Journal of Visualized Experiments

Measuring Real-time Drug Response in Organotypic Tumor Tissue Slices

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE61036R1
Full Title:	Measuring Real-time Drug Response in Organotypic Tumor Tissue Slices
Section/Category:	JoVE Cancer Research
Keywords:	drug screening, Tissue slice culture, organotypic, patient-derived xenograft, real-time viability measurement, luminescence, alternatives to animal testing, high-throughput
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Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
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TITLE

Measuring Real-time Drug Responses in Organotypic Tumor Tissue Slices

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KEYWORDS

tissue slice culture, cancer, drug screening, organotypic, patient-derived xenograft (PDX), real-time viability measurement, luminescence, alternatives to animal testing, high-throughput

ABSTRACT:

We introduce a protocol for measuring real-time drug response in organotypic tumor tissue slices. The experimental strategy outlined here provides a platform to carry out medium-high throughput drug screens on tissue slices derived from clinical or mouse tumors in ex vivo conditions.

SUMMARY:

Tumor tissues are composed of cancerous cells, infiltrated immune cells, endothelial cells, fibroblasts, and extracellular matrix. This complex milieu constitutes the tumor microenvironment (TME) and can modulate response to therapy in vivo or drug response ex vivo. Conventional cancer drug discovery screens are carried out on cells cultured in a monolayer, a system critically lacking the influence of TME. Thus, experimental systems that integrate sensitive and high-throughput assays with physiological TME will strengthen the preclinical drug discovery process. Here, we introduce ex vivo tumor tissue slice culture as a platform for medium-high-throughput drug screening. Organotypic tissue slice culture constitutes precisely-cut, thin tumor sections that are maintained with the support of a porous membrane in a liquid-air interface. In this protocol, we describe the preparation and maintenance of tissue slices prepared from mouse tumors and tumors from patient-derived xenograft (PDX) models. To assess changes in tissue viability in response to drug treatment, we leveraged a biocompatible luminescence-based viability assay that enables real-time, rapid, and sensitive measurement of viable cells in the tissue. Using this platform, we evaluated dose-dependent responses of tissue slices to the multi-kinase inhibitor, staurosporine, and cytotoxic agent, doxorubicin. Further, we demonstrate the application of tissue slices for ex vivo pharmacology by screening 17 clinical and preclinical drugs

on tissue slices prepared from a single PDX tumor. Our physiologically-relevant, highly-sensitive, and robust ex vivo screening platform will greatly strengthen preclinical oncology drug discovery and treatment decision making.

INTRODUCTION:

Cancer cell interactions with the physical and biochemical properties of the surrounding stromal tissue forms the TME. TME can stimulate tumor growth, metastasis, and modulate tumor response to therapy[1]. In conventional preclinical drug development, drug candidates are typically first screened using cultured cancer cell lines, an assay platform that critically lacks the TME[2]. This lack of physiological TME in cell-based prescreening stages may limit the discovery of effective agents in tumor-bearing animal models and may contribute to the high attrition rate of many promising oncology drugs in later clinical stages of development[3].

Despite the importance of TME in modulating tumor drug responses, experimental constraints limit the application of more physiologically relevant systems during the early stages of drug discovery and development. It is impractical to screen hundreds of therapeutic agents on tumors from animal models or patient tumor specimens. Indeed, surgical specimens are scarce resources with varied genetic backgrounds and screening thousands of candidate molecules in animal models is not feasible due to experimental scale, cost, and animal welfare.

Tumor tissue slice culture, where precisely cut, thin tumor sections are cultured ex vivo, can address the limitation of physiological TME in drug screening assays. Historically, the field of neuroscience pioneered and made extensive optimizations of slice culture for brain tissue[4]. Recently, many studies have demonstrated preparation of slices from various types of tumor tissue including cell line-derived tumors, spontaneous tumor models, patient-derived xenografts (PDX), and primary patient tumors. Ex vivo tissue slice culture integrates benefits from both in vivo and in vitro culture[5]. Tumor tissue slices retain intact tissue architecture and variegated cell composition, enabling the study of cancer cells within the TME context.

This protocol introduces an organotypic tumor tissue slice culture system combined with a real-time, highly sensitive viability assay to evaluate drug responses. Drug efficacy tests on organotypic tumor tissue slices in previously introduced protocols rely on measuring changes in cell viability by fluorescent dye incorporation, immunohistochemistry (IHC), or MTT ((3- [4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide) assay[6-9]. However, all of these methods are end point assays and suffer from low sensitivity, long processing time, complex data analyses, narrow signal range, and high experimental error. Our luminescence-based live cell-compatible reagent improves these assays by providing a wide signal range and instantaneous (~5 min) measurement without prior processing and minimal post processing. This reagent is highly sensitive and can coexist in the cell culture media, allowing continuous and time-course measurement of cell viability. This assay system is applicable to high-throughput screening of drug candidates on tissue slices in preclinical drug development.

PROTOCOL:

All mouse experiments were performed according to the recommendations in the Guide for the Care and Use of Laboratory Animals of the Animal Welfare Act and the National Institutes of Health guidelines for the care and use of animals in biomedical research.

1. Preparation of tumor tissue slices

1.1. Prepare tissue slice culture (TSC) medium following the recipe in **Table 1**. Filter the medium with a 0.45 µm vacuum filter unit to sterilize.

1.2. Aliquot 250 µL of TSC medium per well for a 24 well plate, or 1 mL of medium per well for a 6 well plate. Keep the plate in a 37 °C, 5% CO_2 humidified incubator until use.

