Day 4: Perfusion Culture Operation

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Innovation in Organoid Culture and Disease Modeling

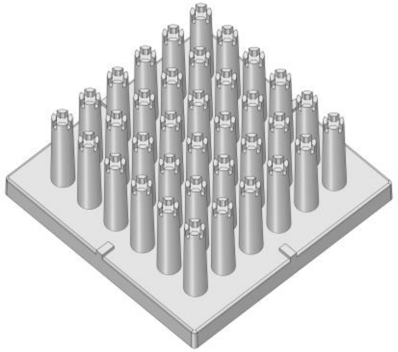
Goal: Learn how to run dynamic 3D cell culture, assemble the full perfusion plate stack, and understand bidirectional perfusion flow principles.

Perfusion mechanism explanation:

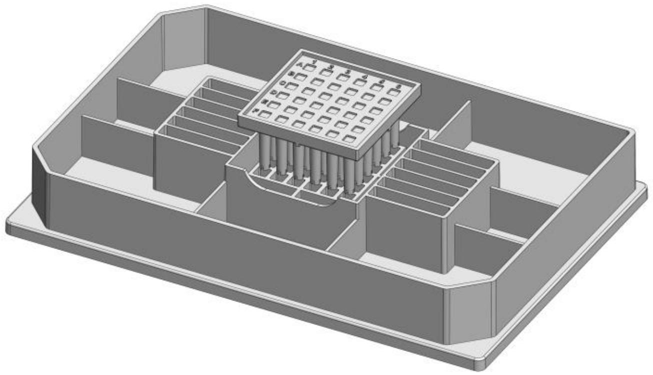
- **Bidirectional perfusion flow by tilting**
- **Flow rate vs. tilt angle**
- **Methods of cell culture media change**

Introduction to the Perfusion Plate setup on a digital rocker

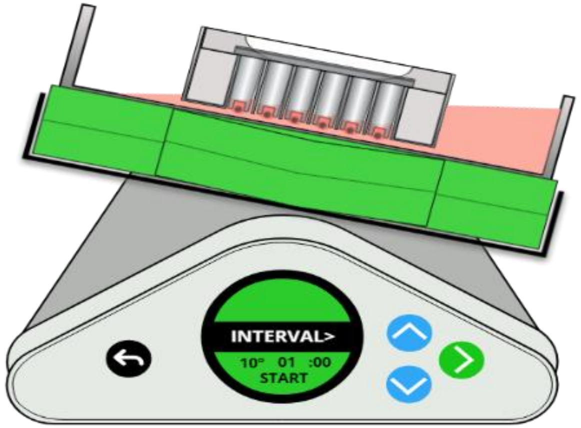
User-Friendly Dynamic Culture



36PillarPlate



36PerfusionPlate



Dynamic culture
in perfusion plate

Following cell encapsulation in hydrogels, the pillar plate can be seamlessly integrated with a perfusion plate, enabling dynamic cultures of 3D cells and organoids.

