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Digital Holography of Intracellular Dynamics to Probe Tissue Physiology

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Abstract

Digital holography provides improved capabilities for imaging through dense tissue. Using a short-coherence source, the digital hologram recorded from backscattered light performs laser ranging that maintains fidelity of information acquired from depths much greater than possible by traditional imaging techniques. Biodynamic Imaging (BDI) is a developing technology for live-tissue imaging of up to a millimeter in depth that uses the hologram intensity fluctuations as label-free image contrast and can study tissue behavior in native microenvironments. In this paper BDI is used to investigate the change in adhesion-dependent tissue response in 3D cultures. The results show that increasing density of cellular adhesions slows motion inside tissue and alters the response to cytoskeletal drugs. A clear signature of membrane fluctuations was observed in mid frequencies (0.1 – 1 Hz) that was enhanced by the application of cytochalasin-D that degrades the actin cortex inside the cell membrane. This enhancement feature is only observed in tissues that have formed adhesions, because cell pellets initially do not show this signature, but develop this signature only after incubation enables adhesions to form.

1. Introduction

Digital holographic techniques provide promising avenues for improved biomedical sensing in the field of deep tissue imaging. Traditionally, imaging through deep tissue has been difficult and *in vitro* tissue assays have favored a two-dimensional (2D) culture model. However, while 2D culture assays have the advantage of simplicity, 2D monolayer cultures feature an artificial environment that modifies cell shape and cell contacts and provides limited connections to the surrounding extracellular matrix (ECM). Furthermore, the mechanical and chemical properties of, and the contacts with, the extracellular environment modify expression of adhesion compounds and adhesion structure [1–4]. Cellular adhesions have been linked to the development and spread of various cancers including colorectal [5, 6], breast [7], ovarian [8], and lung [9], and may contribute to the resistance of tumors to

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chemotherapeutic treatments [10, 11]. Cellular adhesions are an important target of chemotherapy research. 2D cell cultures, with a modified cellular environment, may change how they respond to chemotherapeutic drugs. A more biologically accurate, three-dimensional (3D) tissue model is needed.

Multicellular tumor spheroids closely resemble the macrostructure of *in vivo* cancer tumors [12]. Use of dense 3D tissue models such as tumor spheroids has been limited by the difficulty to obtain information from deep tissue imaging. Biodynamic imaging (BDI) based on digital holographic imaging and analysis techniques obtains biologically relevant information from dense tissue without the need for labels. It uses low-coherence [13–15] digital holography [16] to reduce background and improve sensitivity. The low-coherence holograms enable laser ranging that provides depth-resolved images of regions inside highly scattering media such as biological tissue. This makes it possible to probe processes within the tissue without altering the surrounding microenvironment. In this paper, BDI and dynamic light scattering (DLS) are used to investigate how culture morphology affects cellular adhesions and the measured response of the sample to chemotherapeutic drugs.

2. Biodynamic Imaging

Biodynamic imaging (BDI) combines the depth specificity of off-axis Fourier-domain digital holographic optical coherence imaging (OCI) [17] with the label free sensing of dynamic light scattering (DLS) [18, 19] to measure the biological response of tissue to external stimuli. OCI is a rapid, full-frame, coherence-gated imaging technique that uses short coherence interferometry to depth-resolve images of deep tissue. In OCI (Fig. 1a), light scattered from a target is heterodyned with a distance-of-flight matched reference beam to form a holographic interference pattern at the Fourier plane (Fig. 1, b and c). A CCD pixel array captures this interference pattern, which is then digitally transformed to the image plane (Fig. 1, d and e) through a discrete fast Fourier transform. Because OCI is full-frame, images can be acquired rapidly to capture the dynamics of a living sample as scattered speckle fluctuations. The digital holography system has a lateral resolution of 20 microns that matches the depth resolution set by the coherence length of the broadband light source. The field of view is 1 mm.

