Cellular motility as a novel contrast agent in digital holography of tissue

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Digital off-axis holography with coherence-domain depth gating can measure subcellular motion inside tumors that respond to anticancer drugs affecting the cytoskeleton.

Motility is essential for virtually all cell functions, normal and pathological, including organelle trafficking inside the cytosol (intracellular fluid), cell division during mitosis, and migration in tumor metastasis. Microscopic studies of motility performed on 2D cell cultures lack the natural 3D environment of cellular tissue, and dimensionality affects how cells communicate and respond to stimuli. By combining digital holography with coherence-domain depth gating, the approach known as holographic optical coherence imaging (HOCI) can extract subcellular motion from deep within living tissue.

HOCI is a hybrid technique that uses the depth-selective advantages of coherence-domain imaging combined with the benefits of digital microscopy. Digital off-axis holography provides a natural approach for coherence detection using spatial heterodyning (an interferometric technique), not unlike temporal or spectral heterodyning employed in optical coherence tomography (OCT). Capture of a full frame on a digital CCD camera carries with it a multiplex advantage for improved signal-to-noise performance that offsets finite bit depth on the camera pixels.

We work with a Fourier-domain holography configuration in which the hologram is recorded by the CCD array placed at the Fourier plane of the imaging system, as shown in Figure 1. Image demodulation is accomplished through a simple fast Fourier transform (FFT) that yields both amplitude and phase of the object through the combination of complex-conjugate image pairs. Our approach is somewhat simpler than Fresnel techniques and is compatible with large depth of focus (roughly 0.5mm) at low magnification. With our interest in statistical measurements of cellular motility, as opposed to microscopic imaging, a large field of view works to our advantage.

A 3D visualization of motility in a tumor spheroid is shown in Figure 2. The healthy outer shell, red and orange in the false-color rendering, represents high motility around a blue, low-motility necrotic core. This image clearly indicates active and inactive areas, and such high contrast is exceptionally sensitive to stimuli and assaults on the tumor that directly affect motion. Because of the cytoskeleton’s essential role in cellular motility, we are able to focus on tissue motility in response to certain anticancer drugs.

Antimitotic drugs (AMDs) are common anticancer agents that disrupt cell division. Because cancer cells have unchecked cell cycles, attacking mitosis can preferentially affect them. The most common AMDs impact the cytoskeleton, including microtubules and actin filaments, due to its central role in the mechanics of cell division. We have studied four such drugs, each with a different mechanism of action. Colchicine, naturally derived...
Figure 2. In this volumetric false-color cutaway of a tumor spheroid (680nm diameter), reds and oranges represent high motility in the outer shell surrounding the low-motility region of the necrotic core.

from the autumn crocus, inhibits tubulin polymerization. The synthetic drug, nocodazole, works in a similar manner, but is more potent. Taxol, on the other hand, acts to stabilize microtubules against depolymerization, while cytochalasin D affects actin filaments.

Figure 3 shows the effect of nocodazole on cellular motility, as demonstrated in time-lapse images. The initial frame, prior to application of 33µM, shows strong motility in the outer shell, decreasing in subsequent images, over the course of an hour, at a dose-dependent rate.

In conclusion, we have developed a new imaging modality that uses cellular motility itself as a novel contrast agent. Data are obtained from deep—up to 1mm—within tissue at a range inaccessible to any other imaging technology. This new capability will likely suggest novel applications in the field of cell- and tissue-based assays.

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