https://youtu.be/buXE65N9kAs

Setup of Pillar/Perfusion Plate for Dynamic Culture

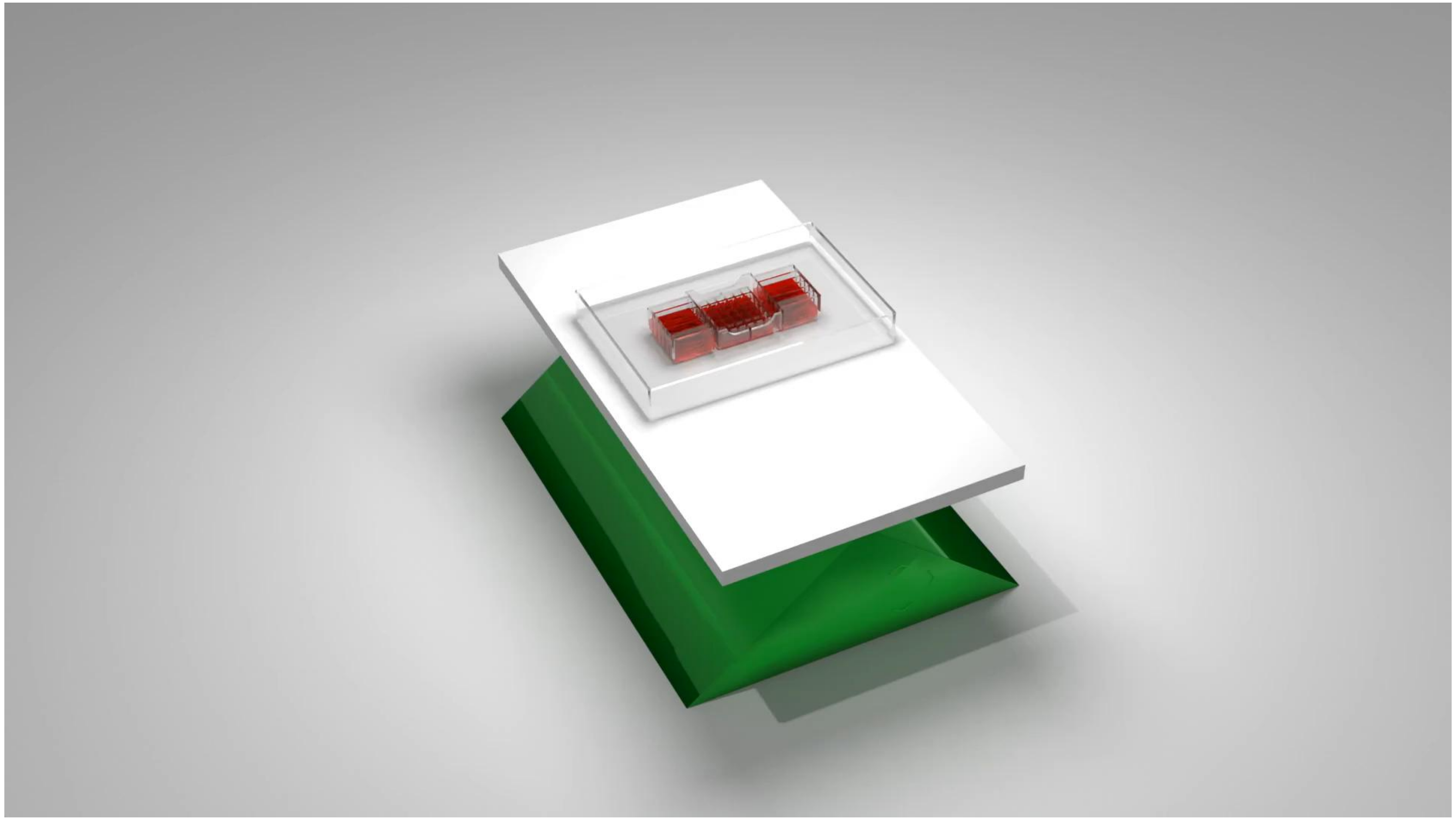
36PillarPlate sandwiched with 36PerfusionPlate

