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Evaluation of two different methods for vascular sampling by three-dimensional power Doppler angiography in solid and cystic-solid adnexal masses

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KEYWORDS: Doppler; ovarian neoplasms; three-dimensional ultrasound

ABSTRACT

Objective *To analyze two different methods for performing three-dimensional power Doppler angiography (3D-PDA) vascular sampling in solid and cystic-solid adnexal masses.*

Methods *Twenty-one 3D-PDA volumes from 18 consecutive and unselected solid or cystic-solid adnexal masses (13 malignant and five benign) were analyzed. A single examiner (J. L. A.) acquired all the volumes according to a predetermined scanning protocol. Two different observers (one inexperienced and the other experienced) calculated 3D-PDA vascular indices (vascularization index (VI), flow index (FI) and vascularization flow index (VFI)) from solid tumor areas. First, manual sampling (Plane A, 15° rotation-step) was performed, and 1 week later 5-cm^3 sphere sampling from the most vascularized area was carried out. The observers made a record of any difficulty that they encountered in delineating the solid areas of tumors or in distinguishing true tumor vessels from pre-existing vessels, the time spent performing each analysis was recorded and inter- and intraobserver reproducibility was evaluated for each method using intraclass correlation coefficients (ICC).*

Results *In four (19.0%) of the 21 volumes sphere sampling could not be performed because it was not possible to obtain a sphere smaller than 5.5 cm^3. This happened in cases in which image zooming was used when acquiring the 3D volume. The inexperienced observer encountered more difficulty, but not significantly more, than the experienced observer when analyzing 3D-PDA volumes both by manual sampling (29% vs. 14% of cases) and 5-cm^3 sphere sampling (35% vs. 18% of cases). The mean time spent by the inexperienced observer was significantly greater ($P < 0.001$) than that spent by the experienced observer both for manual sampling (6.11 min vs. 1.85 min) and 5-cm^3 sphere sampling (2.93 min vs. 2.15 min). Contrary to the findings for the experienced observer, the inexperienced observer required less time to perform sphere sampling than they did manual sampling. Interobserver agreement was high for both methods: ICC for manual volume, 0.993; manual VI, 0.908; manual FI, 0.913; manual VFI, 0.914; sphere volume, 0.949; sphere VI, 0.954; sphere FI 0.850; and sphere VFI, 0.953. Intraobserver reproducibility was also high, with all ICCs above 0.99.*

Conclusions *Manual and 5-cm^3 sphere sampling are reproducible methods for 3D-PDA vascular sampling. Caution is required when image zoom is used at the time of acquiring the volume because this may prevent sphere sampling. Difficulties found in performing both manual and sphere sampling do not seem to significantly affect the reproducibility of Doppler index calculations. Copyright © 2009 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Three-dimensional power Doppler angiography (3D-PDA) has been proposed as a method for predicting malignancy in adnexal masses[1–4]. This new technique allows the objective assessment of tumor vascularization by means of the analysis of power Doppler signals[5]. Using the Virtual Organ Computer-Aided AnaLysis (VOCAL™) software (GE Medical Systems, Zipf, Austria) three vascular indices from a given tissue volume can be estimated: vascularization index (VI), flow index (FI) and

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vascularization flow index (VFI)[5]. This approach has been termed 'vascular sampling'[1].

To date, two different methods for performing vascular sampling in ovarian tumors have been proposed. One method consists of manually outlining the vascularized solid areas of a tumor and then calculating vascular indices from the whole solid area[1]. In the other method a 5-cm^3 spherical sample is obtained from the part of the tumor that appears to be the most vascularized on the basis of subjective evaluation[4].

Both methods have been shown to be reproducible[4,6]. However, there are some methodological questions that still need to be answered, including whether both methods can be applied in all cases, whether one of them is less time-consuming than the other, whether one of them is more easily performed by non-experienced observers, whether both are equally reproducible by the same group of observers and whether there are any factors in the volume acquisition process that may affect the method. In order to try to answer some of these questions we performed a study with the following aims: to identify cases in which either method could not be applied; to determine the percentage of cases in which there is difficulty in delineating the solid area for accurate performance of manual sampling; to determine the percentage of cases in which there is difficulty in distinguishing true tumor vessels from pre-existing vessels in the most vascularized area; to compare the time spent performing calculations using each of the two sampling methods; to determine and compare the interobserver reproducibility for the two methods; and to see if there is a correlation between 3D-PDA indices calculated using each method.

