies, then backscattered signals are received and digitized for processing. We attempted to get whether different layers have difference in the frequency domain to develop the forecast of layers of liposomes.

III. RESULTS

A. simulation and experimental evaluation of concentrations

Results for the second harmonic signals of simulation and experiments for several concentration are plotted in the figure 3, 4, and 5. These results illustrate the changes on the signal strength for different concentrations (circle: the experimental data, line: the simulation data). We compare the results of simulation with the results of experiments under three kinds of cycles.(15, 20, and 25 cycles). When the concentration of liposomes is below around 40% of the concentration (PS: when 100% of the concentration, DSPC is 10^{-4} mole, cholesterol is 5×10^{-5} mole, and DSPE-PEG-OMe is 2.5×10^{-6} mole in 30 ml.), the ultrasound strength is sensitive for the concentration of liposomes. As shown in [5], the linear scattering assumed in the simulation may no longer be valid, multiple scattering would happen and causes apparent discrepancy. In three kinds of conditions, we could observe that in the thinner concentrations there are precise relations between the simulations and the experimental results. In the condition

of 15cycles, the relation is better than others in the all concentrations. And what is more, it is obvious that when the cycles are higher, it excites the nonlinear response of microbubbles more easily, but it is relatively unstable to detect the concentrations. On the contrary, when the cycles are lower, it is relatively stable to detect the concentrations. It is verified that the second harmonic signal is sensitive in different concentrations; Therefore, it is sufficient to prove the possibility of prediction of concentrations by second harmonic signals.

B. The simulation and experimental evaluation of thickness of shell

In the study, we adopted transmitted narrow-band pulse ($f_c = 2\text{MHz}$, cycles = 15 , PRF=1KHz) to simulate 3 different thicknesses of shell. In this study, the thickness of a layer of liposomes is about 3.5 nm under room temperature, the original liposomes generally have 3-5 layer (the thicknesses of shells are 10.5 - 17.5nm), and the handled liposomes generally have 1 layer (the thickness of shell is 3.5nm), so we simulated three kinds of shells (3.5, 10.5, and 17.5nm by thickness).

As a result of the simulation, we could clearly see that the thicker the shells of liposomes, the lower the strength of non-linear response. Therefore, in the figure 6, it is shown that the difference of strength of second-harmonic response between three and five layers of liposomes was 12dB and the difference of strength of fundamental response between three and five layers of liposomes was about 4dB in our experiments.

In the experiments, we reduce the layers of liposomes with the help of a sonicator. Utilizing microscope, it is verified that the layers of shells of liposomes could be reduced to about 1 layer from 3-5 layers.

According to the experiments in the figure 7, we would discover that the second- harmonic response had larger difference than fundamental response whether the center frequency is 2MHz. The difference of strength of second-harmonic response was about 6dB. Similarly, in the actual experiments, it was obviously observed that the thicker the shells of liposomes, the lower the strength of non-linear response. In Figure 7, we could find stronger nonlinear response.

IV. CONCLUSION

To sum up, it is demonstrated that we could get the concentration of liposomes by second harmonic signals under different focal pressures. In the meanwhile, it is obvious that the shell thickness would affect the signal

strength making the concentration prediction confused. It is useful for us to understand the liposome shell affecting drug packaged.

Therefore, we could realize the drug carrier structure and predict the drug dosage by simple volume calculation and drug binding estimation. In the future work, we would build a model to build a set of dosage prediction system.

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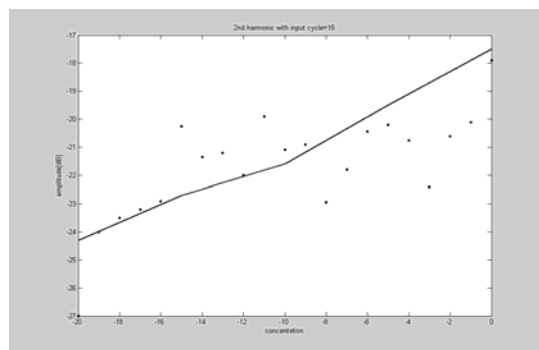


Figure3. The results of simulation and experiments in 15cycles pulse. circle: the experimental data, line: the simulation data.

