Imaging Fluidigm™ C1 Vessels (IFCs) using the Evos™ FL Auto Imaging System

Overview

Fluidigm™ C1 vessels (i.e., integrated fluidic circuits, IFC) allow you to capture and stain single cells for further analysis. Once captured and stained, the cell can be examined by microscopy for viability or for the expression of surface markers or reporter genes prior to downstream RT-PCR or sequencing analysis.

This user bulletin provides instructions for imaging Fluidigm™ C1 (96-cell) and Fluidigm™ C1 HT (800-cell) vessels using the Invitrogen™ Evos™ FL Auto Imaging System (Cat. No. AMAFD1000). For detailed instructions on using the Evos™ FL Auto Imaging System, refer to the Evos™ FL Auto User Guide (Pub. No. MAN0007986), available for download at www.thermofisher.com.

Note: Certain components of the workflow such as loading samples to the Fluidigm™ C1 vessel and protocols for using the Fluidigm™ C1 System for RT-PCR are not available through Thermo Fisher Scientific. Refer to the manufacturer for more information.

Before you begin

Software requirements

Ensure that your Invitrogen™ Evos™ FL Auto software is current. The version must be Version 1.7 Rev. 31966 or later.

Note: Software updates are available from the Evos™ FL Auto Imaging System product page at www.thermofisher.com/evosflauto. For software update instructions, see the Evos™ FL Auto User Guide.

Required materials

- Evos™ Vessel Holder/Adapter for multi-well plates, with retention (Cat. No. AMEPVH022, AMEPVH061, AMEPVH028, or AMEPVH040)
- Fluidigm™ C1 (96-cell) or Fluidigm™ C1 HT (800-cell) IFC (see www.fluidigm.com)
Install the Fluidigm™ C1 vessel (96-cell IFC)

Mount Fluidigm™ C1 vessel (96-cell IFC)
1. Center the microscope stage and lower the Focus Axis using the software controls.
2. Remove any currently installed vessel and vessel holder/adaptor from the instrument.
3. Install an Evos™ vessel holder/adaptor for multi-well plates with retention (e.g. AMEPVH022, AMEPVH061, AMEPVH028, or AMEPVH040) into the X-Y stage base plate and lightly tighten each of the four thumbscrews to secure the vessel holder.
4. Place the Fluidigm™ C1 or the Fluidigm™ C1 HT vessel on the vessel holder. Bias away from the retention clip and verify that the vessel is sitting flat on the stage, making contact with each of the four corner ledges of the vessel holder.
5. Proceed with vessel selection.

IMPORTANT! For best results, run the Calibrate Vessel Alignment function whenever a new vessel is mounted, or the same vessel is removed and then re-mounted (see page 3). Vessel alignment allows the Evos™ FL Auto software to establish internal coordinates for the instrument to properly execute high-resolution imaging and measurement functions.

Select Fluidigm™ C1 vessel (96-cell IFC)
1. Launch the Evos™ FL Auto software.
2. From the Image ▶ Capture tab, click the Vessel Expert button.
3. On the Vessel Selection Wizard, select:
   - Well Plate for Category
   - 96 Well Plate – Fluidigm C1 for Type.
4. Click OK to complete vessel selection.
Calibrate vessel alignment - Fluidigm™ C1 vessel (96-cell IFC)

1. From the Image ➤ Capture tab, select 10X objective.
2. From the System ➤ Basic tab, click Calibrate Vessel Alignment.
3. Using the Vessel Alignment Tool, adjust the alignment positions of the Fluidigm™ C1 vessel (TOP, LEFT, DIAMETER, RIGHT, and BOTTOM) so that the crosshairs on the vessel alignment screen have the positions shown in the example images below.
4. After adjusting the alignment of each position, click Save to lock in the new position.
5. Once you have saved all the positions, click through each of the buttons for TOP, LEFT, DIAMETER, RIGHT, and BOTTOM, to ensure they are correct.
6. Click Finish to complete the vessel alignment procedure.

Note: When calibrating the alignment of the Fluidigm™ C1 vessel (96-cell IFC), you need to adjust the crosshairs for the TOP, LEFT, and DIAMETER positions using Well 1, and the crosshairs for the RIGHT and BOTTOM positions using Well 96.
Capture focus nominal for Fluidigm™ C1 vessel (96-cell IFC)

Focus nominal is the Z-point (i.e., depth) around which the auto-focus function searches to provide focus to the sample. You must first capture the focus nominal value to use the Coarse Auto-focus function for the Fluidigm™ C1 vessel (96-cell IFC).

