



(shown with IonOptix MyoCam - not Included)

Cell Framing Adapter
Model CFA300
Hardware Manual

Cell Framing Adapter Hardware Manual

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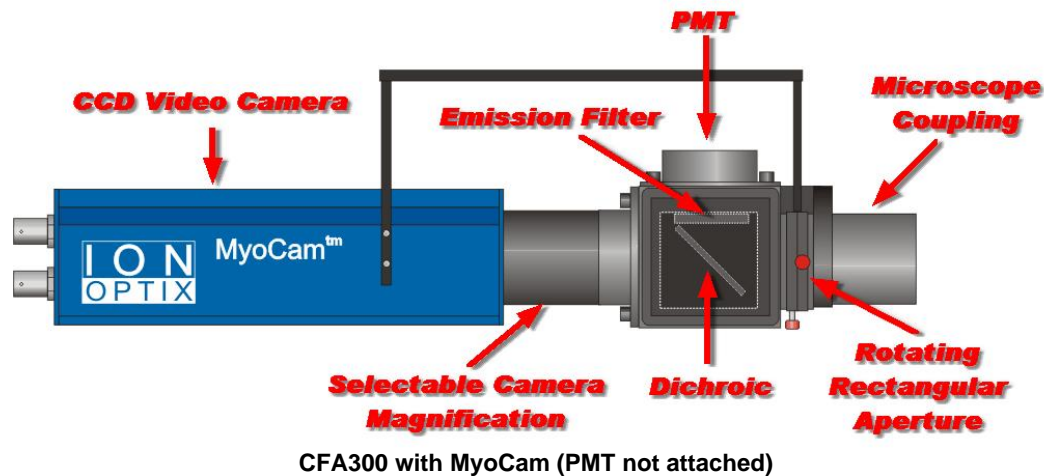
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1 Introduction



The Cell Framing Adapter (CFA) is used to simplify and optimize cell fluorescence recording with a photomultiplier tub (PMT) by integrating a rotatable rectangular aperture and CCD video camera. A combination of the fluorescence emission band and red-filtered transmitted light image enters the CFA from the microscope coupling. A dichroic mirror then reflects the fluorescence emission light through an emission filter and to the PMT adapter while simultaneously passing the red transmitted light image to the camera. An adjustable aperture is used to frame a rectangular area of the microscope field containing the cell which maximizes the cell fluorescence signal by masking background signals. The edges of the aperture are in focus along with the transmitted light image so that you can set the aperture position visually. The standard Cell Framing Adapter comes with a 1x C-mount for the system microscope and the matching mount to connect to the IonOptix PMT Sub-System.

The MyoHandle is a feature specifically designed for cardiac myocyte experimentation. The MyoHandle rotates the camera and the rectangular aperture together to maintain the alignment of the camera and the aperture. This simplifies the process of orienting the long axis of the myocyte with the raster lines of the video; a prerequisite to doing contractility recording.

The CFA300 Cell Framing Adapter includes:

Microscope Coupling - The microscope coupling attaches to the side port or trinocular head of all common microscopes.

Dichroic - An appropriate dichroic mirror for your selected emission band is included.

Options

Option D: Dual Emission - The CFA optics can be stacked to permit dual emission PMT recording.

Option L: Cube lid – Includes an additional optics holding lid to facilitate switching between fluorescence indicators .

Option C: CCD Camera - The CCD camera presents the cell image as a video signal that shows the image area to be recorded by the PMT as framed by the adjustable rectangular aperture.

2 Manual Convention

The following conventions are used in IonOptix manuals:

- Underlined text refers to the names of interface elements shown in the illustrations included in most sections.
- *Italicized* text refers to names given to specific parts of the IonWizard interface. These names can be either IonOptix names, for example *trace bar* or names of Windows controls, like *scroll bar* and are described in various sections of the manual.
- **Bold** text refers to mouse buttons or keystrokes that must be used in order to operate some function.
- The symbol § indicates the following name is a section in the manual.



A note icon indicates an important point that you should know.



An idea icon shows some ideas on how you can use a device or function.



A stop icon indicates a potential for personal injury, equipment damage or data loss.