PATIENTS AND METHODS

Twenty-one stored 3D volume datasets prospectively collected from 18 consecutive women diagnosed as having a vascularized solid ($n = 9$) or cystic-solid ($n = 9$) adnexal mass, evaluated and treated at the Clinica Universitaria de Navarra from September 2007 to December 2007, were retrieved from our database for analysis. In three cases two different volumes from the same tumor were analyzed. Our institutional review board approved the study and all the women gave informed consent.

The mean age of the patients was 57.1 (range, 30–78) years. Six of the women (33.3%) were premenopausal and twelve (66.7%) were postmenopausal, menopause being defined as at least 1 year of absence of menses in women older than 45 years.

All the women underwent surgery for tumor removal, and definitive histological diagnosis was obtained in all cases. Thirteen (72.2%) tumors were malignant (ten primary ovarian cancers and three metastatic tumors to the ovary) and five (27.8%) were benign (one fibrothecoma, one dermoid cyst, one cystadenofibroma, one fibroma and one leiomyoma).

All the women were evaluated prior to surgery by 3D-transvaginal ultrasonography using a Voluson 730 Expert (GE Medical Systems) by one of the authors (J. L. A.) according to a predefined scanning protocol[1]. Briefly, first B-mode ultrasonography was performed to characterize the morphology of the adnexal masses, which were classified as one of the following: unilocular, multilocular, unilocular–solid, multilocular–solid or solid. After B-mode evaluation had been performed, two-dimensional (2D) power Doppler was used to assess tumor vascularization. Power Doppler settings were set to achieve maximum sensitivity for detecting low-velocity flow without noise (frequency, 5 MHz; power Doppler gain, 0.8; dynamic range, 20–40 dB; edge, 1; persistence, 2; color map, 5; gate, 2; filter, L1; PRF, 0.6 kHz). A 3D volume was then obtained of the suspicious areas, e.g. thick papillary projections, solid areas or, in the case of mostly solid tumors, the whole tumor if possible. Once a 3D volume had been obtained, it was stored on a hard disk (Sonoview^{TM}, GE Medical Systems). Volume acquisition time ranged from 2 to 6 s depending on the size of the volume box. In cases in which a given adnexal mass had more than one volume stored because it contained more than one solid area, we used only the first of the stored volumes for further analysis in this study.

The stored volumes were further analyzed using the VOCAL software by two different observers: Observer A (J. L. A.) with 6 years' experience using 3D ultrasound and Observer B (M. P.) with 1 month's experience using 3D ultrasound. Observer B had completed a 2-week training course on the use of the VOCAL software.

The two sampling methods were performed off-line on a personal computer using the 4D-View software version 5.0 (GE Medical Systems). Measurements were first performed using the manual mode. This was done by selecting the 'manual' option in the VOCAL software and then manually outlining the solid area assessed. We chose Plane A and a 15°-rotation step, thus requiring a total of 12 tracings per solid area assessed (Figure 1). The observers made a record of any cases in which they encountered difficulty in delineating the solid area when performing manual sampling.

3D vascular indices of the solid areas were calculated using the histogram facility (Figure 1b). The total 3D volume consists of a number of voxels (smallest unit of volume). Voxels contain gray-scale and color information quantified using an intensity scale ranging from 0 to 100. According to these values, the system obtains the mean grayness and power Doppler indices to evaluate vascularization and blood flow[5]. The vascular indices calculated were: the VI, expressed as percentage, which measures the number of color voxels in the studied volume, representing the blood vessels within the tissue; the FI, which is the average color value of all color voxels, representing the average color intensity; and the VFI, which is the simple mathematical relationship derived from multiplying VI by FI and dividing the result by 100.

