ACOUSTIC CHARACTERIZATION OF MICROBUBBLES FOR IMPROVED QUANTIFICATION

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1 INTRODUCTION

In medical ultrasonics stabilized microbubbles are used as contrast media for imaging of blood flow and perfusion[1]. Their suitability for targeted imaging by coupling ligands for biomolecules of interest to the bubble shell has also been demonstrated[2]. In these molecular imaging applications quantification of the concentration of bound bubbles is of interest for tumour detection and the assessment of response to therapy. A standard method to quantify microbubble concentration in tumors in small animal experiments is the detection of single microbubble destruction events. These events occur when ultrasound pulses with high mechanical index are used to fragment microbubbles bound in the tumor tissue. Bubble destruction can be detected by the decorrelation of echoes by Doppler techniques, e.g. in power Doppler mode. With typically rather high microbubble concentrations it is however difficult to count single bubble destruction events because several microbubbles can be in the focal zone of the transducer at the same time. Reinhardt et al.[3] proposed a method to circumvent this problem: The ultrasound transducer of the imaging system is moved motorized in steps of 10-100µm perpendicular to the image plane. In this way only microbubbles in a slice of 10-100µm thickness are destroyed and the single bubble events can be counted in power Doppler mode. The method is named Sensitive Particle Acoustic Quantification (SPAQ). However, several sources of error influence the resulting accuracy of the method: The destruction threshold of microbubbles is not uniform for all bubbles and thus only a certain percentage of bound bubbles will be destroyed unless very high mechanical indexes are used. Attenuation in the tissue and additionally by the microbubbles leads to varying pressure amplitudes and thus spatially varying destruction rates. Especially in high bubble concentrations this avoids complete bubble destruction even at high mechanical index. Even if these effects can be neglected, multiple destruction events cause errors: within one resolution cell of the power Doppler image still more than one destruction event could have occurred. Thus the quantification result underestimates the true concentration systematically. In this paper we only address this sort of error and discuss statistical corrections of multiple bubble counts which are more precise than the rough approximations made by Reinhardt et al.[3].

2 STATISTICAL CORRECTION OF MULTIPLE COUNTS

Microbubble destruction can be detected by different pulse echo techniques. In principle the subtraction of two consecutively measured scan lines at the same position makes it possible to detect differences due to bubble destruction, especially if non-linear detection techniques are used in combination with the subtraction technique. However, most clinical and preclinical researchers use the power Doppler mode to detect bubble destruction by the Doppler signal due to the observed decorrelation. The amount of bubble destruction without correction is estimated by the ratio of the number N_C of colored pixels to the total number of pixels N. This estimate is assumed to be proportional to the microbubble concentration in the tissue with the constant of proportionality unknown without calibration measurements. In many applications only a ratio of concentrations, e.g.

between targeted and unspecific bubbles or before and after therapy is needed and an absolute calibration not necessary.
When a color pixel is present in the image it indicates that within a certain volume around this pixel at least one microbubble destruction event took place. This volume is determined by system dependent parameters like the resolution cell of the scanner and the sensitivity of the Doppler processing. Here we assume that this volume is unknown but constant over the region of interest. Additionally, we develop the statistical model under the assumption, that all microbubbles in the resolution cell around the pixel are destroyed but their number is unknown.
When at a pixel no color is observed, we assume that no microbubbles were present in the resolution cell around the pixel. In the experiment the volume of interest is sampled at N positions and the number of resolution cells with destruction and without destruction is counted by N_C and $N - N_C$ respectively. If the microbubbles are considered to be uniformly distributed in the volume, the probability to find k microbubbles in a resolution cell is given by the Poisson probability distribution:

$$P(k) = \frac{(C \cdot V)^k}{k!} e^{-C \cdot V}, \tag{1}$$

where C is the volume concentration of microbubbles and V the volume of the resolution cell. For the experiment, we can only estimate the probability of observing no microbubbles in the volume

$$P_0 = P(0) = e^{-C \cdot V} \tag{2}$$

and the probability of one or more microbubbles

$$P_{\geq 1} = 1 - P(0) = 1 - e^{-C \cdot V}. \tag{3}$$

The respective estimators are given by the relative counts, of which one is redundant:

$$\begin{aligned} \hat{P}_0 &= \frac{N - N_C}{N}, \\ \hat{P}_{\geq 1} &= \frac{N_C}{N}. \end{aligned} \tag{4}$$

