Supplementary Information

Vibrational Profiling of Brain Tumors and Cells

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Supplementary Figure S1: Frequency spectrum of the system and the cantilever. Dominant frequency peaks were observed in the system at 120 Hz and higher harmonics. Example dominant peaks observed in a 30 s recording of a dish with EBS only (baseline). (A) Frequency spectrum of EBS only control. (B) Frequency spectrum of EBS only control band-stop filter to remove the power main frequencies. (C) The RMS of signal from A (unfiltered) and B (filtered) were calculated which confirmed the negligible contribution of power mains to subsequent RMS analysis. (D) The two bar graphs show quantified RMS values for baseline (cantilever + media only) and glioma cell line (BT25) at 21°C and 37°C, respectively. Powers of dominant frequency peaks are represented as arbitrary units (A.U.). Error bars are shown as standard error of mean (SEM).



Supplementary Figure S2: Representative images of hippocampal cultures used for vibration recordings at 1 and 2 weeks in culture. At 2 weeks cultures are denser and neurons form many more connections with surrounding cells. Scale Bar = $30 \mu m$



Supplementary Figure S3: Time domain RMS analysis of cerebellum neurons. (A) Example image of cerebellar cultures used for recordings. (B) RMS amplitude of cerebellum neurons is significantly higher than both 30 min, 1mM sodium azide treated neurons and no-cell recordings. *** represent Tamhane post hoc significant differences between groups (P < 0.001). Error bars are shown as standard error of mean (SEM). Scale Bar = 30 µm



Supplementary Figure S4: Effect of hippocampus tissue volume on the frequency pattern. Frequency spectrum analysis was performed on a piece of hippocampal tissue [volume 1X], tissue piece cut in half [volume 0.5X] and cut to quarter [volume 0.25X]. A major frequency peak is present and remains at ~3.4 Hz. The intensity is proportional to tissue volume size.



Supplementary Figure S5: Effect of cantilever-tissue distance on the signal attenuation in time and frequency domains. (A) The dominant frequency peak at ~4 Hz did not shift while the cantilever moved up from the contact (0.25 nN) state to 5, 10, 15 µm. (B) The RMS values of the time domain signal did not significantly differ between tested cantilever-sample distances. Since the Stoke's attenuation rate $(2 \times 10^{-15} - 1 \times 10^{-14})$ is quite small, the exponential coefficient in $A_d = A_0 \exp(-\alpha d)$ will be virtually one and the traveling wave through EBS at distance d from the tissue (A_d) will not be attenuated. Bars represent mean RMS values. Error bars are shown as standard error of mean (SEM). Powers of dominant frequency peaks are represented as arbitrary units (A.U.).



Supplementary Figure S6: Comparison of vibration patterns between live neural tissue and agarose gel. (A) RMS vibrational amplitude of 1.0 % agarose gel is similar to no cell RMS vibration, and it is significantly different from resting hippocampal tissue RMS. Bars represent mean RMS values \pm SEM. *** represent Tamhane post hoc significant differences between groups (P < 0.001). (B) Frequency pattern of 1.0 % agarose gel is shown. The ~4 Hz dominant frequency observed in resting hippocampal tissue is absent for 1.0 % agarose gel. Powers of dominant frequency peak is represented as arbitrary units (A.U.).



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Supplementary Figure S7: Normal probability plots of frequency clusters calculated with Welch's analysis of malignant astrocytoma (3.65, 11.01, 18.48, 28.29 & 36.30 Hz), lateral temporal cortex (3.38 Hz) and meningioma: (4.23 Hz). Welch plots are depicted in (Fig. 2D-E)

Wave files 1-Malignant Astrocytoma, 2-Meningioma, 3-Lateral Temporal Cortex