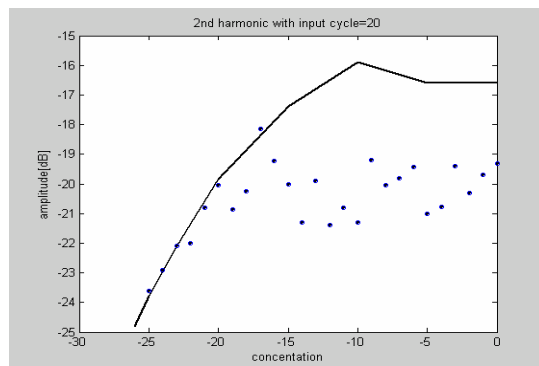


Figure4. The results of simulation and experiments in 20 cycles pulse. circle: exnerimental data. line: simulation data.

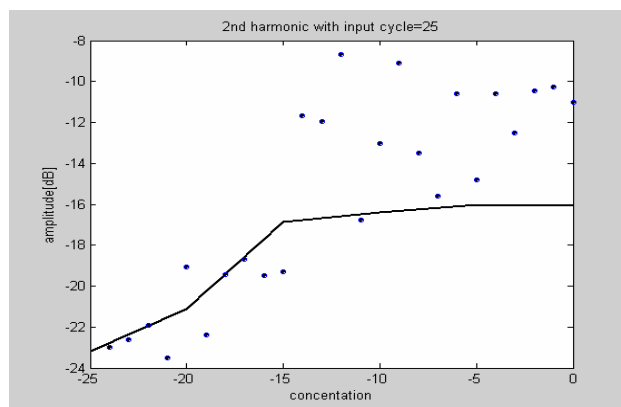


Figure5. The results of simulation and experiments in 25 cycles pulse. circle: the experimental data, line: the simulation data.

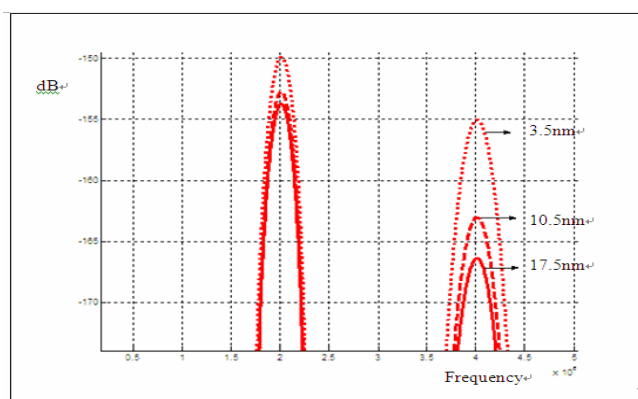


Figure6 .The spectrum of simulation of thickness

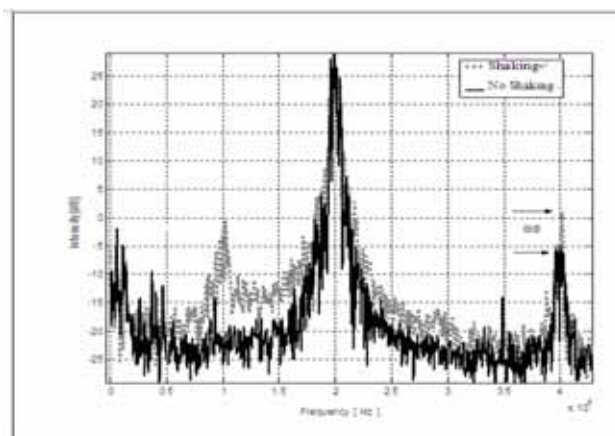


Figure7 .The spectrum of simulation of thickness

Ultrasonic Renal-Stone Tracking with Mesh Regularization

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Abstract- The efficacy of Extracorporeal Shock Wave Lithotripsy (ESWL) depends greatly on the capability to focus shock waves on renal stone. To achieve automatic focusing on moving target, the target must be under tracking. A mesh-based block matching algorithm is proposed for renal stone tracking using ultrasound image sequence. Since multiple targets are tracked together, the mesh-based tracking algorithm can provide a function of contextual regularization for solving the target missing and image degradation problems in renal stone tracking. Recorded ultrasound images of kidney during ESWL treatment are modified for demonstrating the capability of this algorithm.