After manual sampling had been performed a second analysis was carried out using 5-cm^3 sphere sampling from the part of the tumor that appeared to be most vascularized on the basis of subjective evaluation. This was done by selecting the 'sphere' option in the VOCAL

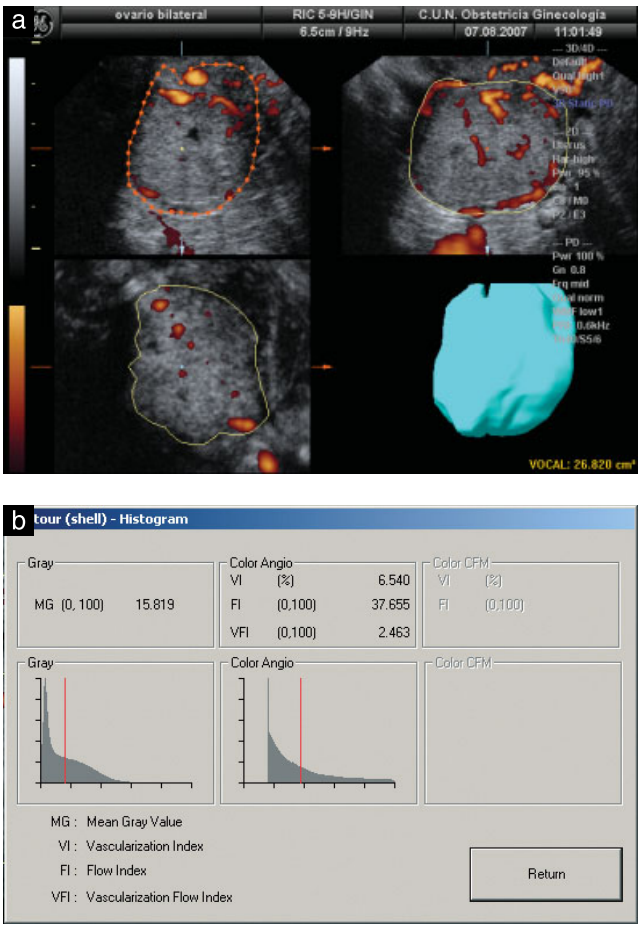

Figure 1 (a) Three-dimensional power Doppler angiography (3D-PDA) volume calculation from a solid adnexal tumor using Virtual Organ Computer-Aided AnaLysis software and manual sampling. A Stage IIb primary serous ovarian cancer was confirmed histologically. (b) Calculation of 3D-PDA vascular indices for the manual sample using the histogram facility in the same case.

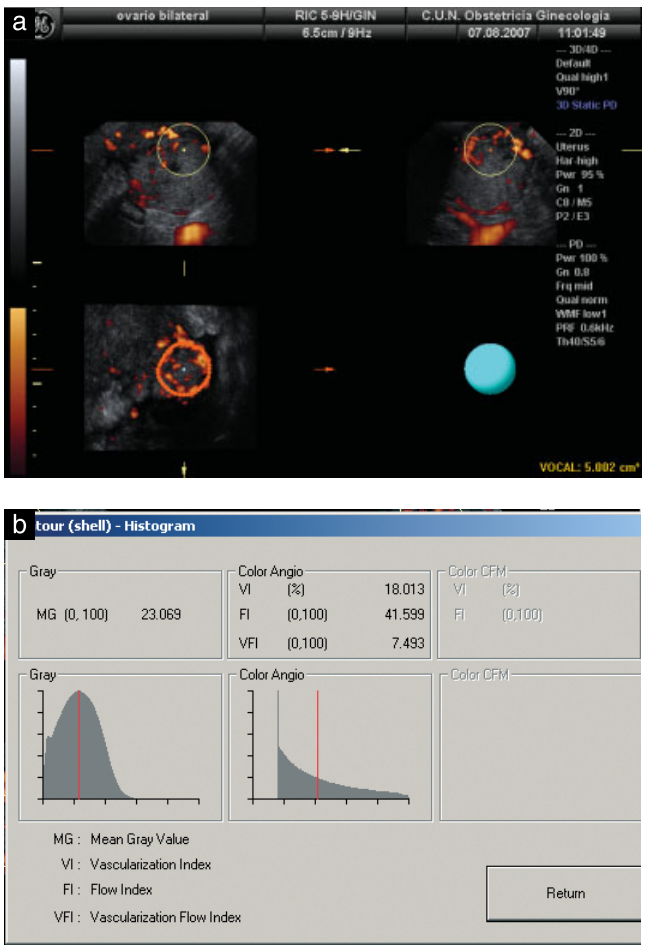

Figure 2 (a) 5-cm^3 sphere sampling from the most highly vascularized area in the same case as that shown in Figure 1. (b) Calculation of three-dimensional power Doppler angiography indices for the 5-cm^3 sphere sample using the histogram facility in the same case.