OrganoFlow L digital rocker for loading perfusion plates

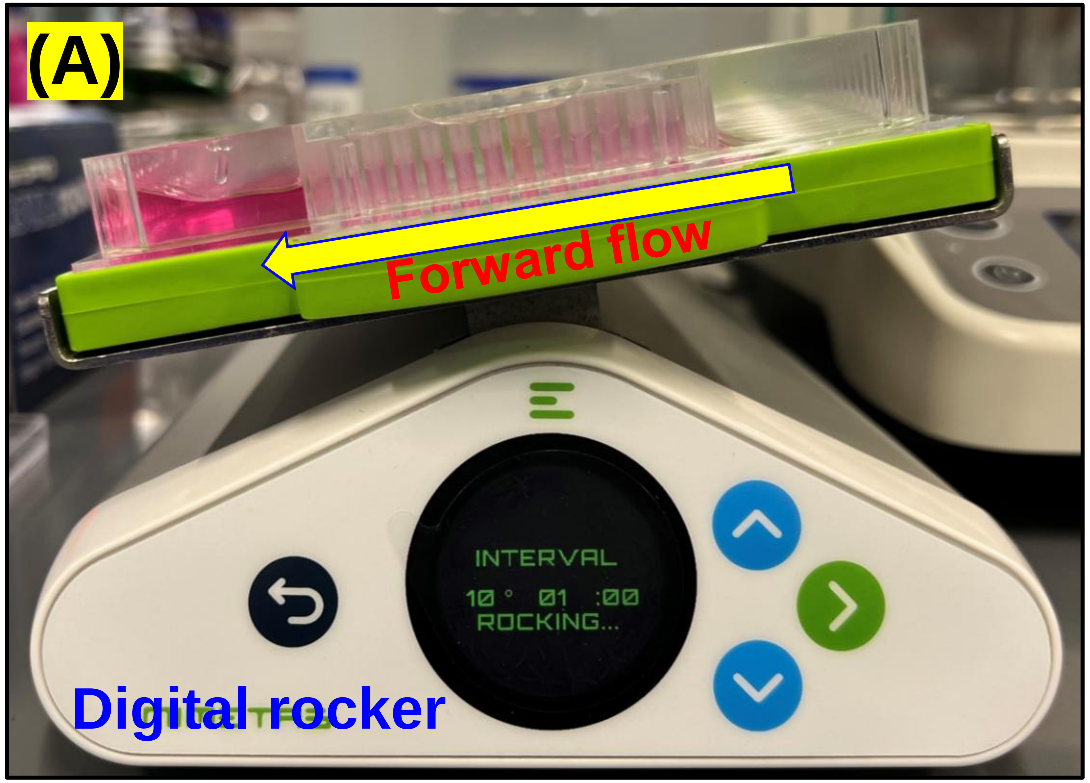
Sandwiched plates loaded on the rocker for dynamic 3D cell culture

