**Line-by-Line Response to Editor’s Comments**

We thank the editor’s careful review of our manuscript. We have revised the manuscript to include further details requested by the editor. Here, we provide a line-by-line response to the editor’s comments and describe changes we made in the revised manuscript.

**Editorial Comments:**

***Comment #1:*** (Line 149) For how long? And at what temperature?

**Response to Comment #1:** The cell suspension is centrifuged for 3 minutes at room temperature.

**Text Changes:** (Line 149-150) Transfer the cell suspension into a 15 mL centrifuge tube and centrifuge for 5 minutes at room temperature, with the speed of 500 x g.

***Comment #2:*** (Line 152) Supernatant?

**Response to Comment #2:** Thank you for the comment. We have changed it in the text.

**Text Changes:** (Line 152) Remove the supernatant and resuspend cells with 4 mL, pre-warmed, culture medium.

***Comment #3:*** (Line 151) How much culture medium (an estimate)

**Response to Comment #3:** About 4 mL.

**Text Changes:** (Line 152) Remove the supernatant and resuspend cells with 4 mL, pre-warmed, culture medium.

***Comment #4:*** (Line 153) Mention cell counting technique

**Response to Comment #4:** We use hemocytometer for cell counting.

**Text Changes:** (Line 153) Pipette one drop of sample on the hemocytometer for cell counting to determine cell concentration.

***Comment #5:*** (Line 154) What is the appropriate concentration?

**Response to Comment #5:** The appropriate concentration for seeding depends on cell lines and the culture plate.

**Text Changes:** (Line 154) Dilute the cells to appropriate concentration for seeding (3000 cells/mL).

***Comment #6:*** (Line 159) How many cells per well? What is the plate size, i.e. how many wells? In how much total volume (of media) should you seed per well?

**Response to Comment #6:** We add 200 µL of cells suspension in each well with the concentration of 3000 cells/ mL. Therefore, it is 600 cells / well.

**Text Changes:** (Line 159) Seed cells into an ultra-low attachment (ULA) round-bottomed multi-well plate. Add 200 µL of cells suspension in each well with the concentration of 3000 cells/mL. Each well has about 600 cells.

***Comment #7:*** (Line 162) Using a plate centrifuge?

**Response to Comment #7:** We use a centrifuge with a plate adapter.

**Text Changes:** (Line 162) Centrifuge the whole plate right after seeding, using a plate adapter, at a speed of 350 x g or the lowest speed available.

***Comment #8:*** (Line 162) For what duration and at what temperature?

**Response to Comment #8:** Centrifuge for 7 minutes and at room temperature.

**Text Changes:** (Line 162) At room temperature, centrifuge the whole plate for 7 minutes right after seeding, using a plate adapter, at a speed of 350 x g or the lowest speed available.

***Comment #9:*** (Line 261) Where is this set? Mention any button clicks if you set this up in software

**Response to Comment #9:** Set the scanning range in the custom software.

**Text Changes:** (Line 261) Set a proper OCT scanning range (e.g., 1 mm x 1 mm) in the custom software to cover the whole tumor spheroid according to its development stages. Click “Save parameters” to save the setting.

***Comment #10:*** (Line 265) Please mention explicit button clicks for software actions.

**Response to Comment #10:** Click “Preview” button to view the preview image and click “Acquire” button to acquire OCT image.

**Text Changes:** (Line 266) Click “Preview” button to view the preview image and click “Acquire” button to acquire OCT image.

***Comment #11:*** (Line 274) Can you show a screenshot of the output? This can be added as a supplementary file for JoVE’s internal use. Without this there is nothing to film in this step.

**Response to Comment #11:** We have presented the output of this step in Figure 4A.

**Text Changes:** (Line 275) See Figure 4A for the output of the generated OCT structural images.

***Comment #12:*** (Line 287) Can you show a screenshot of the output? This can be added as a supplementary file for JoVE’s internal use. Without this there is nothing to film in this step. Unclear how the collage is generated, do you run a script? Can a reference be cited?

**Response to Comment #12:** We have presented the output of this step in the Figure 3 C-E. The collage is realized by arranging each subfigure of 2D OCT images in a predefined location by running a script. No reference is related.

**Text Changes:** (Line 289) See Figure 3C– E for the representative output of collages of spheroid images.

***Comment #13:*** (Line 292) Can you show a screenshot of the output? This can be added as a supplementary file for JoVE’s internal use. Without this there is nothing to film in this step. Unclear how this is done, do you run a script? Can a reference be cited?

**Response to Comment #13:** We included a step-by-step button-click screenshot for the 3D rendering for JoVE’s internal use. Protocol 3.7.1) – 3.7.3) have illustrated the steps to show the 3D rendered image of tumor spheroid. No script is required.

***Comment #14:*** (Line 292) Please remove the product name from the text and add it to the table of materials.

**Response to Comment #14:** Thank you for the comment. We have removed this product name from the text.

**Text Changes:** (Line 294) The following steps show how to obtain the 3D rendering of tumor spheroids using a commercial software.

***Comment #15:*** (Line 295) Unclear which mode is chosen

**Response to Comment #15:** We used the “Blend” Mode to visualize the 3D rendered images.

**Text Changes:** (Line 301) Choose the **Blend** mode to use for 3D rendering.

***Comment #16:*** (Line 302) By using the mouse pointer?

**Response to Comment #10:** Yes, we adjust the viewing angle using mouse pointer.

**Text Changes:** (Line 304) Adjust the viewing angle by dragging the image using the mouse pointer.

***Comment #17:*** (Line 401) Voxels?

**Response to Comment #10:** Thank you for the comment. We have revised in the text.

**Text Changes:** (Line 403) Each OCT data consisted of 400×400×1024 voxels.

***Comment #18:*** (Line 438) From 2d slices?

**Response to Comment #10:** From cross-sectional 2D images.

**Text Changes:** (Line 439) we can also obtain the physiological information of the distribution of dead-cells within the tumor spheroids by analyzing the voxel-by-voxel optical attenuation from 2D cross-sectional images.

***Comment #19:*** (Line 468) Please increase the font size.

**Response to Comment #19:** See the updated Figure 2 for the adjustment of the font size.

***Comment #20*** (Line 490) Do you wish to include the video as a supplementary file? If so please add a legend and cite it in the text.

**Response to Comment #20:** Yes, we would like to include the video as a supplementary file. We have added a legend in the text.

**Text Changes:** (Line 498) Video1: High-throughput OCT imaging of tumor spheroids. A workflow of 3D OCT imaging, basic OCT processing and stage movement was presented in the video with a 5× speed. Previews of processed OCT structural images of spheroids were also presented.