The absolute concentration of microbubbles C can only be estimated, when the volume of the resolution cell V is known:

$$\hat{C} = \frac{-\ln \hat{P}_0}{V} = -\frac{1}{V} \ln\left[1 - \frac{N_C}{N}\right] = \frac{1}{V}\left[\ln N - \ln(N - N_C)\right]. \tag{5}$$

The ratio of two concentrations can be estimated even without knowledge of the volume V, when the color pixels and the total pixels for the two experiments are denoted by N_{C1}, N_{C2} and N_1 and N_2 respectively:

$$\frac{\hat{C}_1}{\hat{C}_2} = \frac{\ln N_1 - \ln(N_1 - N_{C1})}{\ln N_2 - \ln(N_2 - N_{C2})}. \tag{6}$$

These results differ considerably from the statistical corrections proposed in the work of Reinhardt et al.[3]. This is owed to the fact that the probability of coincidence counts that is given correctly in equation (4) as $P_{\geq 1}$ was calculated incorrectly by

$$P + P^2 + P^3 + \ldots = \sum_{k=1}^{\infty} P^k = \frac{P}{1 - P}, \tag{7}$$

with P the probability to destroy exactly one microbubble. It can be easily seen that (7) is incorrect because it results in probabilities larger than one for $P > 0.5$ and goes to infinity for $P \to 1$. The error is the assumption that the occurrence of k microbubbles in the volume can be calculated by P^k with a fixed P when the microbubbles are uniformly distributed in space. Instead (1) holds. Their method led to a correction of the pixel count according to the concentration estimation:

$$\hat{C}V = \frac{N_C}{N}\left(1 + \frac{N_C}{N}\right). \tag{8}$$

3 EXPERIMENTAL VALIDATION

To demonstrate and validate the proposed correction, we used gelatine phantoms with embedded cyanoacrylate microbubbles. For this, 15 g of cold soluble gelatine was dissolved in 500 ml of water at 30°C. The gelatine phantom was cooled down until the mixture was semi-fluid. Contrast agent was added with a syringe under constant stirring. The untargeted microbubbles were prepared at the German Cancer Research Center, Heidelberg, Germany according to recipes published earlier[4]. The microbubble suspension was diluted 1:10 before injection to obtain a more homogenous distribution. The phantom was then refrigerated until it reached a solid state. Different amounts of suspension volume per gelatine phantom volume were prepared with 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml suspension per ml gelatine. The absolute concentrations were not known due the unknown microbubble concentration in the suspension, however, with the current method absolute concentration measurements are not possible anyway and thus concentration ratios are sufficient to evaluate the correction.

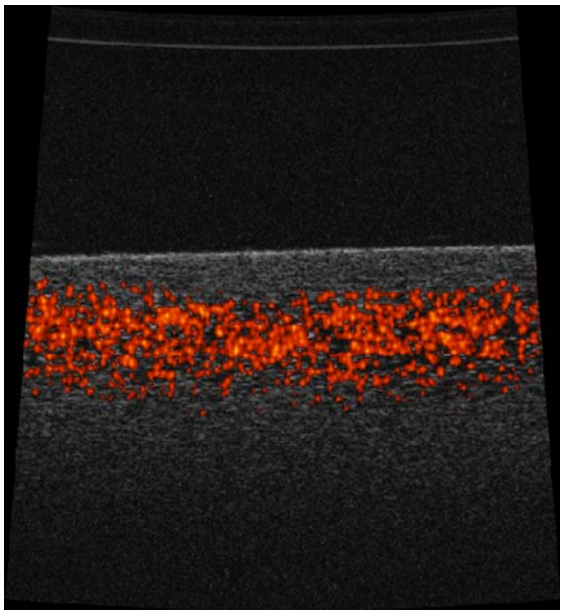

Figure 1: Power Doppler image of bubble destruction in the 32µm overlap region of two scans.

All acquisitions were made with a Vevo770 ultrasound system (Visualsonics, Toronto, ON, Canada) and a RMV704 scanhead at 40 MHz transmit frequency. The images were generated in 3D Power Doppler mode. In each acquisition the scan distance was set to 0.64 mm with a step size of 32 µm. This yields a set of 20 frames, the first one showing the initial destruction of a complete slice and the following 19 the destruction in the 32 µm overlap. The Doppler window was set to the focal

region of the transducer. The display range for the Doppler was held constant at 13dB-40dB. For each phantom ten acquisitions were made of different, nonoverlapping regions of the phantom, directly at the surface of the phantom to avoid acoustic shadowing at high bubble concentrations. Figure 1 shows the power Doppler image of a phantom with 0.2 ml suspension per ml gelatine. It is clearly visible that bubble destruction only occurs in the focal zone of the fixed focus transducer and evaluation has to be limited to the center of this region.