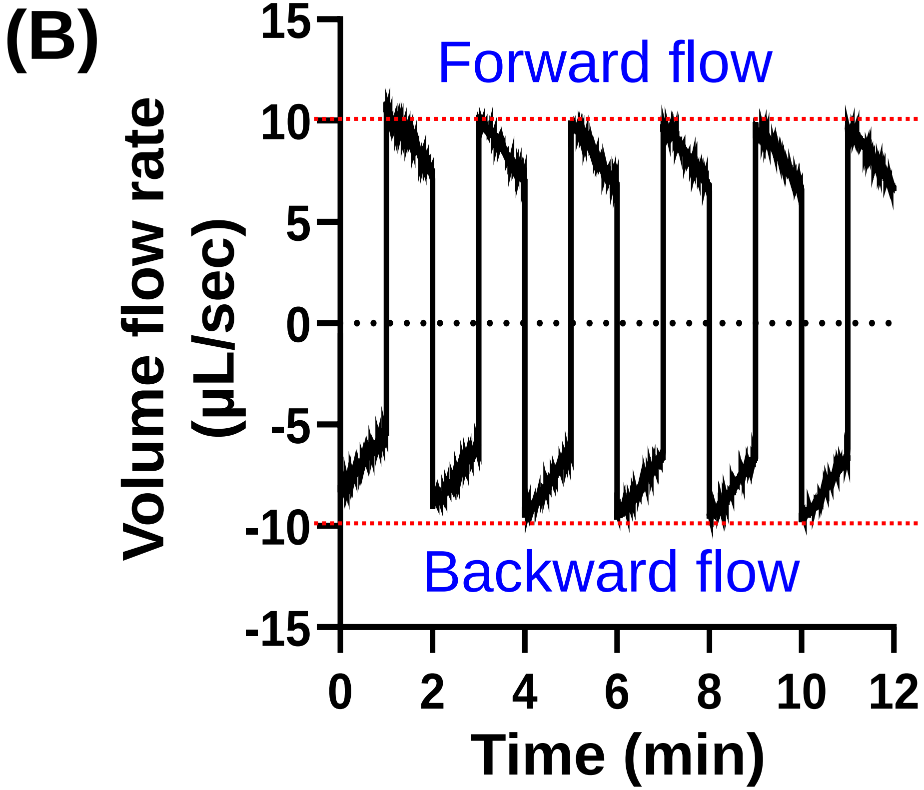
Cell culture media change by tilting

Dynamic 3D Cell Culture with Perfusion Plate



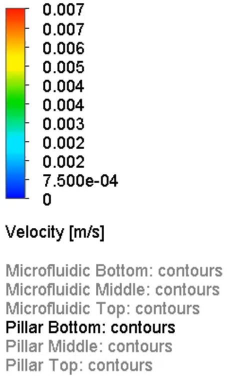
Pillar/Perfusion Plate for Dynamic Organoid Culture

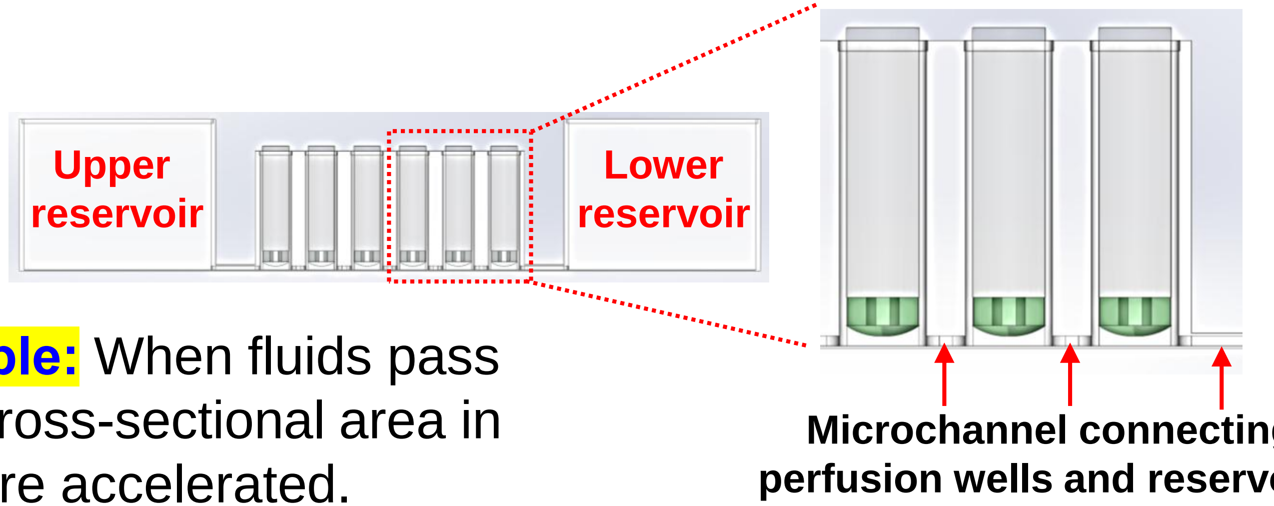




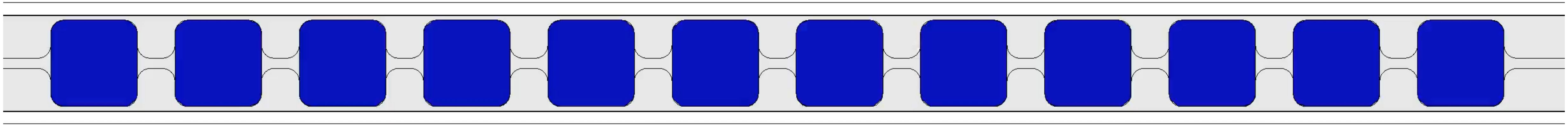
(A) The injection-molded pillar/perfusion plate on a digital rocker for dynamic organoid culture. **(B)** Flow rates in the perfusion plate with 1,500 µL culture media at 10° tilting angle and 1 minute frequency of tilting angle change. SolidWorks simulation and flow rate measurement indicate an average flow rate of 5 - 20 µL/sec, which can be adjusted by changing the tilting angle.