Direct imaging of scattered coherent light was used to directly image a diffuse suspension of cells to compare it against results of the tumor spheroids. Image-domain DLS (Fig. 1f) sacrifices depth specificity for simplicity of setup. Image-domain DLS captures spatial patterns of dynamic speckle that are equivalent to homodyne detection (self-referenced digital holography). There is no need to reconstruct or demodulate the direct images. DLS frames capture an image of the target (Fig. 1g) along with a dynamic speckle pattern from which the desired region of interest (Fig. 1h) can be selected. The lateral resolution of the image-domain DLS system is 30 microns.

Motility contrast imaging (MCI) uses speckle intensity fluctuations as a label-free image contrast to create a false-color image of the sample motility. Sample motility indicates sample health and is induced by all cellular activity. MCI images indicate regions of strong motional differences [20]. Regions of low motility may be due to hypoxia or necrosis within

the sample, a common feature in tumor spheroids of several cell culture lines, or may be due to other structural inhomogeneities such as the presence of stromal tissue in tumor biopsies.

To generate an MCI frame, a sequence of OCI reconstructed images is captured, representing one observation of the living target. By capturing several sequences, changes in the time-dependent behavior of the target are detected. For a single OCI sequence, the temporal normalized standard deviation (NSD) of the intensity I is defined at each pixel (x,y) as

$$NSD(x,y) = \frac{\sigma_I(x,y)}{\langle I(x,y) \rangle} = \frac{\sqrt{[\langle I^2 \rangle - \langle I \rangle^2]}}{\langle I \rangle} \quad (1)$$

where $\sigma_I(x,y)$ is the standard deviation and $\langle I(x,y) \rangle$ is the average of the intensity at that pixel across all OCI reconstructed images in the sequence. This value is used to assign a color to each pixel to create a false-color MCI frame of the motility of the sample during that sequence. One MCI frame is generated for each OCI sequence. Changes in sample health can be inferred from the average NSD of the entire sample during a particular OCI sequence compared with the average NSD of the previous sequence, and the motility maps can be tracked as they change in time in response to perturbations. Fig. 2a shows successive MCI frames from one healthy and one treated (nocodazole) tumor spheroid. Heterogeneous changes in the motility of the treated spheroid can be seen in the MCI frames while relative sample health is quantified by changes in the average NSD over time shown in Fig. 2b.

Tissue dynamics spectroscopy (TDS) [21] uses speckle fluctuation spectroscopy on the reconstructed holographic OCI frames to produce a characteristic power spectrum for the sample. The shape of these power spectra are dependent on the speed and persistence of moving scatterers within the sample (vesicles, mitochondria, nuclei, membranes), and the spectra change shape with changing sample conditions. For a given intracellular process defined by an rms speed v_0 and persistence time t_0 , the diffusive knee frequency of the fluctuation spectrum is

$$f_d = \frac{q^2 v_0^2 t_0}{2\pi} \quad (2)$$

where q is the momentum transfer. Persistence times are around 1 second for active transport. Therefore, the rms speed for a knee frequency of 0.5 Hz is approximately 100 nm/sec, which is a characteristic speed for a nucleus and for active membrane undulations. Speckle fluctuation spectra from living samples are complex with many cellular processes contributing to the shape, but can still be characterized by a general knee frequency, f_k , which is determined by contributions from all motions. The power spectra typically span three orders of magnitude in intensity, and are captured across three orders of magnitude in frequency. Fig. 3 shows the initial reconstructed OCI image (a) and MCI frame (b) for tumor spheroids of two cell lines, HT-29 and PaCa-2, as well as speckle fluctuation spectra (c) for each spheroid before and after an application of nocodazole. The initial PaCa-2 spectrum shows a clear knee frequency at 0.344 Hz, while the knee frequency for HT-29 is less distinct at 0.0175 Hz. Following the application of nocodazole, the knee frequency shifts to

0.199 Hz for PaCa-2 and the HT-29 spectrum becomes more characteristic of a $1/f$ spectrum without a clear knee.

The differential of the power spectrum is calculated by comparing the power spectrum of a sample after a perturbation $S(f, t)$ relative to the average baseline power spectrum $\bar{S}(f, 0)$ for the sample,

$$D(f, t) = \frac{S(f, t) - \bar{S}(f, 0)}{\bar{S}(f, 0)}. \quad (3)$$

Plotting the differential, denoted $D(f)$ in the rest of the paper, on a time-frequency axis creates a drug-response spectrogram that acts as a “fingerprint” of the sample response to that perturbation. Fig. 3d shows two example drug response spectrograms for nocodazole applied to PaCa2 and HT-29 tumor spheroids.

