**Responses to the Editorial Comments**

|  |  |
| --- | --- |
| Article ID: | JoVE59226 |
| Title: | An easy-to-implement method for 3D analysis of multi-cellular response to chemoattractant gradients |
| Author: | Tae-Yun Kang, David Ellison, Sung Hoon Lee, Andrew J. Ewald, Andre Levchenko |

We appreciate the constructive comments of the reviewer. The manuscript has been revised based on these comments. Please find our specific responses to the referee’s comments below.

**Summary of response:**

|  |
| --- |
| We have responded to the comments and revised the manuscript accordingly. |

**Editorial Comment 1:**

|  |
| --- |
| **1. Note that all text written in Equation Editor will be formatted differently from the rest of the text; it is therefore advised that all inline text is formatted as 12 pt Calibri.** |
| **Answer**: All inline text has been formatted as 12 pt Calibri. |

**Editorial Comment 2:**

|  |
| --- |
| **2.1: How is the mammary gland tissue obtained, the same way as in reference 11?** |
| **Answer**: The same method was used in the reference 11. We have added the note mentioning that the details can be found in the reference.  [Section related to the response]: Protocol  **2. Cell preparation-Primary mammary organoid Isolation11** (**Note 1**: The details of mammary organoid isolation can be found in the reference11, **Note 2:** Any kind of single cells or organoids can be prepared according to their isolation/detachment protocols) |

**Editorial Comment 3:**

|  |
| --- |
| **4.4: Which software are you using here? It’s not apparent if it’s in the Table of Materials. Also, how are lengths and angles measured?** |
| **Answer**: Fiji-ImageJ was used for the quantification. We have added the explanation in the protocol and specified the software name in the table of materials.  [Sentence related to the response]: Protocol  4.4. Reconstruct a 3D image from 2D image stacks using either commercial software or custom made program. Measure the length and angle of branches extending from the organoids or migration of individual cells. Here, the quantification was performed by drawing a freehand outline around the organoid body using an open source image processing program.. |

**Editorial Comment 4:**

|  |
| --- |
| **Results/Figure 2A-C: How was the simulation here done? This portion of the Figure doesn’t appear to be from reference 10. Please include at least a brief explanation.** |
| **Answer**: We have added a brief explanation of the simulation in the Result section and specified the software name in the Table of Materials.  [Section related to the response]: Representative results  Both in silico (Fig. 2A-C) and in vitro (Fig. 2D-F) tests demonstrated the formation of a stable linear EGF gradient across the cell culture area which lasted for approximately two days without replenishing the ‘source’ and ‘sink’ reservoirs. The time-dependent diffusion of the proteins was simulated using a commercialized multiphysics software. The 3D geometry of the chamber configuration was recreated on the software and the average concentration within the chamber volume was determined and plotted. |