software. Our previous experience (unpublished data) had shown us that the rotation step (6°, 9°, 15° or 30°) does not affect calculations of Doppler indices when using sphere sampling, so we decided to perform all calculations using a 30°-rotation step. To obtain a 5-cm^3 spherical volume we put the poles as close to each other as possible, including the most vascularized area in between, and then moving them until a 5-cm^3 sphere was obtained (Figure 2). Most of the time it was difficult to obtain an exact 5-cm^3 spherical volume, so we decided to accept volumes ranging from 4.95 cm^3 to 5.05 cm^3 for calculation of power Doppler indices.

The observers made a record of any cases in which they encountered difficulty in distinguishing between true tumor vessels and pre-existing vessels, such as the iliac vessels, in the most highly vascularized area.

The time spent performing the calculations using each sampling method – which can be displayed on the computer screen as calculations are being performed – was measured using multi-timer software. The observers evaluated the volumes blinded to each others' results and also to the histological results. Neither of the observers had previously evaluated the volumes included in the study.

Statistical analysis

The Kolmogorov–Smirnov test was used to assess whether the raw data differed from the normal distribution. Student's *t*-test was used to compare continuous variables that did not differ from the normal distribution and the χ^2 test was used to compare categorical variables. Spearman's rho coefficient (r) was used for analyzing the correlation between 3D-PDA indices calculated by manual and 5-cm^3 sphere sampling. $P < 0.05$ was considered statistically significant.

Interobserver agreement was expressed as the mean difference between the measurements obtained by the two different observers, and limits of agreement (mean difference ±2 SD). Interobserver reproducibility was evaluated by calculating the intraclass correlation coefficients (ICC) with 95% confidence intervals (CI)[7]. Comparison of ICCs was performed by analyzing their 95% CIs; when 95% CIs of ICCs overlap no statistically significant difference exists[8].

To assess intraobserver reproducibility, one observer (M. P.) repeated the 3D-PDA sampling and calculations on the same set of 21 volumes, 1 week after the first

evaluation. As in the interobserver analysis, intraobserver reproducibility was evaluated by calculating the mean difference, 95% limits of agreement and ICC for each variable.

Statistical analysis was performed using the SPSS 15.0 statistical package (SPSS Inc., Chicago, IL, USA).

RESULTS

Manual sampling could be performed in all cases. However, in four (19.0%) of the 21 volumes 5-cm^3 sphere sampling could not be performed because it was impossible to obtain a sphere volume smaller than 5.5 cm^3. We reanalyzed the volumes several times and realized that this happened in some cases in which image zooming had been used when acquiring the volume. In three of these four cases the larger diameter of the solid part was > 5 cm. The use of zooming magnified the tumor even more and it was impossible to obtain a spherical sample less than 5.5 cm^3.

Observer B found more cases difficult to analyze than Observer A, both using manual sampling (29% vs. 14%) and 5-cm^3 sphere sampling (35% vs. 18%), but these differences were not statistically significant. Observer B took more time to perform calculations than did Observer A for both of the sampling methods (Table 1). Observer B performed calculations faster using 5-cm^3 sphere sampling, whereas Observer A performed calculations faster using manual sampling.

There was a significant positive correlation between VI, FI and VFI calculated by manual and 5-cm^3 sphere sampling for both observers (Observer A: VI, $r = 0.532$, $P = 0.028$; FI, $r = 0.708$, $P = 0.001$; VFI, $r = 0.510$, $P = 0.04$. Observer B: VI, $r = 0.640$, $P = 0.006$; FI, $r = 0.836$, $P = 0.001$; VFI, $r = 0.596$, $P = 0.012$). Interobserver agreement was high for both methods. There were no statistical differences between the ICCs for any of the variables analyzed (Table 2, Figure 3). Intraobserver reproducibility was also high (Table 3).

Table 1 Time (min) spent by each observer to perform the calculations using each sampling method

Technique	*Observer A*	*Observer B*	P
Manual sampling	1.85 ± 0.56	6.11 ± 0.57	< 0.001
5-cm^3 sphere	2.15 ± 0.67	2.93 ± 0.32	< 0.001
P	<0.001	<0.001	—

Values are expressed as mean ± SD.