3 Operating Instructions

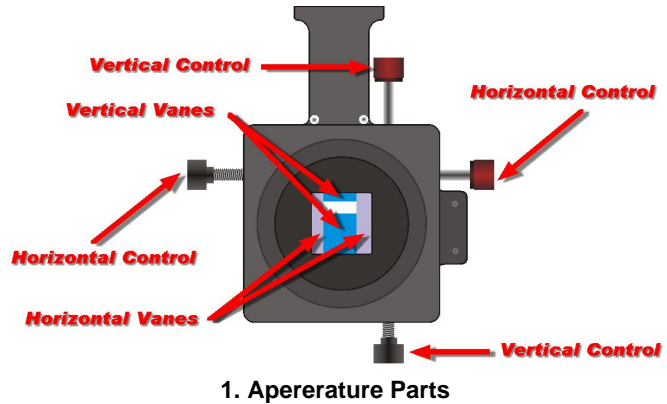
During normal operation, you will usually only need to adjust the aperture and rotate the camera to align it with the cell you are visualizing.

3.1 Aperture Adjustment

The rectangular aperture is adjusted with small knobs that extend on stems from the aperture itself (see figure). Each knob controls two parallel vanes. Pushing or pulling the knob moves the pair of vanes together. Turning the knob move the vanes closer together or farther apart.

Here is a drawing of the "working" parts of the aperture. There are two sets of vanes are oriented at 90 degrees. For each set of vanes there is a control rod that extends out each side with a red knob on one end and a black knob on the other end. The red knob provides the same functions as the black knob - use which ever one is easiest to reach given the current camera rotation.

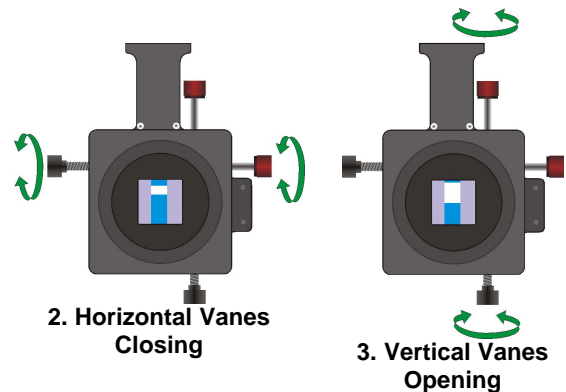
This following figures shows how the vanes move when the controls are operated. In the following figures the horizontal vanes are drawn in purple and the vertical vanes are drawn in blue so that it is clearer what is happening. In the actual CFA both set of vanes are black.



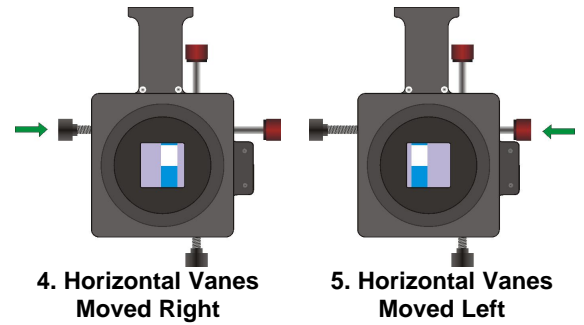
The easiest way to adjust the aperture is to watch the image displayed by the camera as you move the controls.

Rotate the red horizontal control knob counter-clockwise to open the horizontal vanes as shown in figure 2; rotate clockwise to close the vanes. For the black horizontal control knob: clockwise opens and counter-clockwise closes.

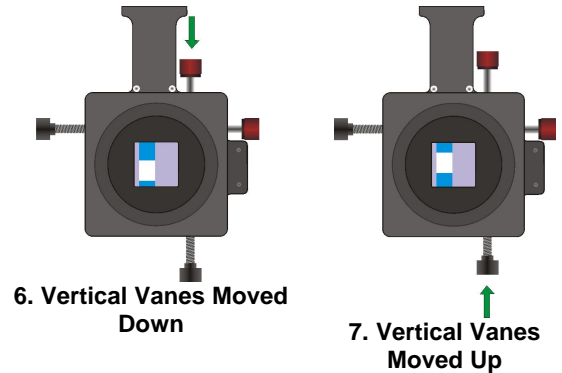
The vertical controls knobs operate exactly the same as the horizontal control knobs: red knob counter-clockwise or black knob clockwise opens and red knob clockwise or black knob counter-clock wise closes. Figure 3 shows the vertical vane being opened.