NOTE: The medium and supplements can be optimized depending on tissue types and experimental goals. The amount of medium should be just enough to soak the porous membrane of a culture insert. Do not exceed the level of the membrane. Adjust the medium volume if the medium level is too low or too high. If researchers are testing immunomodulatory agents, we recommend supplementation of the medium with IL-2[10].

1.3. If using tumor tissue derived from mice, euthanize mice with a recommended procedure, sanitize the exposed skin of mice by spraying 70% ethanol, and dissect tumors with aseptic techniques. Store the tumor tissue in a tube containing ice-cold Hank's Balanced Salt Solution (HBSS).

1.4. If using fresh patient tumor tissues, store in ice-cold HBSS for short term, or ice-cold Belzer University of Wisconsin (UW) solution for longer term storage (up to 24 h) to preserve tissue viability.

NOTE: Slices from a PDX tissue shipped overnight in ice-cold UW solution have been successfully prepared.

1.5. Transfer the tumor tissue into a 10 cm culture plate containing ice-cold HBSS. Cut tumor into halves with a scalpel while submerged in ice-cold HBSS. Shape tumor tissues into cylinders using a 6 mm diameter biopsy punch and trim one side to create a flat surface. Store the tissues in ice-cold HBSS until use.

NOTE**:** Avoid including the necrotic area of the tissue, which is generally at the center and has an opaque and fragile appearance.

1.6. Set up the vibratome. Disinfect all the equipment using 70% ethanol. Place the vibratome buffer tray covered with an acrylic glass lid at the center of the vibratome ice bath. Add ice to the ice bath and fill up the buffer tray with cooled HBSS to keep the tissue cool while slicing. Attach a razor blade to the blade holder.

NOTE: Although keeping vibratomes in a semisterile environment has not affected prior

experiments, it is recommended to store the vibratome under a biosafety cabinet if available. To prepare slices from pathogen-positive or unknown tissues, always work under a biosafety cabinet.

1.7. Carefully lift up a biopsy-punched tumor tissue from the HBSS using toothed forceps and remove excess buffer by dabbing the tissue with a lint-free paper towel.

1.8. Place a drop of medical grade cyanoacrylate glue on the specimen plate and place the tissue on this drop. Air-dry the glue for 2–3 min and place the plate into the buffer tray. Supplement with some HBSS until the tissue is fully immersed in the buffer.

NOTE: Mounting tissues in an upright, stable position is critical to generate uniformly cut slices. If mounted tissues are unstable, either add more glue to the surrounding tissue to retain an upright position or unload the tissue, flatten the bottom, and glue it again. Elastic tissues tend to get pushed and bent more easily during slicing, in which case shorter lengths (<5 mm) and multiple runs may be appropriate. For extremely fragile tissues, paper towels can destroy the tissue. In this case, the tissue can be placed on a plastic surface to wick away some excess HBSS.

1.9. Adjust the vibratome settings. The following settings are used: cutting thickness = 250 µm, amplitude = 3.00 mm, slicing speed = 0.01–0.25 mm/s, and blade angle = 15°–21°.

NOTE: Optimize the slicing settings depending on the stiffness of the tissue. Softer tissues are more easily cut with a deeper blade angle at lower speed. A blade angle of 18°, cutting speed between 0.10–0.18 mm/s, and an amplitude of 3.00 mm works well for mouse 4T1 and PDX HCI010 tumors. Stiffer tissue can be cut with a faster blade speed.

1.10. Set the start and end locations of the blade and adjust the height of the stage. Run the instrument with continuous mode to cut slices for several cycles until the tissues are cut uniformly.

1.11. Continue slicing the tissue. Transfer the slices along with a small amount of HBSS buffer onto the cell culture insert with medium with a wide-tip transfer pipette, using suction to lift the whole slice. Place one slice per insert on a 24 well plate. An insert for 6 well plates can accommodate up to four slices for a replicate assay.

NOTE: If the last edge of the tissue slice is still attached to the uncut tissue, stop the vibratome and separate the tissue slice with two pairs of fine tip forceps without touching or poking the tissue slices. A separate razor blade can be useful in removing hanging tissue.

1.12. Remove any excess buffer with a fine tip transfer pipette.

1.13. When the blade reaches close to the glued tissues, stop the instrument, lower the stage, remove the stiffened tissue with a separate blade, and mount a new tissue. Continue slicing until enough slices have been collected.

1.14. Culture the plates in a humidified incubator set at 37 °C, 5% CO_2. The tissue slices are cultured at the air-liquid interphase on the cell culture insert. The tissue slices are ready for experiments 24–48 h after the initial slice preparation. For long-term cultivation, exchange the medium every 2–3 days.

2. Viability measurement

2.1. For viability measurement of the tissue slice, exchange the medium with a luminescence-based viability measurement reagent containing both luciferase and prosubstrate at 1:1,000 dilution in TSC medium following the manufacturer's instructions. The reagent measures the reduction activity of live cells of metabolized prosubstrate to luciferin[11]. Transfer 50 µL of enzyme-substrate mixture on top of each tissue slice.

2.2. Incubate with gentle agitation on an orbital shaker located inside a humidified incubator set at 37 °C with 5% CO_2 overnight.

2.3. Luminescent signals can be measured using either a microplate reader or an in vivo imaging instrument for tissues cultured in a 24 well plate. To measure the individual viability of multiple tissues on a 6 well plate, an in vivo imaging system should be used.

2.3.1. When using a microplate reader, set the microplate without the lid. Any type of microplate reader capable of measuring luminescent intensity should be suitable for the experiment. We used the following settings: read time = 1 s, measurement from the top, gain = 200.