Velocity Profile of Pillar/PerfusionPlate





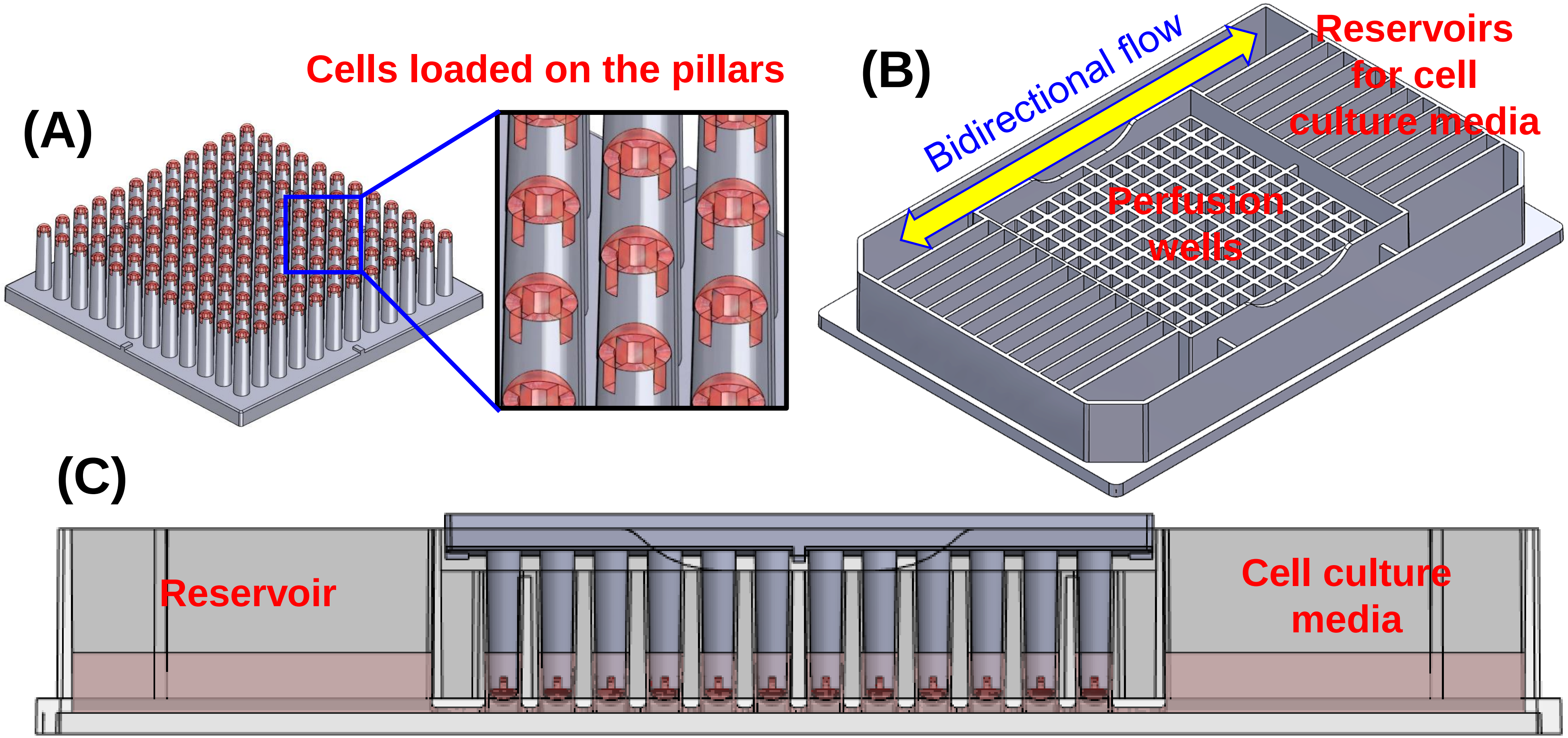
Bernoulli's principle: When fluids pass through a narrow cross-sectional area in a pipe, flow rates are accelerated.