To move both horizontal vanes while preserving the space between them, push or pull either the red or black knob on the horizontal control in the direction you want to go. Figure 4 shows the horizontal vanes being moved to the right by pushing on the black knob. Figure 5 shows the horizontal vanes being moved to the left by pushing on the red knob.



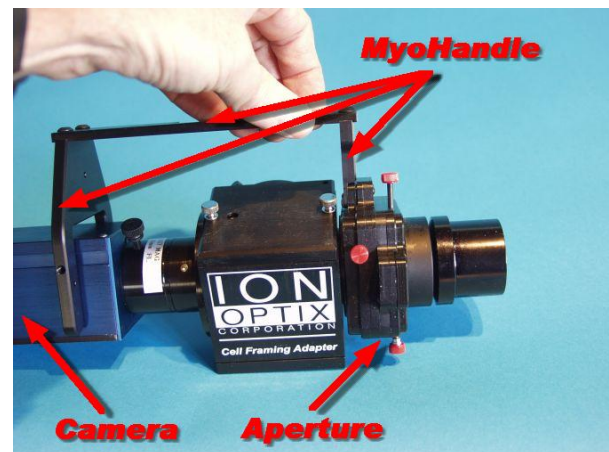
To move both vertical vanes while preserving the space between them, push or pull either the red or black knob on the vertical control in the direction you want to go. Figure 6 shows the vertical vanes being moved to the down by pushing on the red knob. Figure 7 shows the vertical vanes being moved to the up by pushing on the black knob.



3.2 Image Rotation

The rectangular aperture and the camera can be rotated around the center of the visual field of the microscope so that the cells can be horizontally aligned with the camera without rotating the cells on the stage. The MyoHandle connects the aperture and the camera so that the aperture vanes have a fixed alignment with the camera.

The handle rotates through about 270 degrees before it is stopped by the PMT tube (not pictured). If you need more flexibility, you can get independent 360 degree rotation by removing the MyoHandle.



The point of rotation of the camera is the center of the camera sensor which may NOT be the same as the center of the image if you are only sampling the top portion of the lines, such as when using the MyoCam at 240Hz.

4 Setup & Maintenance

The following sections describe how to perform various setup and maintenance tasks for the CFA300.

4.1 Attaching CFA to the Microscope



CFA300 with Nikon Microscope Adapter

The CFA300 has a industry standard C-Mount thread that allows it to be attached in place of a standard camera. The CFA300 can be attached to the microscope horizontally to a side or front port or vertically to a trinocular head. A microscope specific c-mount adapter is provided with the CFA300.



When used horizontally, place the provided scissors-jack under the CFA300 to keep it properly aligned and stable.

4.2 Removing the Emission Dichroic/Filter Cube

The emission filter and dichroic mirror are mounted in a cube that is attached to the lid of the main CFA box. If you have purchased a second lid ([Option L \(12\)](#)) you can quickly change optics by swapping between the two lids. If you are changing the emission filter you will have to remove the lid to get to the cube.

To remove the lid and cube, first remove the two silver thumb screws that hold the lid to the main box.



Then, lift the lid up to remove the lid and cube.