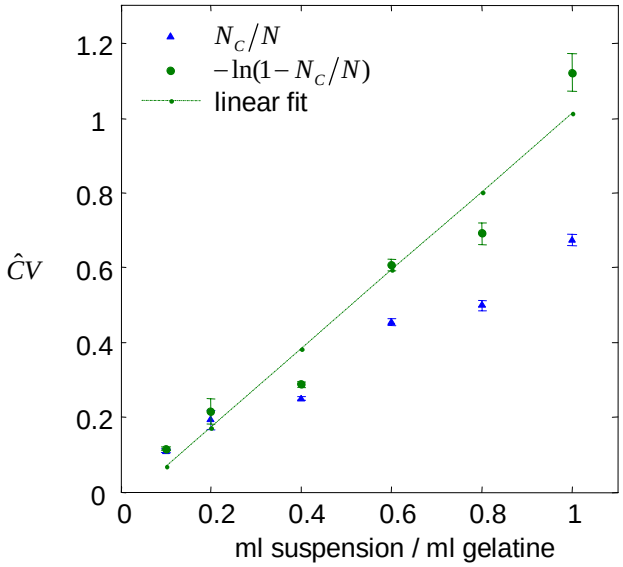

Figure 2: Corrected (green circles) and uncorrected (blue triangles) estimates of the concentration-resolution volume product $\hat{C}V$.

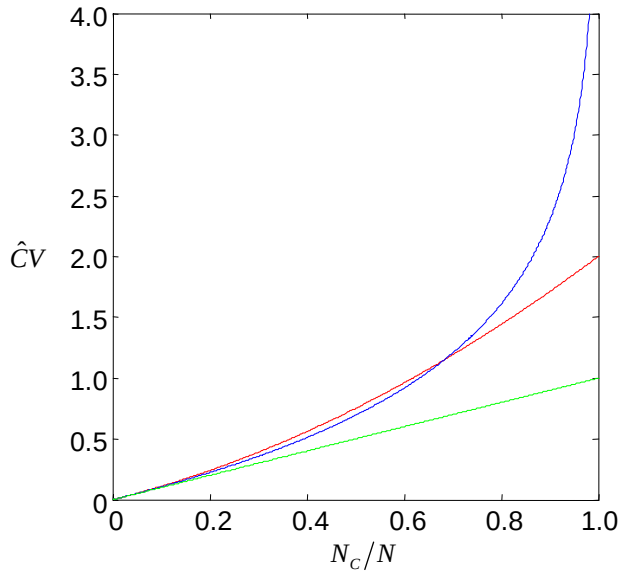

Figure 3: Comparison of the uncorrected results (green) for different ratios N_C/N , the correction according to (5) (blue) and the result of Reinhardt et al. (red).

Image analysis was done with MATLAB (The Mathworks, Inc). The number of pixels above a threshold of 14dB was counted and divided by the number of all pixels within the Doppler window. This ratio was averaged over all nineteen images within one acquisition. Figure 2 shows the mean and standard deviation for the ten acquisitions per phantom for the uncorrected and the corrected

product $\hat{C}V$ of concentration and volume. For absolute concentration estimates the resolution cell volume has to be known. Additionally a linear least squares fit to the corrected values is shown. Additionally, in Figure 3 the correction formula of Reinhardt et al.[3] is compared to the correction according to (5) and the uncorrected result for different ratios of N_C/N .

4 DISCUSSION

The results in Figure 2 clearly show the saturation effect of the uncorrected concentration estimates $\hat{C}V$. In contrast the corrected concentration estimates increase approximately linearly with increasing concentration, which shows the improved proportionality of the estimates by the statistical correction in accordance with (5).
The rather large deviations from the linear fit for some concentrations could be due to inhomogeneous distribution of the contrast media. Currently, we are developing automated optical microscopic concentration measurements of our gelatine phantoms to exclude this source of error in the future.
It can also be seen from the comparison of corrections in Figure 3 that the correction formula of Reinhardt et al. does not cause too large errors up to a filling factor N_C/N of 0.7 and is always better than the uncorrected pixel count. Thus, results in this range are still valid, even when corrected by (8) but it is generally more valid to use the corrections proposed in this paper.

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