I. INTRODUCTION

Extracorporeal Shock Wave Lithotripsy (ESWL) is the clinical procedure for disintegrating renal stones by shock waves from the outside of body [1]. The shock waves must be focused onto the stones. However, stones may move around during the course of treatment due to respiration, patient movement and shock-wave pressure. This will cause miss-hit of shock waves on surrounding tissues and cause trauma to tissues [2]. Respiratory triggering, general anesthesia, and real-time stone tracking [3] are means proposed to keep the stones inside the focal zone of shock wave. The real-time stone tracking approach was proven to be quite effective in reducing miss-hits and thus reduces tissue injury and treatment time [3, 4].

In the renal stone tracking system developed by Orkisz et al. [3], ultrasound image sequence are processed to provide positions of renal stones for real-time automatic shock-wave focusing. A simple block matching algorithm [5] is employed for image tracking. Each track is initialized manually by the physician and terminated when the cross correlation between successive image blocks is lower than a threshold. Tracking ultrasound images is difficult due to tissue deformation, noisy images, motion ambiguities, spatial aliasing, speckle decorrelation, out-of-plane motion, speckle motion artifacts and quantization error[6]. These artifacts make the target trajectory fluctuate fast, since the simple block matching is essentially a speckle tracking technique. The out-of-plane motion happens regularly due to respiration, which makes target disappear temporarily. In addition to these problems, tracking stone under

fragmentation by ESWL is more challenging. Since after fragmentation, stone breaks to be small ones, which makes the stone image change all the time and its contrast degraded.

A mesh-based algorithm was proposed in [6] to cope with the speckle problems in tracking ultrasound image sequences. Unlike the simple block matching used in [3], which uses a single target block to track the stone, mesh-based algorithm uses more blocks to track the motion field over entire image plane. The embedded mesh puts a systematic control on the displacement estimations of the independent image blocks, which is known as mesh regularization.

For renal stone tracking problem, the scenario viewed by the ultrasound scanner is quite stationary over frames separated by 1/30 sec. The pressure caused by respiration makes the stone and its surrounding tissues move coherently to similar directions. Therefore, if more trackers were employed to track the stone and its surrounding tissues simultaneously, the mesh regularization scheme, which enforces the stone and tissues to move consistently, will improve the stability of the trajectory of stone.

It is found in this study that the mesh regularization scheme may provide more than motion regularization, it can provide contextual regularization also. When stone and tissues are tracked simultaneously, the existence of tissues (or background targets) will provide contextual information to infer the existence of stone (target). It is almost impossible that all background targets can disappear simultaneously, therefore, if most of the background targets were tracked properly, the mesh tracker will work properly, even the target disappears for a few frames. That is, the target tracker can be regularized by tissue trackers to tolerate the nonstationary degradation of target images. This helps in the relief of the out-of-plane motion problem and the target fragmentation problem. For the same situation, if there is only a single target tracker, there would be no way to prevent the tracker to switch its track to nearby tissue sites. Once a miss tracking happens, the tracker may keep wrong forever, even the target comes back latter.

The mesh-based tracking algorithm is given in section II. Performances of this algorithm are given in section III. The contextual regularization capability is demonstrated by simulation studies in section III.

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II. THE MESH-BASED TRACKING ALGORITHM

Since mesh-based tracking algorithms are well documented in literature, only a limited amount of description about the algorithm will be given below. For more details, readers may consult the works in [6-9]. The algorithm is a semi-automatic tracking algorithm. It needs manual initialization to supply the target and mesh related information, which will be described after the block matching criteria and mesh regularization are given. Some details about the mesh design will be given in the section of experimental test. After initialization, the algorithm does the *node displacement estimation by block matching* and *mesh regularization on the node displacements* repeatedly for successive frames.

A. Block Matching Criteria

For tracking target with speckled image, it was proposed in [3] to use the normalized texture correlation as the criteria for block matching. Let $\mathbb{M}$ be the set of pixel-positions within a matching block and the sampled data from the k^{th} image frame be $f_k(\mathbf{x}); \mathbf{x} \in \mathbb{M}$, the normalized texture correlation for forward matching is

$$C(\mathbf{d}) = \sum_{\mathbf{x} \in \mathbb{M}} \frac{\varphi_k(\mathbf{x})\varphi_{k+1}(\mathbf{x} + \mathbf{d})}{\sigma_k\sigma_{k+1}}$$

where $\varphi_k(\mathbf{x}) = f_k(\mathbf{x}) - \mu_k$, μ_k and σ_k are the mean and standard deviation of $f_k(\mathbf{x})$, and $\mathbf{d}$ is the displacement vector to be estimated by maximizing $C(\mathbf{d})$ within a limited search range. This is essentially a speckle tracking technique. It suffers from all speckle problems mentioned previously.

