## Supplementary file

# The Reference Phase Correction for the Fluctuated Scanning Lines and the Slope of the Stage in Tissue Characterization by Scanning Acoustic Microscope 

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Figure S1. The slope adjustment stage. The stationary post holder, located at the origin point. The other two translatable post holders (PH2T/M, Thorlabs, New Jersey, USA), aligned along horizontal and vertical axes. The changeable height of the translatable post holders allows for changing the slope of the stage along the axes. The posts and the surface of the stage are linked by the locking ball unit (TRB1/M, Thorlabs, New Jersey, USA), due to the angle between the base and the surface of the stage created by the slope.


Figure S2. Integrated microscope part of the system. The part uses one objective lens to focus on the target. The objective lens holder was mounted on a vertical translator through a post holder to adjust the focus. One right-angle mirror mount (KCB1C, Thorlabs, New Jersey, USA) was used to change the direction of the light head to eye piece.


Figure S3. A pair of linear scanning motors. The top motor is the scanning motor. The bottom motor is the advance motor. The scanning motor is defined as $X$ axis. The advance motor is defined as $Y$ axis.



Figure S4. Schematic illustration of tissue processing. Tumor was collected, fixed in $10 \%$ neutral buffered formalin, dehydrated, embedded with paraffin, sectioned in $20 \mu \mathrm{~m}$ thick, and attached on charged slice glasses. Before scanning, the sample went through the de-paraffin process to remove paraffin.


Figure S5. Average speed of sound. Data were expressed as mean $\pm$ S.D. $\left(\mathrm{n}=3,{ }^{*} p<0.05,{ }^{* *} p<0.01\right)$.


Figure S6. Graphs of the phase values from Table S1. The phase values at 33 MHz (a) and $80 \mathrm{MHz}(\mathrm{b})$, respectively. The red arrows show the value of individual phases, while the purple arrows indicate its average value.

Table S1. The typical values of phase (in degree units) caused by random noise.

| Sample number | Sample 0 |  | Sample 1 |  | Sample 2 |  | Sample 3 |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\mathbf{3 3} \mathbf{~ M H z}$ | $\mathbf{8 0} \mathbf{~ M H z}$ | $\mathbf{3 3} \mathbf{~ M H z}$ | $\mathbf{8 0} \mathbf{~ M H z}$ | $\mathbf{3 3} \mathbf{~ M H z}$ | $\mathbf{8 0} \mathbf{~ M H z}$ | $\mathbf{3 3} \mathbf{~ M H z}$ | $\mathbf{8 0} \mathbf{~ M H z}$ |
| 1 | 68.576 | 208.128 | 64.501 | 195.106 | 65.624 | 201.742 | 56.683 | 188.992 |
| 2 | 63.555 | 197.326 | 61.6 | 196.372 | 69.051 | 195.888 | 64.895 | 188.204 |
| 3 | 63.823 | 196.984 | 60.391 | 187.102 | 62.12 | 188.856 | 57.657 | 192.901 |
| 4 | 64.269 | 202.667 | 69.126 | 212.449 | 63.744 | 201.809 | 58.971 | 185.671 |
| 5 | 71.265 | 204.953 | 56.782 | 190.03 | 62.513 | 194.567 | 62.51 | 191.53 |
| Average | 66.2976 | 202.0116 | 62.48 | 196.2118 | 64.6104 | 196.5724 | 60.1432 | 189.4596 |
| SD | $\pm 3.450612$ | $\pm 4.840541$ | $\pm 4.633014$ | $\pm 9.824691$ | $\pm 2.832761$ | $\pm 5.435522$ | $\pm 3.453606$ | $\pm 2.840707$ |