NOTE: For higher accuracy, use a white-wall microplate to set up the experiment. White-wall, clear-bottom 24 well plates have been tested, and in most cases, clear well plates do not compromise the results.

2.3.2. When using an in vivo imaging system to measure the viability, place the plate at the center of the stage and remove the lid. Acquire normal photographic images followed by luminescent images with a field of stage setting at C, auto exposure time, f-stop = 1, objective setting as microplate with subject height = 0.0 cm.

2.3.3. Quantify the luminescent intensity using the imaging software accompanied with the in vivo imaging system. Draw consistent regions of interest (ROI) around each tissue slice. This protocol uses a 1 cm diameter circle as an ROI (See **Figure 1**). Measure the total flux (p/s) of the area.

3. Evaluation of drug effect on the tissue slices

3.1. Measure baseline viability prior to treatment using the luminescence-based viability measurement reagent as explained in section 2.

NOTE: If several tissues have significantly lower viability compared to others, omit the tissues from the assay.

3.2. Dissolve drugs in dimethyl sulfoxide (DMSO) to make stock solutions. Prepare a 10x drug solution with TSC or other culture medium (25 µL/well for a 24 well plate containing 250 µL of medium) at the desired concentrations. For vehicle control, prepare medium containing an equivalent volume of DMSO.

3.3. Supplement the 10x drug solution to the culture medium at the bottom of the well. Mix by pipetting and transfer 50 µL of the medium on top of the tissues.

NOTE: If several tissue slices are available, test duplicates or triplicates for each condition.

3.4. Incubate overnight with gentle shaking on an orbital shaker in a 37 °C, 5% CO_2, humidified incubator.

3.5. Remove the plate from the shaker and incubate statically in the 37 °C, 5% CO_2, humidified incubator.

3.6. At desired timepoints, measure the luminescent intensity from the tissues using a microplate reader or an in vivo imaging system. Usually, drug effects become detectable after 1–6 days of treatment.

NOTE: The luminescence-based viability reagent is stable for at least 3 days under culture conditions. However, the substrate may be depleted during the assay depending on the metabolic activity of the tissue. If a significant signal drop is observed in tissues with previously high signals, supplement the substrate in all the wells.

3.7. Calculate the remaining viability of the treated tumor tissues using the following equation:

$$Change\ in\ viability(\%) = \frac{Treatment\left(\frac{Luminescence\ after\ incubation}{Luminescence\ before\ incubation}\right)}{Average\ of\ control\left(\frac{Luminescence\ after\ incubation}{Luminescence\ before\ incubation}\right)} * 100$$

The viability of the treated tissue is normalized by its baseline viability and viability shift of control tumor tissues.

REPRESENTATIVE RESULTS:

Here, we demonstrate a time-course, multiple drug efficacy evaluation protocol for tumor tissue slices prepared from mouse 4T1 breast tumor tissues and a breast cancer PDX model, HCI010[12]. We successfully prepared tissue slices from several mouse-derived tumors, PDX, and fresh patient tumors[10]. The overall workflow of the tumor tissue preparation and drug efficacy test is described in **Figure 1**. In general, we could prepare 20–40 tissue slices from a single bulk tumor

of 1,000–1,500 mm^3 in volume.

For viability measurements, we exploited a luminescent-based, live cell-compatible viability reagent. Treatment of a multi-kinase inhibitor, staurosporine, at 1 µM concentration for 4 days reduced the signal intensity by 100x, compared with tumor tissues treated with DMSO control (**Figure 2A**). The tissue slices prepared from the 4T1 tumor were maintained for at least 21 days with occasional medium exchange (**Figure 2B**). Most of the drug response measurements were performed within 7 days from tissue slice preparation. Because the live cell-compatible reagent does not require processing or fixation before measurement, it allowed us to carry out time-course measurements. Time-dependent changes in the viability of tumor tissue slices from the identical 4T1 tumor tissue are summarized in **Figure 2C**. Staurosporine at 500 nM decreased luminescence from day 1 and reached the lowest level at day 4, remaining low for each subsequent timepoint. In contrast, the luminescent intensity from the control group of tissues supplemented with DMSO remained stable until day 6. Additionally, tissue slices prepared from identical 4T1 tumors were treated with serial doses of staurosporine (0–1 µM) for 4 days, and showed dose-dependent changes in viability and EC_{50} (**Figure 2D**).

We tested chemotherapeutic drugs on tissue slices prepared from an orthotopic PDX model of breast cancer, HCI010. PDX tissue slices were treated with doxorubicin, a standard chemotherapeutic drug, at doses of 0–5 µM for 6 days, providing dose-dependent changes in viability (**Figure 3A**). Further, the efficacy of 17 drugs, including preclinical and clinically approved drugs, was tested in triplicate on tissue slices prepared from a single bulk HCI010 tumor. The drugs were applied at 0.5 µM for 4 days in triplicate (**Figure 3B**). These results provide proof-of-concept that medium-throughput drug screening can be performed on tumor slices with native TME.

FIGURE AND TABLE LEGENDS:

Figure 1: Schematic showing tissue slice preparation followed by drug efficacy evaluation. Tumor tissues were processed into slices 6 mm in diameter and 250 µm thick and cultured in the air-liquid interphase with support from cell culture inserts. Tissue viability was measured using a bioluminescent reagent both before and after drug treatment.