4.3 Changing Emission Filter

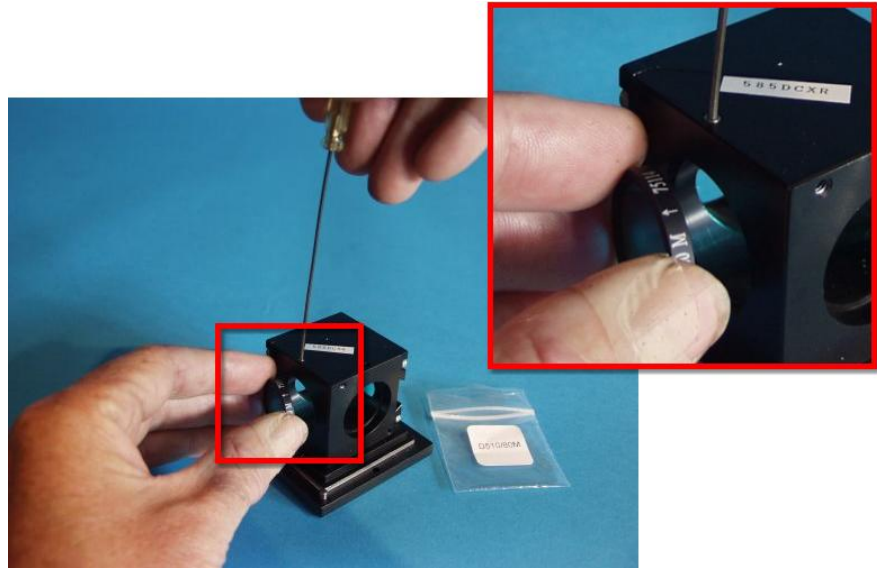
If you want to use a fluorescence indicator with a different emission frequency, you will have to change the emission filter. The easiest way to do this is to purchase a second lid with a complete set of the required optics ([Option L](#)^[12]) and swap lids as needed (see [previous section](#)^[6]). Another option is to change the emission filter in your existing cube.



The new filter must be optically compatible with the existing dichroic mirror!

To change the emission filter, remove the lid and cube as detailed in the [previous section](#)^[6] to get access to the emission dichroic/filter cube.

Next, place the lid/cube upside-down on the table and loosen, but don't remove, the set screw that holds the filter into the cube. You should then be able to remove the filter from the cube.



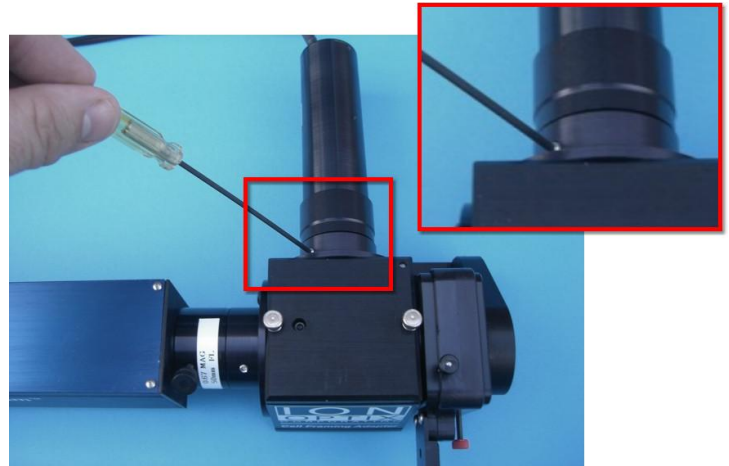
Insert the new filter and lightly tighten the set screw to hold it into place.



Be sure to follow the filter manufacturer's instructions to assure that the filter is inserted facing the correct direction. There should be an arrow on the outer edge that indicates the proper direction. Unfortunately, not all filter manufacturers point the arrow in the same direction.

4.4 Removing the Photomultiplier Tube

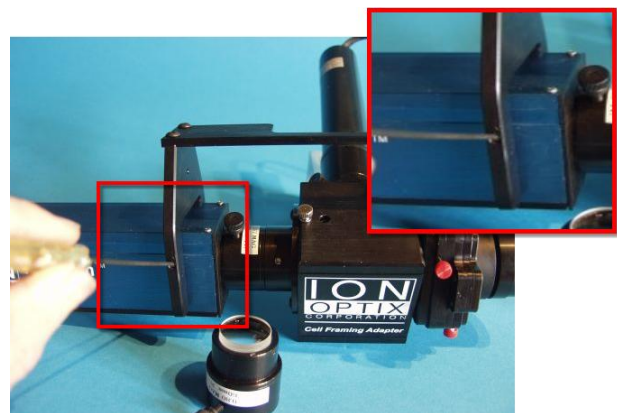
To remove the photomultiplier tube, loosen, but don't remove, the set screw that holds the tube into the coupler on the side of the main dichroic box.