A. OCI Sequence

A diagram of the OCI setup is shown in Fig. 1a. The OCI system uses a 20.5 mW broadband super-luminescent diode (Superlum) centered on 847 nm wavelength to generate the incident low-coherence beam. The CCD camera used was a Rolera EMC2 from QImaging. Capture was triggered externally through a function generator controlled by a custom developed LabVIEW (National Instruments) program. Camera frames were recorded using StreamPix 5 (Norpix).

OCI frames of tumor spheroids were captured in cycles every 20–24 minutes, on average. Each capture sequence consisted of 15–45 seconds travel time to allow the system to transition between tumors, followed by frame capture: 10 background frames captured at 10 frames per second (fps) with the reference beam not zero-path matched, then 50 frames at 0.5 fps and 500 frames at 25 fps with the reference beam zero-path matched. All frames were captured with a 10 msec exposure time. Tumor spheroids were observed for 12 cycles (4.8 hours) to allow the tumors to stabilize. Half of the growth medium (~0.5 mL) was removed from each well and replaced with a solution of drug compound and growth media. Tumors were then observed for 27 cycles (10.8 hours).

A. Image-domain DLS

A diagram of a separate image-domain DLS system is shown in Fig. 1f. The incident beam at 840 nm wavelength was supplied by a high-coherence 106 mW Melles-Griot CW diode laser (56DOL817). The CCD camera used was a Retiga EX from QImaging. Capture was triggered externally through a function generator controlled by a custom developed LabVIEW (National Instruments) program. Camera frames were recorded using StreamPix 5 (Norpix). Another custom LabVIEW program controlled the pump (NE-500, New Era Pump Systems) used for drug injection.

Frames were captured in 4-minute cycles, capturing 250 frames at 10 fps followed by 100 frames at 0.5 fps. All frames were captured with a 10 msec exposure time. Cells in suspension were observed for 46 cycles (~3 hours) to allow the sample to stabilize. Then 2 mL of control drug was injected into the flow cell over 8 minutes to completely replace the

growth medium. Samples were observed for 180 cycles (12 hours). Frame capture was continuous with 4 cycles given to the drug injection. At the end of the experiment, samples were fixed by injecting 3% glutaraldehyde into the flow cell and were observed for another 16 cycles (~1 hour).

3. Experimental Procedure

A. Cell Cultures

Cell lines are from American Type Culture Collection (ATCC) Manassas, VA. Cells were cultured in their respective growth media (Table 1) at 37 °C in a humidified CO₂ incubator. All growth media contain 10% fetal calf serum (Atlanta Biologicals), penicillin (100 IU), and streptomycin (100 µg/mL).

B. Cytoskeletal Drugs

Cytochalasin-D, colchicine, nocodazole, paclitaxel, and dimethyl sulfoxide (DMSO) were obtained from Sigma. Each drug was prepared to twice the desired concentration to allow for later mixing inside the sample well. DMSO dissolved in growth medium was used as the negative control in all experiments, and all drugs are each dissolved in DMSO before being added to the growth medium to improve solubility.

C. Tumor Spheroids

Spheroids were created in a 50 mL rotating bioreactor (Synthecon, Houston, TX). Bioreactors were incubated for 7–10 days, or until tumor spheroids were just visible to the naked eye (~150 µm), before spheroids began being used in an experiment. Typical spheroid size was 200–600 µm. Spheroids were immobilized in 8-well chamber slides.

Slides were glued to a 2-axis translation stage in the OCI system to prevent sample movement during observation. Eight tumors were selected from each slide for testing, typically one tumor from each well. Growth medium was heated to 37 °C and pipetted into each well until the well was full and any bubbles were removed.