1. Verify that 96 Well Plate – Fluidigm C1 is selected.
2. From the Image ▶ Capture tab, select the objective and light channel that you will most commonly use with the Fluidigm™ vessel.
3. Turn the light on.
4. Move to one of the areas of interest near the center of the plate.
5. Adjust the focus until you obtain an in-focus image.
6. Navigate to the System ▶ Basic tab, and click the Calibrate Vessel Alignment button.
7. Click OK on the confirmation message that appears. This sets the Focus Nominal and completes the calibration of the Coarse Autofocus for the vessel.

**Note:** For best results, set the Focus Nominal again whenever a new vessel is mounted, or the same vessel is removed and then re-mounted.
1. Go to Scan ▸ Routines tab, and then click Create New Routine to open the Scan Wizard. The Scan Wizard will guide you through the process of creating your own scan routine as you make a series of onscreen selections. For more information on creating a scan routine, see the Evos™ FL Auto User Guide.

2. When prompted, select 96 Well Plate - Fluidigm™ C1 from the list of available vessels.

3. Work your way through the Scan Wizard to the area selection page, which prompts you with the question How would you like to select areas to scan? On this page, select Select wells on vessel image.

4. On the next page, click All Wells to select all the wells.
   After you select all the wells, the Scan Wizard screen should look like the example on the right.

5. Once all the wells have been selected, proceed through the Scan Wizard as described in the Evos™ FL Auto User Guide.

Note: You can also select and deselect an individual well by directly clicking on it in the virtual vessel map. Use the Zoom tool below the virtual vessel for a better view of the wells.
Use the Fluidigm™ C1 vessel (96-cell IFC) in Time Lapse

1. Go to the Time Lapse ➔ Routines tab, and then click Create New Routine to open the Time Lapse Wizard.
   
   The Time Lapse Wizard will guide you through the process of creating your own time lapse routine as you make a series of onscreen selections. For more information on creating a time lapse routine, see the Evos™ FL Auto User Guide.

2. When prompted, select 96 Well Plate - Fluidigm™ C1 from the list of available vessels.

3. Work your way through the Time Lapse Wizard to the beacon placement page.
   
   On this page, verify that Fluidigm™ C1 vessel is selected.

4. Adjust lighting and focus settings.

5. Click the Populate Wells button. This will automatically place a beacon at the center of each well.
   
   After you populate the wells, the Time Lapse Wizard screen should look like the example shown below.

6. Once beacons have been created, proceed through the Time Lapse Wizard as described in the Evos™ FL Auto User Guide.
Install the Fluidigm™ C1 HT vessel (800-cell IFC)

Mount Fluidigm™ C1 HT vessel (800-cell IFC)

1. Center the microscope stage and lower the Focus Axis using the software controls.
2. Remove any currently installed vessel and vessel holder/adaptor from the instrument.
3. Install an Evos™ vessel holder/adaptor for multi-well plates with retention (e.g. AMEPVH022, AMEPVH061, AMEPVH028, or AMEPVH040) into the X-Y stage base plate and lightly tighten each of the four thumbscrews to secure the vessel holder.
4. Place the Fluidigm™ C1 HT vessel on the vessel holder. Bias away from the retention clip and verify that the vessel is sitting flat on the stage, making contact with each of the four corner ledges of the vessel holder.
5. Proceed with vessel selection.

IMPORTANT! For best results, run the Calibrate Vessel Alignment function whenever a new vessel is mounted, or the same vessel is removed and then re-mounted (see page 8). Vessel alignment allows the Evos™ FL Auto software to establish internal coordinates for the instrument to properly execute high-resolution imaging and measurement functions.

Select Fluidigm™ C1 HT vessel (800-cell IFC)

1. Launch the Evos™ FL Auto software.
2. From the Image ▶ Capture tab, click the Vessel Expert button.
3. On the Vessel Selection Wizard, select:
   - Well Plate for Category
   - 800 Well Plate – Fluidigm C1 L or 800 Well Plate – Fluidigm C1 R for Type (see Note below).
4. Click OK to complete vessel selection.

Note: The center panel of the Fluidigm™ C1 HT vessel (800-cell IFC) is divided into two sections, left and right, with 10 columns of 40 capture sites in each section that are loaded through the corresponding cell inlets. However, the Evos™ FL Auto Imaging System can image the cells in one section at a time (left or right). Therefore, you need specify the section of the vessel (L or R) you wish to image during vessel selection.
1. From the Image ▶ Capture tab, select 10X objective.
2. From the System ▶ Basic tab, click Calibrate Vessel Alignment.
3. Using the Vessel Alignment Tool, adjust the alignment positions of the Fluidigm™ C1 HT vessel (TOP, LEFT, DIAMETER, RIGHT, and BOTTOM) so that the crosshairs on the vessel alignment screen have the positions shown in the example images below.
4. After adjusting the alignment of each position, click Save to lock in the new position.
5. Once you have saved all the positions, click through each of the buttons for TOP, LEFT, DIAMETER, RIGHT, and BOTTOM, to ensure they are correct.
6. Click Finish to complete the vessel alignment procedure.