Figure 2: Tissue slice viability measurement and response to the drugs. (**A**) Representative images of luminescence-based viability measurements in breast tumor tissue slices. Tissue slices from 4T1 tumor were incubated with 1 µM staurosporine or DMSO control together with luminescent viability assay reagent for 4 days. Images were obtained using an in vivo imaging system and analyzed by accompanying image analysis software. Red circles indicate the ROI measured for bioluminescence. Bottom = A plot showing luminescent signal from tumor tissue slices treated with staurosporine or control as measured by an in vivo imaging system. The bar indicates the mean of the four tissue slices shown as circles (control) or square (staurosporine treated). (**B**) A plot showing viability of 4T1 tumor tissue slices cultured for 21 days from preparation. Viability at day 21 measured by an in vivo imaging system was normalized to that of day 3. Each dot indicates measurement from a single slice; the bar indicates the mean; the box shows quartiles, and whiskers show the minimum to maximum value of the measurement. (**C**)

Time-course measurements of tissue viability after the staurosporine treatment. The viability of tissue slices from the 4T1 tumor treated with staurosporine (500 nM) or equivalent volume of DMSO as a control were measured over time. Luminescence intensities were measured by a microplate reader over the indicated timepoints. Each dot illustrates a datapoint from an individual tissue slice, and the bar graph indicates mean ± SEM. (**D**) Dose-dependent treatment of staurosporine on tissue slices. Tissue slices prepared from 4T1 tumor were supplemented with serial doses of staurosporine or DMSO as a vehicle control. The remaining viability based on luminescence was measured by an in vivo imaging system at 4 days after treatment initiation. Relative viability was calculated using the equation shown in the protocol.

Figure 3: Drug screen in tissue slices prepared from a breast cancer PDX model. (**A**) A plot showing changes in viability in response to various doses of doxorubicin. The tissue slices were prepared from an orthotopic breast cancer PDX tumor, HCI010. Viability was measured in slices treated with titrated doses of doxorubicin. After 6 days, luminescence was measured using an in vivo imaging system. Bar = mean ± SEM. (**B**) A small-scale chemotherapeutic drug screen on tumor tissue slices from an orthotopic breast cancer PDX tumor. The bar graph = mean ± SEM of triplicate experiment. These data have been published previously[10].

Table 1: TSC medium recipe.

DISCUSSION:

In this protocol, we demonstrate a platform for quantitative, and real-time drug efficacy studies on organotypic tumor tissue slices. The tissue slice culture system provides distinct advantages over traditional cell-based in vitro methods by capturing cell heterogeneity and physiological characteristics of the native tumor microenvironment. This platform also enables higher throughput for drug efficacy testing, helping to bridge the gap between cell culture studies and in vivo experiments.

To obtain accurate and consistent results, it is imperative to minimize tissue damage during preparation. Tissue slices should be handled with wide tip plastic pipettes to avoid damage caused by physical contact. The culture medium volume must be maintained to allow the tissue slice to contact both the air and medium for the duration of the experiment.

The culture medium should be optimized depending on the tissue type and experimental purpose. The TSC medium used in this protocol is a serum-free medium that was originally developed for primary hepatocyte culture[13]. This medium has also been used to maintain several types of tumors, including breast, liver, colon, and pancreas[10]. Further, we demonstrated enhanced immune cell survival using an optimized DMEM-based medium[10]. Vibratome settings should be optimized based on tissue texture to obtain intact and uniform tumor tissue slices. Slicing softer and/or loosely consolidated tissues is typically made easier by using deeper blade angles, slower cutting speed, and thicker slices. Occasionally, tissues are too soft to cut, in which case researchers should consider embedding the tissue within low melting point agarose, a technique common in brain tissue slicing[14,15].

Previously, several studies introduced therapeutic drug testing approaches on cultured tissue slices. These studies relied on fluorescent dye incorporation, immunohistochemistry, or MTT assays to evaluate tissue viability[6-9]. In this protocol, we used the luminescence-based, live cell-compatible reagent RealTime-Glo[11] for viability measurement. Luminescence-based assays have several benefits over previously used methods, including increased sensitivity, wider signal range, and the ability to rapidly make real-time viability measurements. Measuring time is short for both microplate readers (~1 s/well) and an in vivo imaging system (~1 min/plate) and can provide signal intensity readings directly with minimal processing. Faster acquisition of the signal and fewer postprocessing steps are necessary features in high-throughput drug screening, allowing luciferin-based viability measurements to enable a much greater sample throughput in tissue slice culture. While a luciferase-based assay provides an accurate, quantitative measurement of the whole slice viability, it is unable to detect drug-induced cell type-specific changes in the tissue. However, coupling luminescence-based viability measurements with end point IHC on the same tissue slice will enable cell type-specific measurements.

While the tissue slice culture system is a powerful tool in bringing tumor tissue complexity to a high-throughput screening platform, it has several drawbacks. Inherent heterogeneity within a tumor tissue may cause differential drug responses among tissue slices even from the same bulk tumor. On occasion, we observed relatively large variations in drug response among tissue slices in our experiments. We can partially overcome this variation by averaging multiple tissue slices prepared from different sites within the same tumor. Although tissue slice culture can be maintained at baseline level of viability for at least 21 days (**Figure 2B**), cell populations change over time. We have described shifts in immune cell population during the course of tissue slice culture experiments[10]. Therefore, we recommend tissue slices be examined over shorter culturing time intervals to recapitulate native tissue characteristics.