DISCUSSION

3D-PDA is a relatively new technique that allows an objective assessment of ovarian tumor vascularization. Using this technique virtual vascular sampling of a given part of a tumor can be performed. The rationale for this technique is based on the fact that malignant ovarian tumors have a higher microvascular density than do benign tumors[9]. Also, 3D power Doppler-derived vascular indices are thought to reflect tissue vascularity; namely, VI is thought to reflect vascular density and FI is thought to reflect blood flow within those vessels[5]. Our initial study on this topic, therefore, aimed to answer the question of whether malignant ovarian tumors showed higher 3D-PDA vascular indices than did benign ones[1]. We found that solid components in malignant tumors had higher VI, FI and VFI values than those in benign lesions, and concluded that this new technique could be useful for discriminating between and benign malignant vascularized complex adnexal tumors[1]. We called such an approach 'vascular sampling' since our idea was to assess the vascularization from those solid portions of a given tumor. Methodologically, our approach was based on the manual outlining of solid areas using the VOCAL software.

Subsequent studies using a similar approach confirmed our preliminary data[2,3]. More recently, Jokubkiene *et al.* proposed an alternative method based on the use of a 5-cm^3 spherical sample automatically calculated using the VOCAL software. They proposed taking this 5-cm^3 spherical sample from the part of the tumor that appeared to be most vascularized based on subjective assessment[4]. This study also showed that 3D-PDA indices

Table 2 Interobserver reproducibility of measurements of volume and vascular indices

		Differences between two observers		
Parameter	*Median (range)*	*Mean (95% CI)*	*Limits of agreement*	*ICC (95%CI)*
Manual sampling				
Volume (cm^3)	28.860 (0.760–266.300)	−0.600 (−6.620 to 5.410)	−23.140 to 21.940	0.993 (0.981–0.998)
VI (%)	4.654 (1.129–31.533)	1.758 (0.589 to 2.927)	−0.622 to 4.138	0.908 (0.745–0.967)
FI	34.481 (24.770–46.943)	−1.419 (−2.490 to −0.348)	−3.457 to 0.619	0.913 (0.760–0.969)
VFI	1.520 (0.086–14.564)	0.606 (0.141 to 1.071)	−1.084 to 2.350	0.914 (0.762–0.969)
Sphere sampling				
Volume (cm^3)	5.001 (4.950–5.050)	−0.007 (−0.022 to 0.008)	−0.571 to 0.557	0.949 (0.804–0.985)
VI (%)	15.190 (1.716–34.227)	3.761 (1.654 to 5.867)	−2.145 to 9.667	0.954 (0.873–0.983)
FI	36.995 (25.480–52.101)	−4.136 (−6.514 to −1.758)	−11.060 to 2.788	0.850 (0.587–0.946)
VFI	5.130 (0.521–14.210)	1.249 (0.370 to 2.129)	−0.051 to 2.549	0.953 (0.869–0.983)

FI, flow index; ICC, intraclass correlation coefficient; VFI, vascularization flow index; VI, vascularization index.

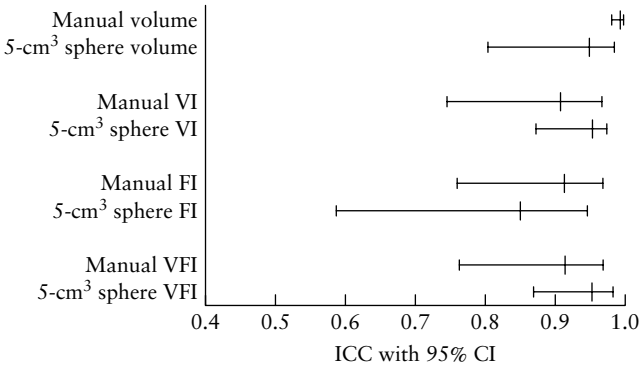

Figure 3 Interobserver intraclass correlation coefficients (ICC) with their 95% confidence intervals (CI) for each parameter assessed. FI, flow index; VFI, vascularization flow index; VI, vascularization index.

in the 5-cm^3 sphere sample were significantly higher in malignant tumors as compared with benign lesions. Thus, both methods seem to be promising for distinguishing malignant from benign ovarian tumors. However, since some methodological differences exist we wondered whether one method could be easier to apply, more reproducible and faster than the other, and whether this would depend on the level of experience of the examiner who performed the calculations. To the best of our knowledge, this is the first study to address these questions. We would like to stress that our aim was not to compare the diagnostic performance of the two methods, but rather to ascertain whether methodological differences between them are relevant or not.