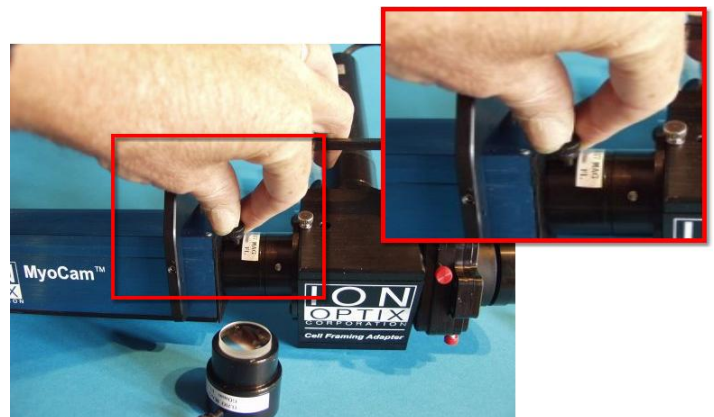
4.5 Removing the Camera

The following instructions demonstrate removal of the camera from the CFA. Note the pictures show the IonOptix MyoCam, but other cameras will be similar.

First loosen, but don't remove, the screws that hold the MyoHandle fork to the camera body as shown. It is usually enough to loosen one side. If not, there is a 2nd screw on the other side of the fork (not pictured).



Next loosen the screw that holds the camera to the output end of the magnification adapter. Use your hand to loosen the plastic screw as shown.



With the MyoHandle and magnification adapter loosened, the camera should slide out of the magnification adapter.



When you reattach the camera to the magnification adapter, do not tighten the plastic screw completely unless you want to prevent the camera and aperture from rotating.

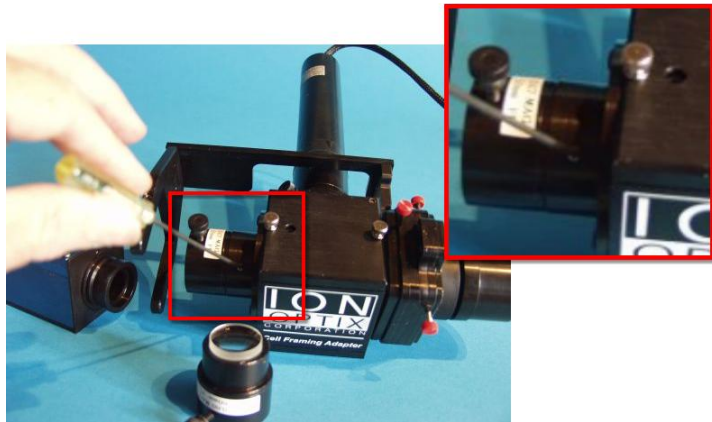
4.6 Changing the Camera Magnification

IonOptix provides a small range of magnification options to help you can fine tune the size of of your cells as seen by the camera. The camera magnification is controlled by the small tube that sits between the camera and the CFA300 and holds different lens at the correct distance from the camera sensor.

To change the magnification adapter, follow these steps:

First, remove the camera as described in the [previous section](#) ⁸.

Next, loosen the set screw that holds magnification adapter to the dichroic holder.



Slide the magnification adapter out and insert the new magnification adapter in its place.



Reverse the procedure to reconnect the camera and MyoHandle to the new magnification adapter.