Tissue slice culture is poised to fill a critical gap in drug discovery and development between high throughput cell culture screening and animal experiments. Hundreds of drugs can be evaluated on slices produced from a single tumor biopsy, adding increased physiological relevance prior to animal studies. This system could help to reduce the number of animal experiments required to bring drugs to market. Furthermore, tissue slices prepared from patient tumors have informative potential in guiding treatment plans in the clinic. Over a hundred therapeutic drugs are available today, yet there are few biomarkers to guide their selection. Moreover, heterogeneity in patients also results in disparities in response. Tissue slices from a patient biopsy can be used to test multiple drugs before treating systemically, providing valuable information on drug efficacy within a week's time. Overall, real-time and rapid testing of drugs using tissue slice culture systems has great potential in cancer drug development and therapeutic decisions.

ACKNOWLEDGMENTS

This work was supported by the NIH/NCI [K22CA201229, P30CA015704], and Sidney Kimmel Foundation [Kimmel Scholar Award], Lung Cancer Discovery Award (LCD-505536). We would like to thank Dr. Alana Welm (University of Utah) for providing the breast cancer PDX tumor. We would also like to thank the staff at Comparative Medicine, Fred Hutchinson Cancer Research Center (FHCRC) for maintenance of the PDX model and members of the Gujral lab for helpful

discussions. N.N.A is supported by the JSPS Overseas Research Fellowship and Interdisciplinary Training Grant from FHCRC. A.J.B. is supported by the Chromosome Metabolism and Cancer Training Grant from FHCRC.

DISCLOSURES:

The authors have nothing to disclose.

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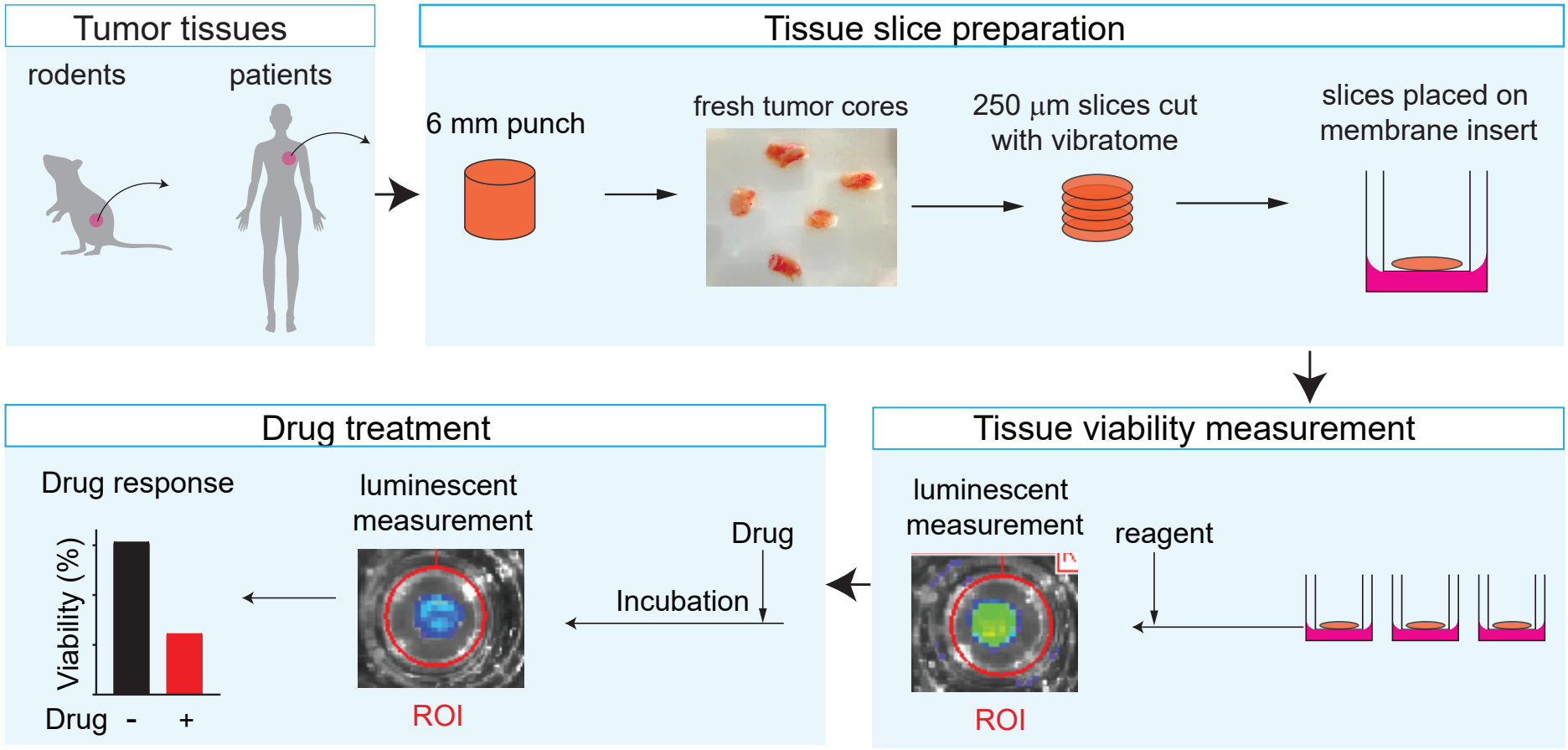