D. Cell Suspensions

Cells were grown in flasks until about 80% confluent. Cells were then trypsinized and pipetted into a 1.5 in. diameter petri dish at 10⁵–10⁶ cells/mL. Cells and agarose were gently mixed and the dish was incubated for ~4 hours prior to experimentation. Each sample was cut into roughly rectangular shape (~10mm × 5mm) using a razor blade and then placed inside a flow cell (49IR1, NSG Precision Cells). The lid of the flow cell held the sample in place. Growth medium was heated to 37 °C and between 3 and 5 mL were injected through the flow cell using a syringe and tubing.

E. Cell Pellets

8-well chamber slides of cell pellets were prepared from centrifuged cell cultures and embedded in low-temperature gelling agarose. Slides received either no incubation, or 24 hours of incubation, prior to testing to allow adhesions to form. Slides of cell pellets were otherwise handled in the same manner as those of tumor spheroids.

4. Results

A. Cell Line Trends in Cellular Adhesions

Three cell lines were chosen to investigate the effects of cell adhesion density on the performance of biodynamic imaging. The holographic MCI images (Fig. 4a) of human colorectal adenocarcinoma HT-29 demonstrated the strongest adhesions. They form round spheroids and exhibit a low-motility core indicative of hypoxia even in small tumors ($< 100 \mu\text{m}$). Human pancreatic carcinoma MIA PaCa-2 (PaCa-2) showed the weakest adhesions, forming loose cell conglomerates instead of spheroids with no low-motility core even in larger tumors ($> 300 \mu\text{m}$). Rat osteosarcoma UMR-106 has adhesion density falling between the two extremes, forming round spheroids but exhibiting low-motility cores in larger tumors.

Fig. 4b shows microscope images of cells suspended in agarose for each cell line. HT-29 cells formed heterogeneous suspensions composed of cell clumps and individual cells. UMR-106 and PaCa-2 formed fairly homogenous suspensions. PaCa-2 formed slightly larger cell clumps due to rapid cell growth.

Sample responses were measured under the four cytoskeletal drugs (Table 2). Fig. 5 shows average tissue response spectrograms for the three cell lines under the four drugs. Response to cytochalasin-D for the three cell lines showed an enhancement in the mid-frequencies, generally between 0.1 and 1 Hz (shown in white square on the figure). This frequency band is associated with undulations in the cell membrane [22] and its enhancement is consistent with increased movement of the cell membrane due to a decrease in cell adhesions.

The intensity and duration of the mid-frequency enhancement (Fig. 6) caused by cytochalasin D were proportional to the relative cell adhesion strengths of the three cell lines as predicted from the MCI images. The center frequency describes the frequency about which the enhancement is centered which decreases with increasing relative cell adhesion strength, indicative of the resistive influence that cell adhesions have on the motion of the cell membrane.

The response to colchicine, nocodazole, and paclitaxel (Fig. 7) in UMR-106 and PaCa-2 showed a low-frequency enhancement that shifts to lower frequencies with increasing relative adhesion strength. Significantly, HT-29 response to these microtubule drugs had almost no low-frequency enhancement, but does exhibit enhanced motion above 1 Hz. This high-frequency enhancement was observed in the UMR-106 and in PaCa-2 at or above 5 Hz.

B. Influence of Growth on Cellular Adhesion Formation

To test the effect of different growth techniques on drug responsiveness, tissue response was measured for three different cell culture growth environments: tumor spheroids, cell pellets, and cells suspended in agar.

The response in UMR-106 to colchicine and paclitaxel (Fig. 7) is consistent between tumor spheroids and cells in suspension. Colchicine shows a characteristic low-frequency (0–0.1 Hz) enhancement that reflects changes in cell shape due to reduction in cytoskeletal stiffness

by inhibition of microtubule polymerization. The features of the response spectrogram shift from 0.05 Hz in the cell suspension to 0.1 Hz in the tumor spheroids due to the increased degree of multiple light scattering.