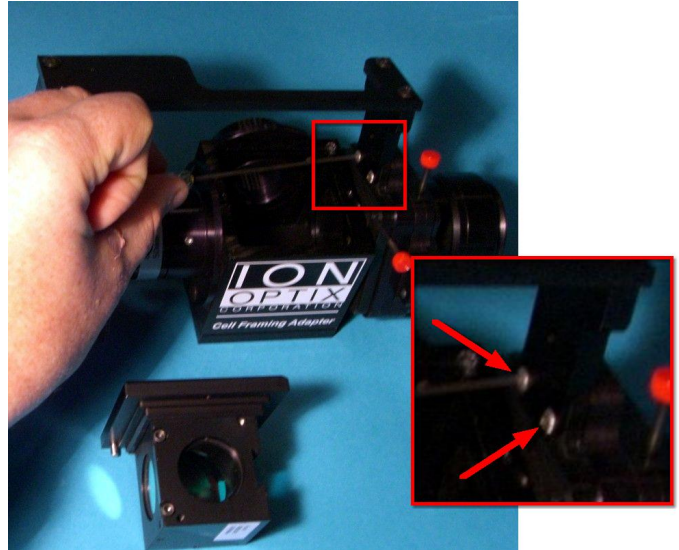
4.7 Removing the MyoHandle

If you need independent 360 degree rotation of the camera and the rectangular aperture, you can remove the MyoHandle.

First loosen, but don't remove, the screws that hold the MyoHandle fork to the camera body (as shown in the [Removing the Camera](#) ⁸ section).

Next, remove the lid with the filter/dichroic cube (as shown in the [Removing the CFA Dichroic/Filter Cube](#) ⁷ section).

Next remove the two screws (indicated by the arrows) that hold the short "arm" of the MyoHandle to the SIDE of the aperture arm.



5 Options

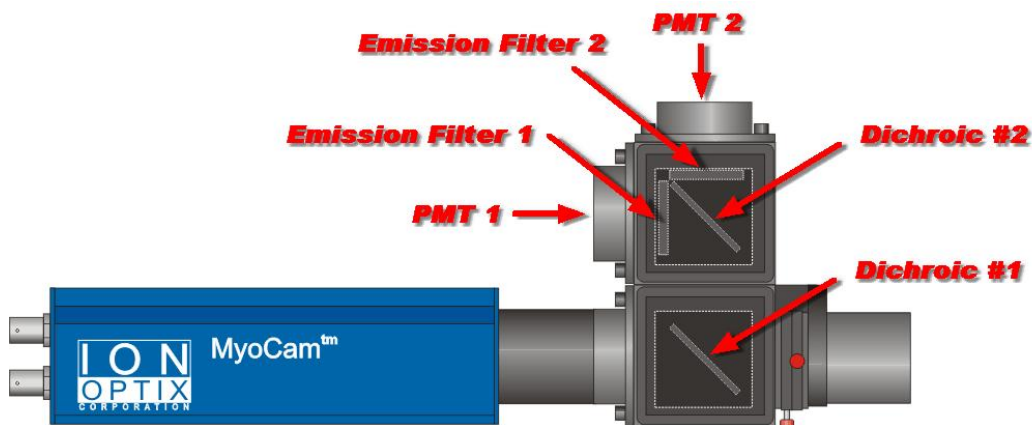
The following options are available for the cell framing adapter.

5.1 Option D - Dual Emission



Dual Emission CFA300 with MyoCam

The Dual Emission Option for the CFA adds a second dichroic/emission filter holder assembly. The filter/dichroic cube in the second holder is used to split the dual emission wavelengths to individual PMTs. The optical components in a dual emission CFA are shown below:



Optical Components for CFA300 w/option D (MyoHandle removed)

Dichroic/filter cube #1 holds a single dichroic mirror (and no filters) that reflects both lower emission wavelengths to the second dichroic/filter cube and passes the red transmitted light to the camera. Dichroic/filter cube #2 holds a dichroic mirror that reflects the lower emission wavelength through emission filter 1 to PMT 1 and passes the higher emission wavelength through filter 2 to PMT 2.

5.2 Option L - Extra lid w/dichroic/filter cube

Option L provides you with a 2nd emission filter and dichroic cube with lid. It also includes the appropriate optics for a user-specified fluorescence indicator. To switch between fluorescence indicators, follow the instructions in the [Removing the Emission Dichroic/Filter Cube](#) ⁶ section.

5.3 Option C - Aperature Visualization CCD Camera

Option C provides a basic CCD video camera and black and white monitor to visualize the aperture for systems that are not configured with a MyoCam for simultaneous edge detection or sarcomere spacing measurements. The camera provided has a white plastic spacing ring that makes it the proper size to be held in the MyoHandle "fork" which is sized for the MyoCam.