Figure 2

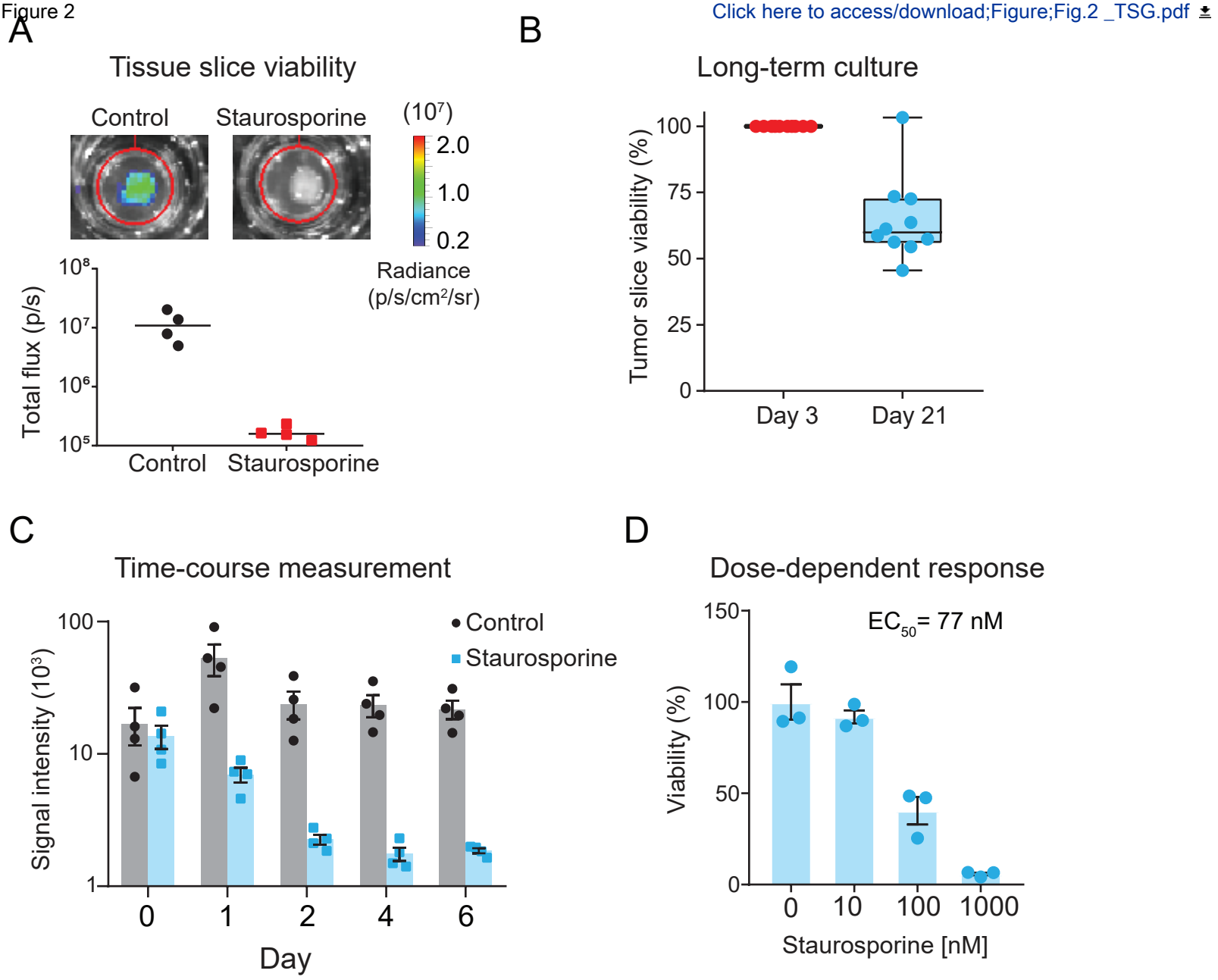

Figure 3

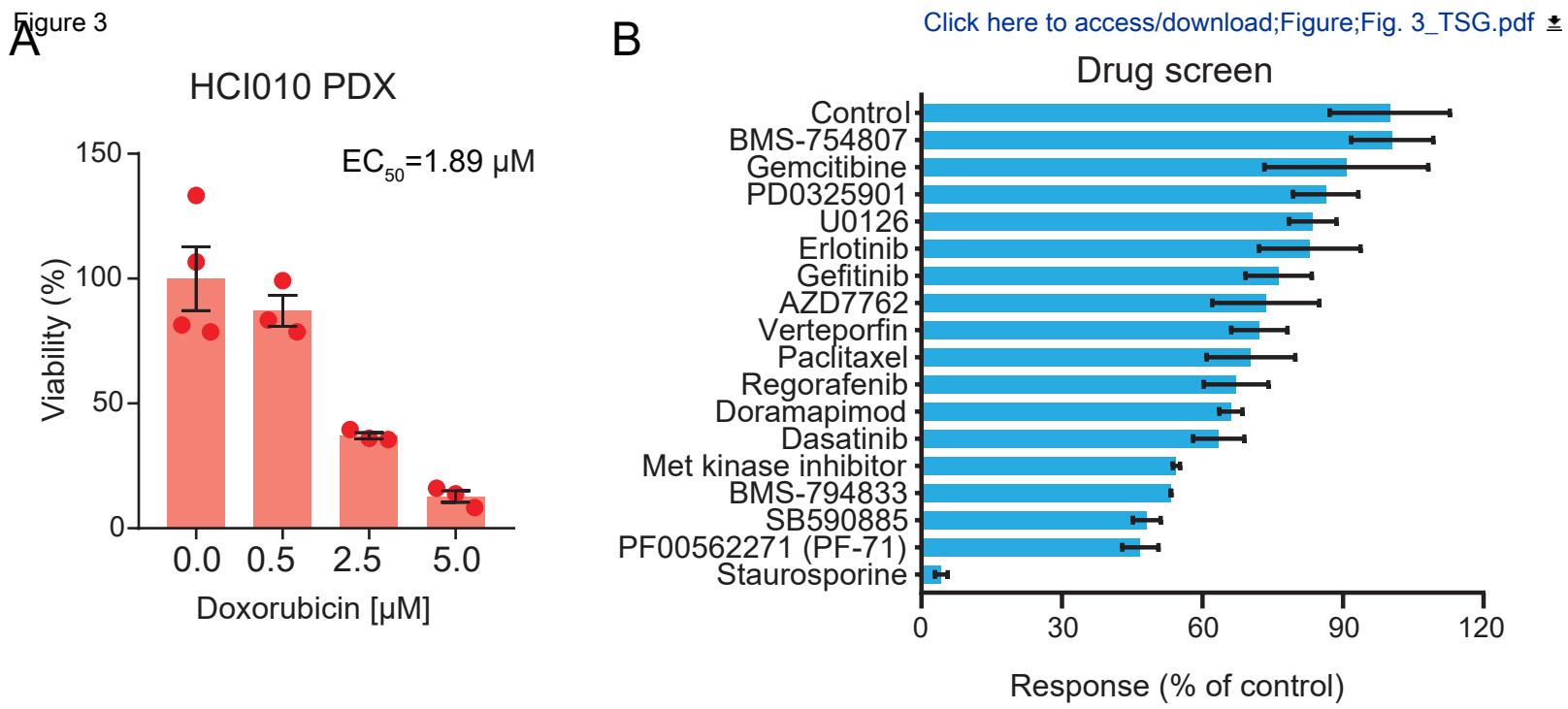

Table 1

Material	mL	Final Concentration
William's Medium E	500	
Nicotinamide	6	12 mM
Ascorbic Acid	6	10 mM
Sodium Bicarbonate	15	2.25 mg/mL
HEPES Buffer	10	20 mM
Glucose	10	50 mg/mL
Sodium Pyruvate	5	1 mM
L-Glutamine	5	2 mM
ITS + Premix	5	
Penicillin-Streptomycin	2	40 IU/mL Pen, 40 ug/mL Strep
Recombinant EGF	0.5	20 ng/mL

Stock

1 M stock of Nicotinamide in Williams' Medium E, sterilized
0.21 g/50 mL (>10 mM stock) of Ascorbic Acid in William's Medium E, sterilized
7.5% (w/v) solution
1 M stock solution
250 mg/ml stock, steralized
100 mM stock
200 mM stock
Contains human recombinant insulin, human transferrin (12.5 mg each), selenous acid (12.5 µg), BSA (2.5 g), and l
10,000 IU/mL Pen, 10,000 ug/mL Strep
20 µg/mL stock

inoleic acid (10.7 mg)

Name of Material/Equipment	Company
10 cm dish	Corning
24-well dish	CytoOne
4T1	ATCC
6 mm diameter Biopsy punch	Integra Miltex
6-well plate	CytoOne
Ascorbic Acid	Sigma-Aldrich
Belzer UW cold storage solcution (UW solution)	Bridge to Life
Corning Matrigel Membrane Mix	Fisher scientific
DMSO	Corning
Double Edge Stainless Steel Cutting Blades	TED PELLA
Doxorubicin (hydrochloride)	Cayman Chemical
FBS	Gibco
Fine tip forceps	ROBOZ
Fine tip forceps	ROBOZ
Glucose	Sigma-Aldrich
HBSS without Calcium, Magnesium or Phenol Red	Gibco
HCI010	Dr. Alana Welm, University of Utah
HEPES Buffer Solution	Gibco
ITS + Premix	Fisher Scientific
IVIS Spectrum	PerkinElmer
Leica VT1200S Vibratome	Leica
L-Glutamine	Gibco
Millicell Cell Culture Insert, 12 mm, hydrophilic PTFE, 0.4 µm	Millipore