B. Mesh Regularization

A mesh can be considered as a connected spring system [8,9]. Given a set of N nodes located at $\mathbf{x}_n = (x_n, y_n)^T$ and their connectivity, a mesh can be defined. Let $\mathbf{u}_n = (\Delta x_n, \Delta y_n)$ be the deformation (i.e., displacement) of the n^{th} node, all of the node deformations can be collected to be a deformation vector

$\mathbf{U} = (\Delta x_1, \Delta y_1, \dots, \Delta x_n, \Delta y_n, \dots, \Delta x_N, \Delta y_N)^T$. Based on the finite element theory, a stiffness matrix $\mathbf{K}_e$ can be found for computing the deformation energy of the mesh as $J_m(\mathbf{U}) = \mathbf{U}^T \mathbf{K}_e \mathbf{U}$.

Based on the theory of active mesh [8], the optimal estimate of the displacement vector $\mathbf{U}$ should maximize the global (ensemble) correlation of all N blocks of image data

$J_c(\mathbf{U}) = \sum_{n=1}^N C(\mathbf{u}_n)$, as wells as the deformation energy of mesh model $J_m(\mathbf{U})$. Since $J_c(\mathbf{U})$ is mathematically untraceable, there is no efficient algorithm to find the

optimal solution. Yet, a suboptimal solution can still be constructed [6, 7], which is to estimate the independent displacements of each node first and then use them to find a mesh-regularized solution of $\mathbf{U}$ latter.

Let $\mathbf{d}_n = (dx_n, dy_n)^T$ be the displacement estimate of the n^{th} node based on block matching, which maximizes $C(\mathbf{d}_n)$ already. For all mesh nodes, we have the observed displacement vector $\mathbf{D} = (dx_1, dy_1, \dots, dx_n, dy_n, \dots, dx_N, dy_N)^T$ based on the image data. The mesh-regularized solution can be found by minimizing the sum of squared difference between $\mathbf{U}$ and $\mathbf{D}$ and the mesh deformation energy $\mathbf{U}^T \mathbf{K}_e \mathbf{U}$. Using the Lagrange technique, the object function to be minimized is

$$J_{CLS}(\mathbf{U}) = \sum_{n=1}^N \|\mathbf{u}_n - \mathbf{d}_n\|^2 + \lambda \mathbf{U}^T \mathbf{K}_e \mathbf{U} \\ = \|\mathbf{U} - \mathbf{D}\|^2 + \lambda \mathbf{U}^T \mathbf{K}_e \mathbf{U}$$

where λ is a regularization parameter. This is essentially a constrained least square solution, which tries to minimize the discrepancy between the mesh-regulated displacement $\mathbf{U}$ and the observed node displacement $\mathbf{D}$. If $\mathbf{D}$ is estimated by block matching, it will result in a mesh-regulated block matching algorithm for estimating $\mathbf{U}$. By differencing $J_{CLS}(\mathbf{U})$, it is easy to show that the solution for minimizing $J_{CLS}(\mathbf{U})$ can be found by solving a matrix of equations and its solution is

$$\mathbf{U}_{CLS} = (\mathbf{I} + \lambda \mathbf{K}_e)^{-1} \mathbf{D},$$

where $\mathbf{I}$ is an identity matrix of size $2N \times 2N$.

C. Algorithm Initialization

There are two major works to be done in algorithm initialization. They are the node allocation and the node labeling to specify the target and tissue nodes. Usually the mesh nodes are placed at positions of image feature points, such as edge points, corners, or points with high texture energy[6-9]. By so doing, it will result in an adaptive mesh, which has irregularly shaped mesh elements. Since the feature points change all the time, positions and labels of the mesh nodes must be updated for each frame and the stiffness matrix must be recomputed also. Furthermore, this will require a separate algorithm for target identification to keep the labels of each node.

For ESWL application, stone must be tracked in real-time, the use of adaptive mesh may cause computation problems. Therefore, a fix-sized regular mesh with quadrilateral elements is adopted in this study. In this way, the mesh nodes will be allocated only once in the initialization step of the tracking algorithm. Thus the mesh model is time invariant, the regularization operator $(\mathbf{I} + \lambda \mathbf{K}_e)^{-1}$ need to be computed also once only.