Velocity profiles underneath the pillars in the 144PerfusionPlate over time simulated with SolidWorks with 1500 µL of water at 10° tilting angle, 1 min frequency, and total 4 min running time. The unique design, where pillars are sandwiched into perfusion wells, facilitates rapid mixing of cell culture media, effectively minimizing diffusion limitations for nutrients and oxygen.

https://youtu.be/irLQlB6Ljnk

Pillar/Perfusion Plate for Dynamic Organoid Culture



Our 144PillarPlate and 144PerfusionPlate platform. (A) The pillar plate with a 12 x 12 array of pillars for cell loading. **(B)** The perfusion plate with a 12 x 12 arrays of perfusion wells and a 12 x 2 array of reservoirs connected by microchannels. **(C)** The pillar plate sandwiched onto the perfusion plate for dynamic cell culture.

Some Precautionary Steps to Avoid Common Errors

- The optimum volume of cell culture media in the 36PerfusionPlate is 800 µL per channel (1,600 µL per channel for 144PerfusionPlate & 70 µL per well for 384DeepWellPlate).

- Dispense the cell growth medium from the far end of the reservoirs (away from the microchannels) to prevent overflow in the perfusion wells due to capillary action.

- Ensure that all perfusion wells are filled with cell culture media and that the flow rates are uniform in each channel on a digital rocker.

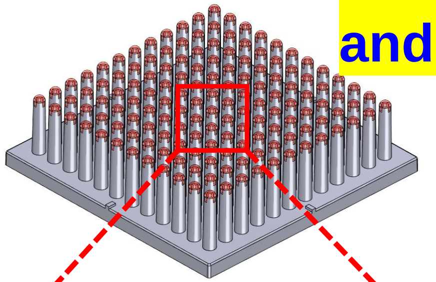
- Adjust the tilting angles of the digital rocker to range between 5 and 15 degrees to prevent overflow.

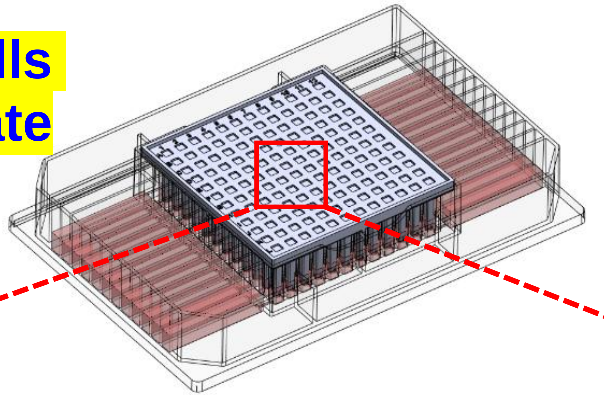
- To avoid overflow, drain the old medium in two steps into the lower reservoirs using a well plate lid and a tip box, removing 600 µL per channel from the 36PerfusionPlate. Add the same volume of fresh medium (600 µL) during the medium change.

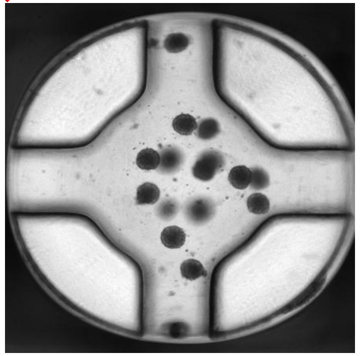
- Do not remove the pillar plate from the perfusion plate during media change process.

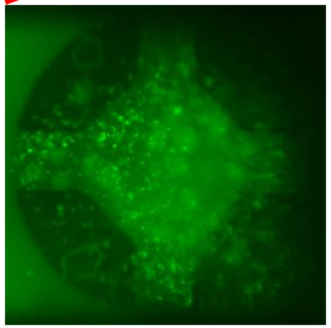
Dynamic Immune Cell-Mediated Killing of Liver Tumor Cells in the Pillar/Perfusion Plate

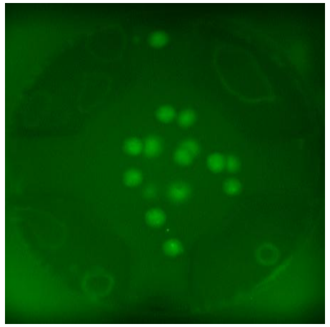
Bidirectional flow of NK-92 cells and berberine in perfusion plate