On the other hand, the response to cytochalasin-D in UMR-106 (Fig. 7) is inconsistent between tumor spheroids and cells in suspension, and the mid-range (0.1–1 Hz) spheroid enhancement response is not present in the response of the cells in suspension. This lack of mid-range enhancement was found to be independent of the dose of cytochalasin-D, although the cells in suspension do exhibit a dose-dependent response (Fig. 8).

Tumor spheroids and cell suspensions of PaCa-2 (Fig. 9) show a difference in their respective drug responses that is similar to the difference in drug response of UMR-106, with the cell suspensions lacking an enhancement feature in the mid-frequencies that occurs in the drug response of the tumor spheroids. Spectrogram features for PaCa-2 appear at higher frequencies than UMR-106 in both tumor spheroids and cell suspensions, which is consistent with the hypothesis that the lower adhesion density of PaCa-2 allows for faster cell membrane motion when the cytoskeletal drugs are applied.

To investigate the transition in response between the adhesion-poor environment of the cells in suspension and the adhesion-rich environment of the tumor spheroids, we measured the response of dense cell pellets formed from cell cultures. These pellets are optically dense like tumor spheroids, but initially they have fewer adhesions. Incubating the pellets allows adhesions to form. Cell pellets of PaCa-2 were treated with cytochalasin-D and their response was measured to rule out sample density and scattering frequency as the cause of this difference in drug response. Differing levels of cell adhesions were allowed to develop by changing the time over which the pellets were incubated. Pellets that received no incubation prior to testing showed a drug-response similar to that of the cell suspensions. After 24 hours of incubation, cell pellet drug-response showed a moderate enhancement in the mid-frequencies, becoming more like the tumor spheroid response, indicating increased formation of cell contacts prior to drug injection (Fig. 9).

5. Discussion and Conclusion

Predicting cancer response to chemotherapies requires culture models that accurately mimic the native tissue microenvironment and requires assays that can measure cell response within that environment. In the present study, we used digital holography in the form of biodynamic imaging to study the response of multicellular tumor spheroids, a common 3D tissue model, to several cytoskeletal drugs. We also investigated how this response is altered by differences in the level of cellular adhesions present in the culture model.

Tumor spheroids of the three cell lines—HT-29, UMR-106, and PaCa-2—displayed strong changes in cellular motion in response to disruption of the cytoskeleton. For the UMR-106 and PaCa-2 cell lines, changes to the microtubules resulted in similar alterations in the cellular motion irrespective of whether the disruption was due to polymerization or depolymerization. The changes due to microtubule disruption for HT-29, however, are significantly different from those of UMR-106 and PaCa-2 over the frequencies measured.

Cohen [23] showed that microtubule disruption in HT-29 induced cell differentiation and was accompanied by assembly and reorganization of the actin microfilaments, which suggests that HT-29 displays a response to nocodazole, colchicine, and paclitaxel that is physiologically different from that of UMR-106 or PaCa-2.

Tumor spheroids and cells in suspension exhibit significantly different behavior in response to cytochalasin-D for the UMR-106 and PaCa-2 cell lines. Drug dosage does not account for this difference, with dose only influencing how quickly a drug affects the cells. While cytochalasin-D appears to induce cell death in both the tumor spheroids and cells in suspension, only the adhesion-rich tumor spheroids showed significant increase in membrane motion accompanying the actin microfilament depolymerization. Ujihara found that actin disruption by cytochalasin-D significantly decreased adhesion area in fibroblasts while microtubule disruption by colchicine had a much smaller effect [24]. Greater decrease in adhesion area would account for the stronger difference in tissue response due to cytochalasin-D relative to colchicine observed between adhesion-rich and adhesion-poor culture models.

Holographic biodynamic imaging measurements on cell pellets with variable incubation demonstrate a transition from adhesion-poor to adhesion-rich environments. This suggests that the difference in response shown by the cell suspensions and tumor spheroids is not due to differences in sample density, but is caused by changes in the microenvironment during incubation and is a strong indication of the influence of cell-cell contacts and the extracellular matrix on overall sample response to chemotherapeutic drugs.