Millicell Cell Culture Insert, 30 mm, hydrophilic PTFE, 0.4 µm	Millipore
Nicotinamide	Sigma-Aldrich
PELCO Pro CA44 Tissue Adhesive	TED PELLA
Pen Strep	Gibco
Penicillin-Streptomycin	Fisher Scientific
RealTime-Glo MT Cell Viability Assay	Promega
Recombinant Mouse EGF	BioLegend
RPMI1640	Gibco
Single Edge Industrial Razor Blades	VWR
Sodium Bicarbonate	Corning
Sodium Pyruvate	Fisher Scientific
Staurosporine	Santa Cruz Biotechnology
Synergy H4	BioTek
Tooothed forceps	ROBOZ
Transfer pipettes	Fisher scientific
Transfer pipettes	Samco Scientific
William's medium E, no glutamine	ThermoFisher Scientific

Catalog Number	Comments/Description
430293	
CC7682-7524	
CRL-2539	
33-36	
CC7682-7506	
A8960-5g	For TSC medium
500 mL	
356234	
MT-25950CQC	
121-6	For slicing
15007	
26140-079	
RS-4974	
RS-4976	
G5767-500g	For TSC medium
14-175-103	
	Breast cancer PDX
15630080	For TSC medium
354352	For TSC medium
124262	
VT1200S	
25030164	For TSC medium
PICM01250	
PICM03050	
N0636-500g	For TSC medium
10033	Glue
15140-122	
15140163	For TSC medium
G9711	
585608	For TSC medium
11875135	
55411-050	For removing glued tissues
25-035-CI	For TSC medium
BW13115E	For TSC medium
sc-3510A	
RS-5155	
13-711-7M	Wide tip
235	Fine tip
12551032	For TSC medium

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Dec 16, 2019

Dr. Phillip Steindel,
Review Editor
JoVE

Dear Phillip,

Thank you for your positive response to our manuscript entitled "*Measuring Real-time Drug Responses in Organotypic Tumor Tissue Slices*". We would especially like to thank you and the editorial board members for the comments, and all the reviewers for their thoughtful consideration and insightful recommendations.