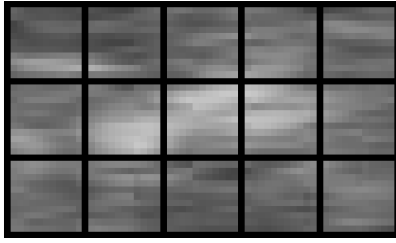


Fig. 1 The image blocks used for displacement estimation, the block size is 11 by 11 pixels

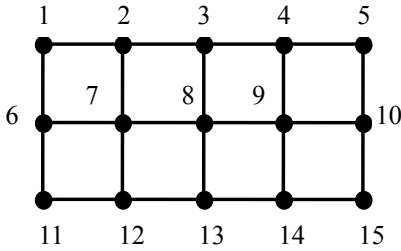


Fig. 2 The mesh model used for displacement regularization

III. RESULTS

A. Experimental Test

An image sequence recorded during ESWL treatment is used for testing the proposed tracking algorithm. It contains sixty thousand frames of ultrasound images of a renal stone inside pelvis. The size of the stone is about 10 by 30 pixels (= 3.75 by 11.25 mm) as shown in Fig.1. This makes the choice of element size of mesh to be 11 by 11 pixels, then the target will have 3 nodes. To make the target nodes be surrounded by a ring of tissue nodes, the mesh must have at least 3 by 5 nodes. The image blocks used for displacement estimation is given in fig.1 and the mesh model of 15 nodes is given in fig.2. The nodes-7, 8 and 9 are target nodes. All 15 nodes are tracked simultaneously using the proposed mesh-based block matching algorithm. The stiffness matrix of the mesh is computed by the finite element method. The elasticity properties of the mesh model are set with Poisson's ratio = 0.5 and Young's modulus = 40kPa. The search range of block matching is ± 4 pixels.

The (x,y) positions of node-8 estimated by the mesh-based block matching algorithm are recorded as the trajectories of the stone, which are shown in Fig. 4 and 6. For comparison, the (x,y) trajectories of tracking using the simple block matching are shown in fig. 3 and 5. As expected, the mesh regulation scheme makes the trajectories stable. For example, the marked fast movement at frame# 610 in fig.3 has been suppressed, which is erroneous since it has a speed of 9cm/sec. The two x-trajectories has larger difference than the difference of the y-trajectories. This is because that the x-axis resolution is lower than the y-axis resolution, and the interference from the ESWL system is much serious in x direction.

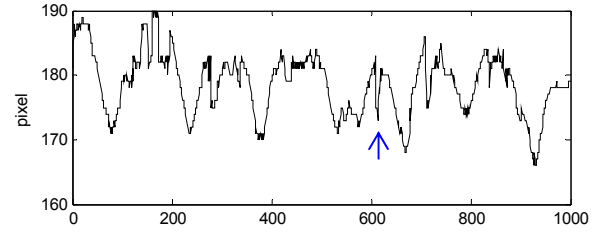


Fig. 3. The x-trajectory of renal stone using simple block matching.

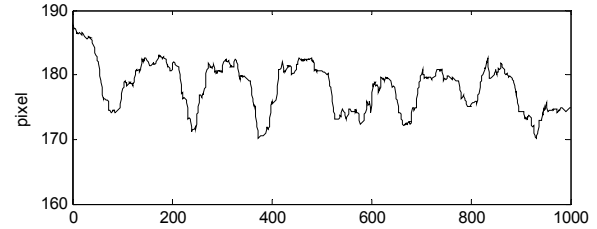


Fig. 4 The x-trajectory of renal stone using mesh regularization.

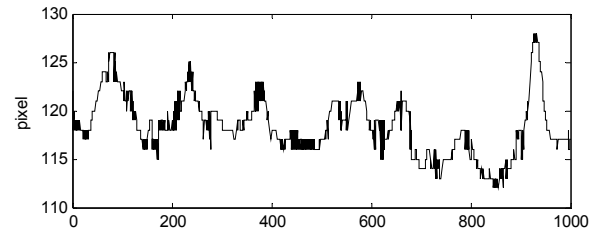


Fig. 5 The y-trajectory of renal stone using simple block matching.