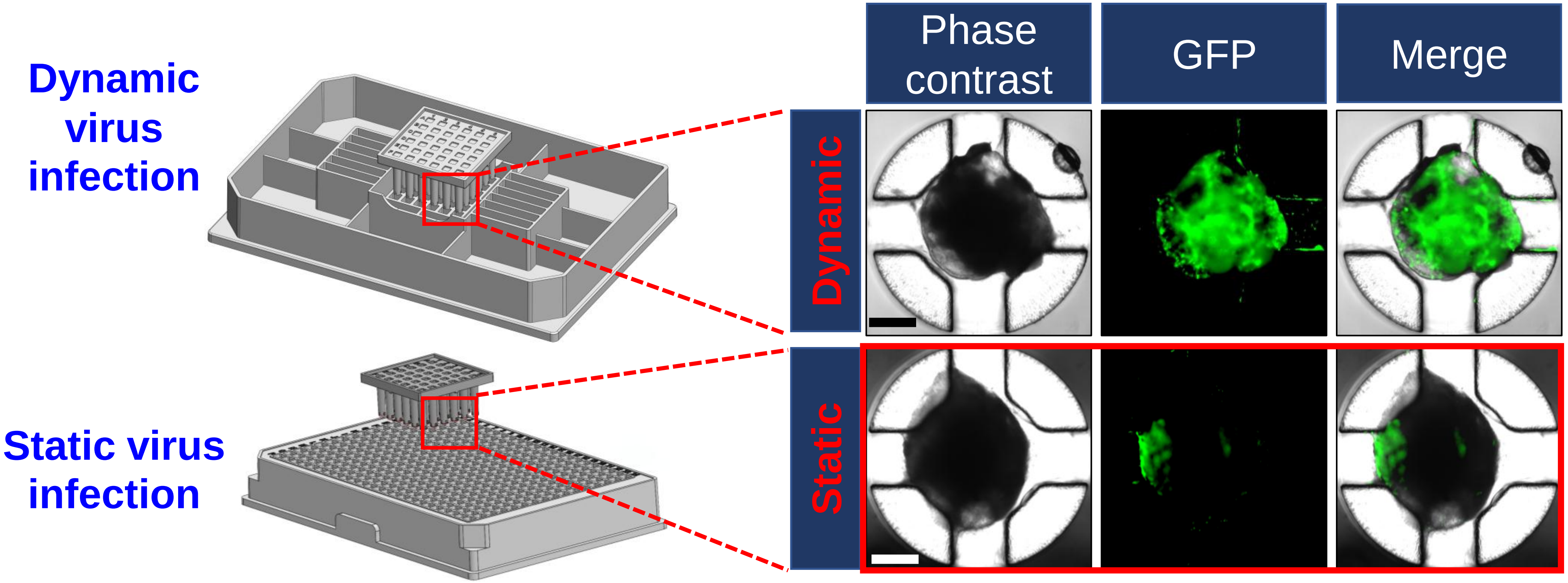
Hep3B spheroids in Matrigel printed on the pillar plate

CellTracker Green-stained NK-92 cells in perfusion wells

Infiltration of NK-92 cells into Hep3B spheroids encapsulated in Matrigel

Berberine-enhanced NK-92 cytotoxicity against Hep3B tumor spheroids under dynamic conditions in the pillar/perfusion plate. A 1:1 ratio of Hep3B and NK-92 cells was treated with 5 µM berberine over 3 days. Immune cell infiltration into liver tumor spheroids, followed by immune cell-mediated cytotoxicity, was observed in the pillar/perfusion plate.

High Efficiency of Dynamic Virus Infection in Brain Organoids



Day-35 brain organoids infected with AAV9 carrying GFP under dynamic and static conditions in the pillar/perfusion plate. Scale bars: 500 µm. A viral load of 1.74 x 10^8 genomes was applied per organoid. The pillar/perfusion plate demonstrated high efficiency in organoid virus infection, highlighting its critical role in gene editing for human organoids and disease modeling.