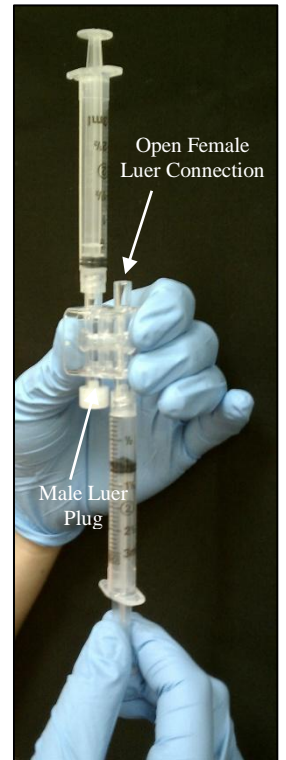


3DKUBE™ *In Situ* Confocal Microscopy

Image your samples directly within 3DKUBE™ 3D Cell Culture Plasticware. Using the [Universal Microscope Stage Adapter](#) accessory, the 3DKUBE can be used in any standard microscope. *In situ* 3D confocal microscopy captures the cells exactly as they are without the disruption that is caused by sample manipulation.

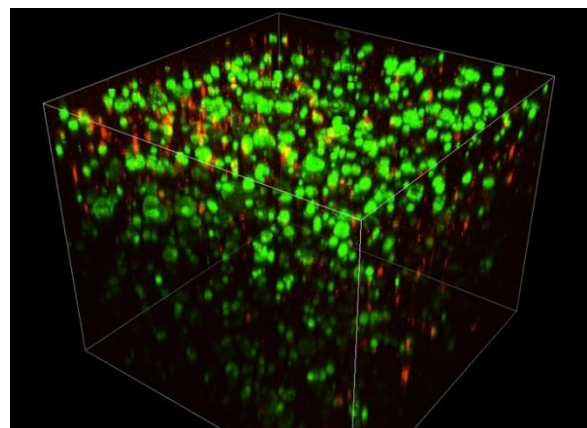
In Situ Staining:

1. Remove the 3DKUBE from its perfusion flow circuit(s) and carefully draw off any residual media from the cell culture chambers using a 3ml syringe.
2. Prepare stain according to the manufacturer's guidelines.
3. Using up to a 3 ml syringe and positioning the 3DKUBE upright (see right), slowly inject 0.5-1.0 mL prepared stain solution into one chamber to decrease air bubble formation and cap the opposing end with a standard male luer plug when the entire chamber is filled (syringe remains attached).
4. Invert the 3DKUBE and repeat for the opposing chamber (see right).
5. Incubate according to manufacturer instructions.
6. Remove prepared stain solution and slowly flush chambers with 1 mL sterile phosphate buffered saline (PBS) using a syringe.
7. Using the above inverting technique to eliminate air bubbles, fill each chamber with sterile PBS and cap BOTH ends with male luer plugs (see bottom left).



In Situ 3D Confocal Microscopy:

1. Transfer capped 3DKUBE to a [Universal Microscope Stage Adapter](#) (bottom left) and position in the microscope stage.
2. Using 3D confocal laser microscopy with up to a 10x objective (100x total magnification), create a z-stack penetrating into the scaffold material (bottom right). Depth of penetration depends on the optical properties of the scaffold material.
3. Flip the 3DKUBE within the [Universal Microscope Stage Adapter](#) to image the opposing chamber.



(Left) Universal Stage Adapter with capped 3DKUBE. (Right) *In situ* 3D confocal image of MCF7 breast cancer cells in alginate beads (100x total magnification).