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Longitudinal, quantitative monitoring of therapeutic response in 3D *in vitro* tumor models with OCT for high-content therapeutic screening

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ABSTRACT

In vitro three-dimensional models of cancer have the ability to recapitulate many features of tumors found *in vivo*, including cell–cell and cell–matrix interactions, microenvironments that become hypoxic and acidic, and other barriers to effective therapy. These model tumors can be large, highly complex, heterogeneous, and undergo time-dependent growth and treatment response processes that are difficult to track and quantify using standard imaging tools. Optical coherence tomography is an optical ranging technique that is ideally suited for visualizing, monitoring, and quantifying the growth and treatment response dynamics occurring in these informative model systems. By optimizing both optical coherence tomography and 3D culture systems, it is possible to continuously and non-perturbatively monitor advanced *in vitro* models without the use of labels over the course of hours and days. In this chapter, we describe approaches and methods for creating and carrying out quantitative therapeutic screens with *in vitro* 3D cultures using optical coherence tomography to gain insights into therapeutic mechanisms and build more effective treatment regimens.

1. Introduction

Therapeutics have traditionally been chosen through a process of trial and error, where the selection of potential candidates can be time consuming, laborious, and expensive. While trial and error methods can be acceptable in the fight against some slow-growing cancers, in many cases there is a pressing need to select optimal therapeutics; ineffective therapies can lead to the loss of precious time in fighting growing cancers, while treatments with only moderate success can drive the evolution of therapeutic resistance in patients. For many patients diagnosed with fast-growing or advanced stage cancers, such as metastatic ovarian cancer, the use of emergent technologies to build personalized therapies on a case-by-case basis are sorely needed.

Live-cell assays utilizing patient-derived samples can provide important insights into how an individual will respond to therapy. Cultured cells have been extensively used for therapeutic screening, with readouts including traditional metrics of cell death (necrosis, apoptosis), gene expression changes, and morphologic and phenotypic cellular alterations. These approaches have revealed insights into different stages of treatment response, and can be used to identify windows of opportunity for targeted therapy [1]. The downside to single-cell approaches is that the artificial testing environment insufficiently recapitulates the disease *in vivo*. Therapeutic response in monolayer cellular cultures often overestimates treatment efficacy, and can miss the effects of key parameters affecting treatment outcome including microenvironmental variables such as perfusion, hypoxia, and acidosis.

Three-dimensional (3D) cultures are far more biologically relevant in that they provide many of the important cell–cell and cell–matrix interactions that occur within tumors and can be engineered to replicate crucial microenvironmental features found *in vivo*. These complex culture systems come in many varieties, including suspension, overlay, and embedded models [2–6], as well as natural and synthetic scaffold-based cultures [7]. Cancer cells grown into 3D cultures can be curated to develop into nodules that replicate morphological parameters of clinical disease, such as the formation of hollow lumens [4] and hypoxic microenvironments in the cores of large tumors [8,9]. With the ability to replicate important, therapeutically-relevant features and parameters of disease

Abbreviations: 3D, three-dimensional; 4D, four-dimensional; CCD, charge-coupled device; GFR, growth factor reduced; FD-OCT, frequency domain OCT; FDML-OCT, frequency domain mode-locked OCT; FWHM, full width at half maximum; ECM, extracellular matrix; NA, numerical aperture; OCT, optical coherence tomography; OFDI, optical frequency domain imaging; PDT, photodynamic therapy; PS, photosensitizer; SNR, signal-to-noise ratio; SD-OCT, spectral domain OCT; TD-OCT, time-domain OCT; TL-OCT, time-lapse OCT.

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in vivo, 3D culture systems closely replicate the dose-dependent treatment responses observed in patients, making them a valuable tool for evaluating and optimizing novel therapeutic regimens [10].

As 3D cancer culture systems are typically heterogeneous, optical imaging has been used effectively to monitor treatment response, enabling analysis of the complex information they provide. Many approaches, such as histology and fluorescence staining, however, can miss critical dynamic treatment response information, as they require either fixation or the introduction of cytotoxic compounds. Live-cell stains and autofluorescence imaging can achieve non-perturbative imaging, but the limited penetration depth of fluorescent agents and light into 3D cultures makes examining large (>500 μm diameter) nodules difficult.

Optical coherence tomography (OCT) [11,12] offers considerable advantages for monitoring therapeutic response in 3D tissue culture systems. As an optical ranging technique, OCT is analogous to ultrasound and is used to rapidly generate 3D image volumes of samples. OCT systems can be made to probe millimeters deep into samples with subcellular spatial resolution, allowing for detailed cellular analysis throughout entire *in vitro* cultures. The contrast in OCT derives from the scattering of light from samples, and as such, does not require the use of exogenous labels. Using only a few hundred microwatts of infrared light, time-lapse OCT (TL-OCT) can continuously capture dynamic processes such as treatment response in 3D cultures over the course of days and even weeks. Moreover, OCT data can be readily mined and quantified, allowing for comprehensive, 3D mapping of treatment response over time.