**Note:** When calibrating the alignment of the Fluidigm™ C1 HT vessel (800-cell IFC), you need to adjust the crosshairs for the TOP, LEFT, and DIAMETER positions using Well 1, and the crosshairs for the RIGHT and BOTTOM positions using Well 400.
Capture focus nominal for Fluidigm™ C1 HT vessel (800-cell IFC)

Focus nominal is the Z-point (i.e., depth) around which the auto-focus function searches to provide focus to the sample. You must first capture the focus nominal value to use the Coarse Auto-focus function for the Fluidigm™ C1 HT vessel (800-cell IFC).

1. Verify that 800 Well Plate – Fluidigm C1 L or 800 Well Plate – Fluidigm C1 R is selected.
2. From the Image ➤ Capture tab, select the objective and light channel that you will most commonly use with the Fluidigm™ vessel.
3. Turn the light on.
4. Move to one of the areas of interest near the center of the plate.
5. Adjust the focus until you obtain an in-focus image.
6. Navigate to the System ➤ Basic tab, and click the Calibrate Vessel Alignment button.
7. Click OK on the confirmation message that appears. This sets the Focus Nominal and completes the calibration of the Coarse Autofocus for the vessel.

Note: For best results, set the Focus Nominal again whenever a new vessel is mounted, or the same vessel is removed and then re-mounted.

Imaging both sections (L and R) of the Fluidigm™ C1 HT vessel (800-cell IFC)

When using the Fluidigm™ C1 HT vessel (800-cell IFC), the Evos™ FL Auto Imaging System can image the cells in one section at a time, left or right (see Note on page 7).

If you wish to load and image both sections (L and R) of the Fluidigm™ C1 HT vessel without dismounting and remounting the vessel between runs, first calibrate the vessel alignment for the 800 Well Plate – Fluidigm C1 L and then repeat the alignment procedure for the 800 Well Plate – Fluidigm C1 R without dismounting the vessel.

After you have calibrated the vessel alignment for both sections (L and R) without dismounting the vessel, capture focus nominal for the 800 Well Plate – Fluidigm C1 L and proceed with your experiments using the left section.

After you have completed your experiments using the left section, repeat the capture focus nominal procedure for the 800 Well Plate – Fluidigm C1 R and proceed with your experiments using the right section of the vessel.
Use the Fluidigm™ C1 HT vessel (800-cell IFC) in Scan

1. Go to Scan > Routines tab, and then click Create New Routine to open the Scan Wizard. The Scan Wizard will guide you through the process of creating your own scan routine as you make a series of onscreen selections. For more information on creating a scan routine, see the Evos™ FL Auto User Guide.

2. When prompted, select 800 Well Plate – Fluidigm C1 L or 800 Well Plate – Fluidigm C1 R from the list of available vessels.

3. Work your way through the Scan Wizard to the area selection page, which prompts you with the question **How would you like to select areas to scan?**
   On this page, select **Select wells on vessel image**.

4. On the next page, click **All Wells** to select all the wells.
   After you select all the wells, the Scan Wizard screen should look like the example on the right.

5. Once all the wells have been selected, proceed through the Scan Wizard as described in the Evos™ FL Auto User Guide.

**Note:** You can also select and deselect an individual well by directly clicking on it in the virtual vessel map. Use the Zoom tool below the virtual vessel for a better view of the wells.
Use the Fluidigm™ C1 HT vessel (800-cell IFC) in Time Lapse

1. Go to the Time Lapse Routines tab, and then click Create New Routine to open the Time Lapse Wizard.

   The Time Lapse Wizard will guide you through the process of creating your own time lapse routine as you make a series of onscreen selections. For more information on creating a time lapse routine, see the Evos™ FL Auto User Guide.

2. When prompted, select 800 Well Plate – Fluidigm C1 L or 800 Well Plate – Fluidigm C1 R from the list of available vessels, depending on whether you wish to image the left or the right section of the Fluidigm™ C1 HT vessel (800-cell IFC).

3. Work your way through the Time Lapse Wizard to the beacon placement page.

   On this page, verify that 800 Well Plate – Fluidigm C1 L or 800 Well Plate – Fluidigm C1 R vessel is selected.

4. Adjust lighting and focus settings.

5. Click the Populate Wells button. This will automatically place a beacon at the center of each well.

   After you populate the wells, the Time Lapse Wizard screen should look like the example shown below.

6. Once beacons have been created, proceed through the Time Lapse Wizard as described in the Evos™ FL Auto User Guide.