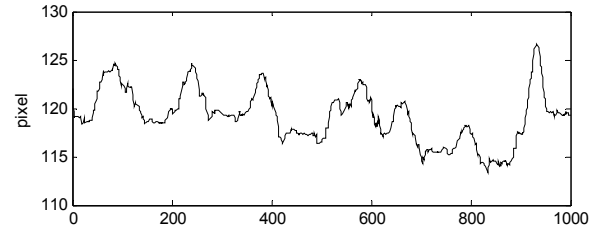


Fig. 6 The y-trajectory of renal stone using mesh regularization.

B. Simulation Study

To test the capability of the mesh regularization scheme, the displacement estimator at node 8 is interfered artificially by adding zero-mean Gaussian noise to the observed displacement d_{y_8} . The artificial interference has a standard deviation of 2 pixels and the modified observation is truncated to be within the search range of ± 4 pixels, when the interference is too large. The artificial interference starts from frame-300 and ends at frame-500. The interfered displacement vector D is then used for solving the regularized displacement estimate U_{CLS} . The modified observation d_{y_8} is shown in Fig.7. The mesh-regularized

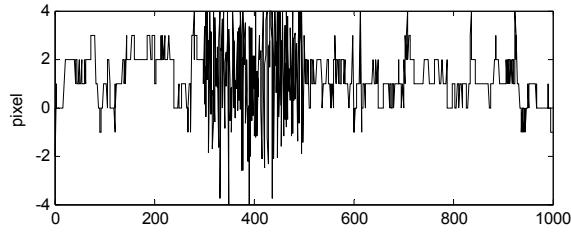


Fig.7 The observed displacement d_{y_8} with artificial interference from frame-300 to 500.

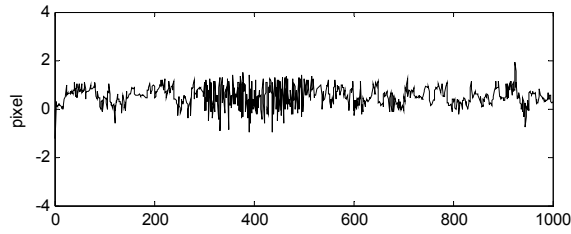


Fig.8 The regularized displacement u_{y_8} with $\lambda = 1$.

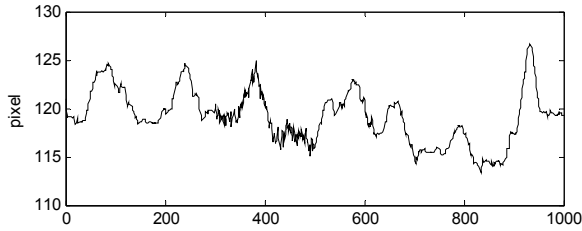


Fig.9 The mesh regularized y-trajectory of renal stone with $\lambda = 1$.

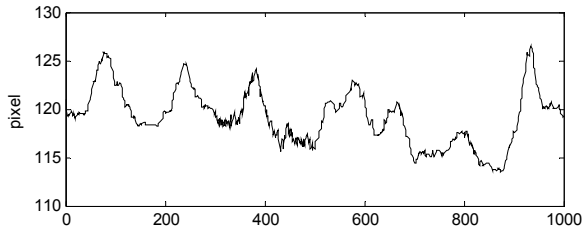


Fig.10 The mesh regularized y-trajectory of renal stone with $\lambda = 2$.

displacement u_{y_8} with regularization parameter $\lambda = 1$ is shown in Fig.8. It is apparent that the extra error in d_{y_8} is suppressed to a compatible level as those frames without interference. The mesh regularized y-trajectory of renal stone is shown in Fig.9. It can be found that the interference does cause errors to the regularized trajectory estimate, however, comparing it to Fig.6, the stone is still under tracking properly. This result justifies that the mesh-regularized block matching algorithm may allow some node to be out-of-track through the function of contextual regularization.

For comparison, the mesh regularized displacement u_{y_8} with regularization parameter $\lambda = 2$ is shown in Fig.10. Compared it to Fig.9, it shows that the interference can be suppressed more by increasing λ . However, there is a trade-off between the discrepancy error $\|U - D\|^2$ and the degree of regularization.

IV. CONCLUSION

Mesh regularization can reduce the tracking error due to speckle. The mesh-based algorithm has the potential to provide contextual regularization for solving the target missing and image degradation problems in renal stone tracking. Through the use of the contextual information from tissue trackers, it might save the requirement of target recognition for ensuring that the ESWL device is shooting the stone instead of tissues.

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