In conclusion, digital holography using a low-coherence light source enables depth-gated (coherence-gated) imaging up to a mm inside tissue. The holographic detection has high sensitivity to phase modulation caused by intracellular motions in living tissue. Therefore, holographic capture of backscattered light provides a valuable means to investigate changes in intracellular dynamics induced by applied drugs. In this paper, a systematic study was performed of dynamic light scattering signals as a function of the adhesion strength among the cells in tissues grown from different cell lines and different growth environments. A clear signature of membrane fluctuations was observed in mid frequencies (0.1 – 1 Hz) that was enhanced by the application of cytochalasin-D that degrades the actin cortex inside the cell membrane. This enhancement feature is only observed in tissues that have formed adhesions, because cell pellets initially do not show this signature, but develop this signature after incubation enables adhesions to form. Digital holography, and in particular biodynamic imaging that uses digital holography to capture tissue dynamics, provides a new analysis technique to assess drug effects in natural 3D tissue environments.

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Fig. 1.

Fourier-domain OCI and image-domain DLS setup and output. In off-axis Fourier-domain OCI (a), lenses and waveplates (not shown) shape low-coherence light from the superluminescent diode before it is split by a polarizing beamsplitter (PBS) into object and reference beams. The object beam is scattered by the target and then collected and projected onto the Fourier-plane (FP) at the camera (CCD) by a system of lenses (L1) with a $3\times$ demagnification. The reference beam passes through the delay stage (dotted box) and is directed onto the CCD by means of the mirrors (M). Lenses (not shown) are used to shape the reference beam to match the beam profile of the scattered object beam at the CCD. The OCI Fourier frame (b) captures a holographic intensity pattern (c) when the optical path length difference between the object and reference beams is within the coherence length of the source. The delay stage can adjust the optical path length difference to probe different depths within the target. The corresponding image and conjugate image (d) are reconstructed from the OCI frame through a fast Fourier transform. An image of the target, a tumor spheroid, can be seen (e). In image-domain DLS (f), incident light from a CW laser scatters off a target and then is collected and projected onto the image-plane (IP) at a CCD by lenses (L1 and L2). Focal lengths of the two lenses are 5 mm for L1 and 20 mm for L2, resulting in a $4\times$ magnification of the object plane at the CCD. The DLS frame captures an image of the

object (g) as a dynamic speckle pattern. Dynamic measurements are made on a small portion of the sample (h), indicated by the white box in (g).

Fig. 2.

MCI and average NSD as indicators of sample health. Successive MCI frames (a), spaced 18 minutes apart, for two HT-29 tumor spheroids—one healthy under growth medium, and one treated with nocodazole at 10 $\mu\text{g/mL}$ —show how the drug affects motility. Each MCI frame was generated from a separate sequence of OCI reconstructed images. The scaled NSD (b) of the healthy spheroid remains constant over 10 hours while the scaled NSD of the drugged spheroid rapidly decreases during the first two hours following application of the drug.

Scaled NSD sets the initial average NSD of each spheroid to 1 to account for differences in the initial NSD values of the samples.

Fig. 3.

Example BDI results for HT-29 and PaCa-2 tumor spheroids. Reconstructed holographic OCI frames of the spheroids (a) capture dynamic speckle patterns that encode information about scatterers in the sample volume. MCI frames (b) use NSD to contrast regions of high and low motility. Speckle fluctuation spectra (c) show the power spectral density of the different frequencies of motion of the scatterers, which change in response to outside stimuli. The spectral knee frequency, f_k , shifts from 0.0175 to less than 0.01 Hz for HT-29 and from 0.344 to 0.199 for PaCa-2 as the tumor spheroids respond to an application of nocodazole (10 $\mu\text{g/mL}$). Drug response spectrograms of two tumor spheroids (d) plot the difference, $D(f)$, between the average initial power spectrum of the spheroid before treatment and each successive power spectrum calculated after treatment on a time-frequency axis, and indicate enhanced/suppressed scatterer motion at those frequencies. Arrows in (c) and (d) indicate the zero-difference contour of the response, which separates increase from decrease in the power spectral density of the response.

Fig. 4.