We are now submitting a revised version of the manuscript that addresses the points raised by the reviewers. I hope that we have responded in a satisfactory manner and that the revised manuscript is now suitable for publication in JoVE.

Thank you for your consideration. I look forward to your response

Sincerely,

Taran Gujral, PhD

Editorial comments:

General:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

We have carefully proofread the manuscript to correct for any grammatical errors.

2. Please ensure that the manuscript is formatted according to JoVE guidelines–letter (8.5" x 11") page size, 1-inch margins, 12 pt Calibri font throughout, all text aligned to the left margin, single spacing within paragraphs, and spaces between all paragraphs and protocol steps/substeps.

We have changed the formatting of our manuscript to follow the description above.

3. Please provide at least 6 key words or phrases.

We have included more key words in the Keywords section.

4. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please limit the use of commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.
For example: RealTime-Glo, IVIS

We have rephrased KimWipes, RealTIme-Glo and IVIS to appropriate words.

Protocol:

1. If necessary, please include an ethics statement before the numbered protocol steps, indicating that the protocol follows the guidelines of your institution's human research ethics committee.

An ethics statement has been provided before the numbered protocol.

2. There is a 10 page limit for the Protocol, but there is a 2.75 page limit for filmable content. If revisions protocol to be more than 2.75 pages, please highlight 2.75 pages or less of the Protocol (including headers and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

Our protocol section is approximately 3 pages long. We have highlighted filmable content of our procedure in yellow.

3. For each protocol step/substep, please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. If revisions cause a step to have more than 2-3 actions and 4 sentences per step, please split into separate steps or substeps.

We have carefully proofread our manuscript to follow the instructions and made appropriate changes highlighted with 'track changes'.

Figures:

1. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit

permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."

We have included the phrase "These data have been published previously[10]." in the legend of Fig. 3 (B).

2. Please provide 1 file per figure (3 in total). Please remove 'Fig. 1' etc. from the Figures themselves.

Updated figure files are attached.

Acknowledgment and Disclosures:
1. Please include a Disclosures section, providing information regarding the authors' competing financial interests or other conflicts of interest. If authors have no competing financial interests, then a statement indicating no competing financial interests must be included.

Disclosure section was included before the Reference section.

References:
1. Please do not abbreviate journal titles.

We have revised the name of the journal titles to full terms.

Table of Materials:
1. Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.
2. Please remove trademark (™) and registered (®) symbols from the Table of Materials.

We have removed ™ and ® from the revised Table of Materials.

Reviewers' comments:
Reviewer #1:
Manuscript Summary:
This contribution from Gujral et al provides an important new method and another highly needed comparative data set for the discovery of small molecule potential as cytotoxic agents versus cancer models. The screening of cancer cell lines versus approved and investigational drugs is a key first step in the development of a new drug or the repurposing of existing therapies. However, for solid tumors the use of cultured cells may not alway recreate the key disease features that predict in vivo outcomes. The tumor slice method, while sacrificing throughput, is likely going to allow for a greater predictive outcome.
Major Concerns:
None
Minor Concerns:
It would be good to see additional models/outcomes. But others will surely follow.

We appreciate your positive comments on our protocol. We hope our protocol will be utilized by many researchers studying other types of cancers and models.

Reviewer #2:

Manuscript Summary:

The authors state that the assay is applicable for medium to high throughput assays. However, I don't see high throughput as a possibility (thousands to millions of compounds). Therefore, I would say low to medium throughput.

We appreciate the reviewers careful reading and thoughtful comments to improve our manuscript. We agree that the tissue slice culture system itself is incapable of screening on the order of millions of compounds. However, we expect screening of hundreds of inhibitors is possible if the system becomes automated, or by utilizing our computational modeling-based inhibitor screening (PNAS, 2014, Nat Comm, 2017 and our manuscript in preparation). Therefore, we hope to keep this phrase as is.

Major Concerns:

It would be much more compelling if a more extensive characterization of the culture conditions would be added. For example, do slices maintain proliferation (e.g. by doing EdU staining), how much apoptosis develops (e.g. TUNEL staining), is tissue morphology still intact (HE staining), does immune cell content change over time (specific immunostainings), etc. I understand that this manuscript mainly describes the procedures, but without these baseline parameters it is very difficult for readers to decide whether it would be a good assay to be used.

We and our collaborators have extensively characterized organotypic tissue slices using histological characterizing from pancreatic ductal carcinoma (Jiang et al., 2017) and liver tissue (Wu at al., 2018), proliferation by staining Ki67+ (Jiang et al., 2017), as well as apoptosis by caspase-3 (Jiang et al., 2017), and immune cell population (Jiang et al., 2017, Wu at al., 2018, Sivakumar et al., 2019). Proliferation of cells in tissue slices by EdU or equivalent proliferation markers was also evaluated by several other publications during optimization of tissue slice culture conditions (Naipal et al., 2016, Nagaraj et al., 2018, Vesci et al, 2015). We have cited most of these references in our manuscript. Therefore, we consider tumor tissue slice culture to have been well-characterized, and it is not necessary to repeat in this protocol. In the context of this manuscript, we would like to introduce a new method for real-time evaluation of drug efficacy of tissue slices.

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