The first finding that we report here is that it is not possible to perform 5-cm^3 sphere sampling in some cases in which image zooming was used in real time when acquiring the 3D volume. This was not a problem for manual sampling. Thus the first lesson learned is that image zooming should not be used when acquiring a 3D volume if the intention is to perform 5-cm^3 sphere sampling later.

Regarding reproducibility, our present study confirms previously published data[4,6]. We found that both methods are highly reproducible between observers. The new information that we provide in this study is that both methods are equally reproducible, since we did not find any statistically significant differences between the ICCs calculated for any variable.

We found some interesting results regarding the amount of time spent by each observer performing the calculations using each sampling method. The non-experienced observer spent longer than the expert observer performing both sampling methods. This is not surprising, since the experienced observer is more accustomed to dealing with 3D volume datasets. However, what was surprising was that the non-experienced observer was faster using 5-cm^3 sphere sampling whereas the experienced observer was faster using manual sampling. This could suggest that less experienced practitioners should use 5-cm^3 sphere sampling rather than manual sampling.

As might be expected, the non-experienced observer found a higher percentage of volumes difficult to analyze, both by manual and 5-cm^3 sphere sampling. However, the differences were not statistically significant. Interestingly, the fact that the non-experienced observer encountered difficulties in more cases in delineating solid areas or identifying true tumor vessels does not seem to have affected the reproducibility of calculations made using either sampling method. In our opinion, this could be considered an advantage of 3D-PDA ultrasound.

It should be stressed that we tested the reproducibility of measurements made on stored volumes. We did not test the reproducibility of the whole procedure, i.e. including volume acquisition. This might be a potential source of bias in our study.

In conclusion, both manual and 5-cm^3 sphere sampling of 3D-PDA volume datasets are reproducible methods. Both methods are less time-consuming when performed by an experienced observer, but for an inexperienced observer sphere sampling was found to require less time than manual sampling. Caution should be used when image zooming is used before acquiring the volume because this may prevent sphere sampling. Difficulties encountered in performing both

Table 3 Intraobserver reproducibility of measurements of volume and vascular indices

		Differences between two measurements made by the same observer		
Parameter	*Median (range)*	*Mean (95% CI)*	*Limits of agreement*	*ICC (95% CI)*
Manual sampling				
Volume (cm^3)	32.116 (8.618–270.080)	−4.661 (−9.534 to 0.202)	−23.599 to 14.270	0.996 (0.988–0.998)
VI (%)	11.360 (1.335–34.167)	0.194 (−0.192 to 0.580)	−1.308 to 1.696	0.998 (0.994–0.999)
FI	35.251 (25.096–46.188)	0.277 (−0.308 to 0.860)	−2.003 to 2.551	0.990 (0.972–0.996)
VFI	4.030 (0.386–14.654)	0.098 (−0.063 to 0.258)	−0.526 to 0.722	0.998 (0.994–0.999)
Sphere sampling				
Volume (cm^3)	5.020 (4.950–5.050)	0.005 (−0.046 to 0.014)	−0.031 to 0.041	0.970 (0.918–0.989)
VI (%)	15.826 (2.058–34.227)	−0.477 (−1.193 to 0.238)	−3.261 to 2.207	0.995 (0.987–0.998)
FI	36.202 (25.480–44.517)	−0.003 (−0.839 to 0.819)	−3.253 to 3.247	0.973 (0.924–0.990)
VFI	5.572 (0.529–14.210)	−0.189 (−0.473 to 0.093)	−1.291 to 0.193	0.995 (0.987–0.998)

FI, flow index; ICC, intraclass correlation coefficient; VFI, vascularization flow index; VI, vascularization index.

manual and sphere sampling do not seem to significantly affect the reproducibility of Doppler index calculations.

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