Holographic motility contrast images (MCI) (a) showing the shape and motility of tumor spheroids of different sizes for three cell lines along with microscope images (b) of cell suspensions for the same cell lines. HT-29 formed tight, round tumor spheroids with large regions of low motility in the center even in tumors of less than 200 μm diameter. UMR-106 formed round tumor spheroids, with low-motility central regions typically appearing in spheroids of greater than 200 μm diameter. PaCa-2 formed irregularly shaped tumors that showed no low-motility regions even at sizes greater than 500 μm .

Fig. 5.

Biodynamic tissue response spectrograms of tumor spheroids responding to different cytoskeletal drugs. All drugs were administered at 10 $\mu\text{g/mL}$ concentrations. BDI of HT-29 and PaCa-2 spheroids used a faster frame rate (25 fps) compared with BDI of UMR-106 (10 fps). The response of HT-29 to the microtubule-affecting drugs—paclitaxel (Taxol), nocodazole (Noco.), and colchicine (Colch.)—was noticeably different from the response of PaCa-2 or UMR-106. The white lines highlight the zero-contour of the relevant feature.

Fig. 6. Response to cytochalasin-D. Center frequency, amplitude, and duration of mid-frequency enhancement measured from tissue dynamic response spectrograms for cytochalasin-D applied to different cell lines. As the relative adhesion strength of the cell line increases, the enhancement shifts to lower frequencies, suggesting slower motion, and has longer amplitude and duration.

Fig. 7.

Comparison of tissue response spectrograms for UMR-106 to cytoskeletal drugs. Response spectrograms for cells in suspension (Susp.) under colchicine (Colch.) and paclitaxel (Taxol) present features (marked by the white lines) consistent with those for the tumor spheroids (Spher.) under the same drug. However, the response spectrogram for cells in suspension under cytochalasin-D (Cyto.) lacks the mid-range (0.1–1 Hz) enhancement seen in the tumor spheroid (white square). The white lines and square highlight the zero-contour of the relevant feature.

Drug Dose ($\mu\text{g/mL}$)	0.	0.01	0.1	1	10
Saturation Time (hr)	>340	20.9	6.1	0.9	0.4

Fig. 8.

Dose dependent response to cytochalasin-D for UMR-106 cells in suspension. Tissue response spectrograms for UMR-106 cells in suspension under cytochalasin-D at concentrations of 0, 0.01, 0.1, 1 and 10 $\mu\text{g/mL}$ lack mid-range (0.1–1 Hz) enhancement at all doses. However, the average of the differential response shows an overall suppression whose saturation time decreases monotonically with increasing concentration. The saturation time estimates how quickly the cytochalasin-D affects the cells in suspension. Average differential response is averaged over frequencies from 0.005 to 1 Hz. The samples

under 0 and 0.1 $\mu\text{g/mL}$ cytochalasin-D have an slight initial enhancement relative to the baseline (0.095 and 0.126, respectively) that offsets the overall suppression measured.

Fig. 9.

Tissue response spectrograms of PaCa-2 to cytochalasin-D and colchicine for cells in suspension (Susp.), cell pellets, and tumor spheroids (Spher.). Cell pellets were tested after 0 and 24 hours of incubation. The response to colchicine consistently shows a strong low-frequency range enhancement. The response of the cell pellets to cytochalasin-D demonstrated a transition in responses with the response of samples without incubation (0 hr) being similar to the response of cell suspensions, lacking the enhancement associated with changes in the cellular adhesions. After 24 hours of incubation the response becomes closer to that of the tumor spheroid and begins to show the expected enhancement (indicated by the white oval).

Table 1

Cell Culture Information

Cell Line	Cell Origin	Growth Medium
HT-29	Human colon	McCoy 5A
UMR-106	Rat bone	DMEM
MIA PaCa-2	Human pancreas	DMEM

Table 2

Cytoskeletal Drug Information

Drug	Abbr.	Mechanism of Action
Cytochalasin-D	Cyto.	Inhibit actin polymerization
Colchicine	Colch.	Inhibit microtubule polymerization
Nocodazole	Noco.	Inhibit microtubule polymerization
Paclitaxel	Taxol	Inhibit microtubule depolymerization