In this chapter, we will introduce methods and provide guidance in demonstrating how to create and optimize OCT systems for use with 3D cultures, integrate such a system into a commercial microscopy platform, and carry out high-content therapeutic screens using biologically-relevant 3D culture models. In addition, we will demonstrate an approach for analyzing the generated data sets to reveal insights into therapeutic response mechanisms.

2. Selecting and building OCT systems for *in vitro* screening

OCT is based on low-coherence interferometry, where depth information is collected by interfering light reflected from two separate but equal path lengths. Most OCT systems are configured with a Michelson interferometer design: broadband input light is divided by a beam splitting optic into two orthogonal paths, here called the sample arm and the reference arm. Light reflected from the ends of these arms travels back to the beam splitter; if the paths are equal, a strong interference signal is observed. OCT takes advantage of the fact that light can be reflected off of many different surfaces in a sample, each of which creates a contribution to the interferometric signal. Early OCT systems operated in the so-called time-domain: a movable mirror at the end of the reference arm would oscillate over the depth scan range to generate interferometric fringes corresponding to each reflecting surface in the sample [13,14]. While these time-domain OCT systems (TD-OCT) had much success, they have been superseded by faster spectral (SD-OCT) or frequency (FD-OCT) OCT systems that offer lower noise operation [15]. In spectral domain systems, the reference arm length is held constant, and instead the entire spectrum of the interferogram is collected on a camera. The depth scan is recovered by simply Fourier transforming the spectral interferogram. Swept-source spectral domain OCT systems have recently been created, including optical frequency domain imaging (OFDI) [16] and frequency domain mode-locked OCT (FDML-OCT) [17], that offer significant speed, depth and sensitivity enhancements.

Like any optical system, OCT imaging platforms can be designed for different goals and end users. OCT systems developed for *in vitro* screening have different requirements than those typically built for high-speed *in vivo* imaging. For most screening requirements, *in vitro* imaging systems rarely require high data acquisition rates or multiple millimeters of imaging depth for 3D culture systems. *In vitro* culture systems are typically no more than 1–2 mm in thickness, with experiments generally focused on monitoring growth, migration, and cellular death. For monitoring such processes, high resolution is paramount, necessitating the use of broadband light sources. In OCT, the spectral interferogram and depth scan are related by a Fourier transform. Thus, the spectral bandwidth of the light source is inversely related to the axial resolution of the system: the broader the light source, the more axial resolution afforded. Commercial light sources for OCT are available in three main wavelength regimes: 750–960 nm, 1000–1100 nm, and 1250–1400 nm ranges. Of these three regimes, light sources centered around 800 nm generally offer the greatest bandwidth, and thus axial resolution. For example, a recent μOCT system utilizing extreme broadband light from a supercontinuum source [18] was capable of visualizing cilia *in vitro* with < 1 μm of axial resolution.

Another important design criteria for *in vitro* OCT systems is imaging depth, for which there are two considerations. First, the OCT imaging range is limited via the Nyquist sampling theorem by the spectral interference frequency resolution. As interferometric modulation frequency increases with distance from time-zero, higher interference frequency resolutions allow for greater ranging depths in samples. In SD-OCT systems the sampling frequency is simply the spectral bandwidth divided by the number of lateral elements in the detection camera. For swept source systems, the sampling frequency is the instantaneous linewidth of the wavelength-tunable laser. While the spectral sampling frequency sets the maximum theoretical imaging range of the system, the penetration depth itself is governed by light scattering in the turbid 3D cultures. Longer wavelengths encounter the least amount of scattering, with systems operating at 1300 nm offering more than 2 mm of potential imaging depth. Unfortunately, current long wavelength systems do not offer large bandwidths, with many 1300 nm-centered systems offering about 7 μm of axial resolution.

For most *in vitro* time-lapse or longitudinal imaging experiments, the most important metric is stability: system alignment as well as the light source power and spectra need to be stable over days and even weeks for quantitative imaging. In general, the most stable systems are those with as few moving parts as possible. For this reason, time-domain OCT systems may not be ideal, and swept-source OCT systems (OFDI or FDML) need to be rigorously vetted for long term stability. For our *in vitro* therapeutic screens, we chose an SD-OCT system based on a superluminescent diode light source and grating/camera pair, as similar devices have been found to offer excellent stability over periods spanning a week or more [19].

For the protocols described here, we used an overlay culture system [4,10,19,20] to model the disseminated micrometastatic lesions found in ovarian cancer. These model tumors can grow to sizes of 500–1000 μm in diameter, requiring optical ranging of about 1 mm. 3D tumor nodules are moderately light-scattering, which can occasionally lead to difficulties imaging with 800 nm-centered OCT systems at depths exceeding 900 μm. Typical growth and treatment response changes occur over the course of hours and days, requiring a highly stable system that can be left to run continuously if desired. Treatment response in model tumors encompasses large-scale changes, such as fragmentation and matrix invasion, as well as cellular-level alterations, such as apoptosis and senescence, requiring resolutions better than 3–4 μm. To achieve these goals, we chose to build an SD-OCT system (Fig. 1)