MultiCell system

User's Guide

Manual version 4 - IW release 7.5.1.
March 2021

CYTOCYPHER

Contents

1 Introduction

1.1 Overview

The MultiCell High Throughput System is a microscope that can be used to measure cardiomyocyte calcium and contractility automatically in combination with IonWizard equipment.

This manual is intended to describe the basic connections and operations of the MultiCell system

1.2 User's Guide Conventions

The following stylistic conventions are used throughout this and other CytoCypher manuals.

`[Courier New text within brackets]` refers to specific named items in button selections

Bold Italic text refers to other manual sections.

2 Quick start guide

1. Turn on all equipment (Microscope, FSI, MyoPacer)
2. Open IonWizard
3. File → New, Collect → Experiment → Select/Add Experiment (must include advanced sequencing)
4. Press `[Open Cell Finder]` to open MultiCell window
5. Bring dish into focus by adjusting z-focus or use autofocus settings
6. Press `[Start]` in IonWizard
7. Adjust cell finding settings or select a profile
8. Press `[Find and measure]` or `[Add Measurement]`
9. Check found cells and discard cells if necessary in `Free view mode`
10. Press `[Complete Step]` → if sequence only consists of one Epoch, MultiCell will close. Save your file in IonWizard
11. Press `[Automatic measure]` to perform measurements as defined by the 2nd step of your sequence. Repeat until last step when MultiCell window closes. Save your file in IonWizard

3 Software

3.1 Software versions

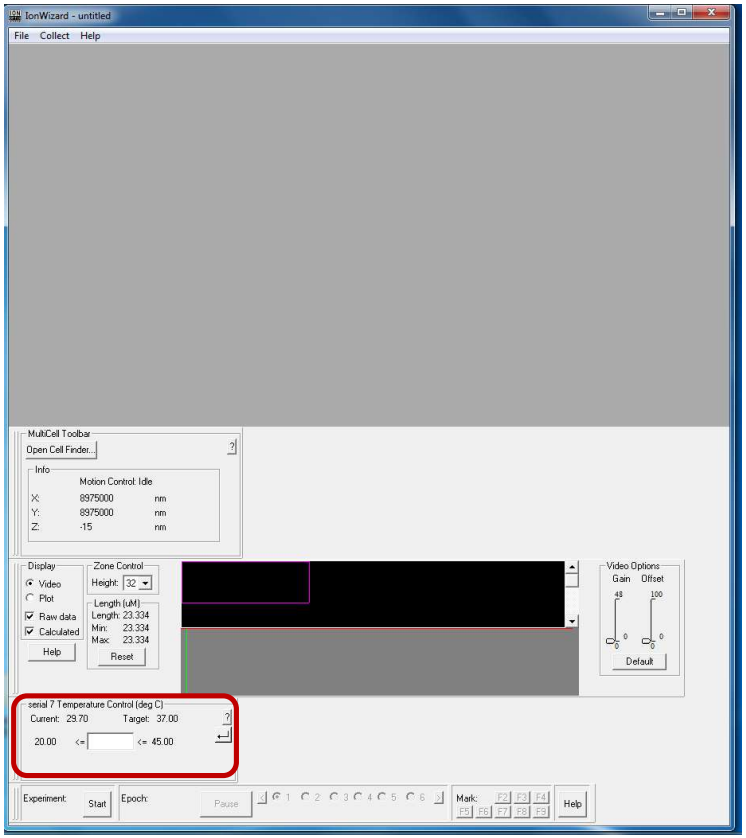
MultiCell is integrated in IonWizard. We assume that the user is familiar with IonWizard software and only describe the specific alterations for the combination with MultiCell. A full IonWizard Acquisition manual can be found here: http://www.ionoptix.com/wp-content/uploads/2014/07/Acquisition.pdf

3.2 Start

Turn on all equipment (Microscope, FSI, Myopacer) and wait for the Microscope to finish initializing. Should take ~5 minutes and is indicated by a green light next to the Power button (only on microscopes with SN > 012)

Start IonWizard. select File → New

N.B. Warming up the system to 37 degrees Celsius takes ~ 30 minutes. Current and target temperature is indicated in the main IonWizard screen (*red box*).



3.2.1 Hardware configuration

Prior to first use/installation the following configuration of all hardware components needs to be performed:

Select Collect → Hardware → Add root

- CytoCypher Microscope → select instance → `[Add]` → Description: "Microscope"
- MCC PCI Card → select instance → `[Add]` → free devices: select "FSCI0"→ `[Attach]`
- Select "Mark In"→ Free devices: select "Digital Source" → `Attach`. Description: "Mark"
- IonOptix Myocam → select instance → `[Add]` → Press `[OK]`

- Select <Time Sync>: Available sockets → Select [start Out] on FSCI0 → [Connect]

For fluorescence experiments add:

- Hyperswitch → DCA settings (~2700, ~3700, ~2000)
- PMT

Press [OK]

Calibrate camera:

- Select Collect → Hardware
    - Go to MyoCam → Press [Specify] → [Calibrate] (~takes about 5 minutes) → [OK]

Calibrate microscope:

- Place ruler on microscope → Press [Open Cell Finder] → Bring ruler into focus and align striation → Close MultiCell window
- Select Collect → Task Manager → Sarcomere Length → [Edit] → [Collect] → add point e.g. 50um and place indicators at 50um → Add another point e.g. 100um and place indicators at 100um. Press [OK]. Copy pixel/um ratio
- Select Collect → Task Manager → MultiCell → [Edit] → Camera: copy ratio.

Set fluorescent diameter (recommended 100um):

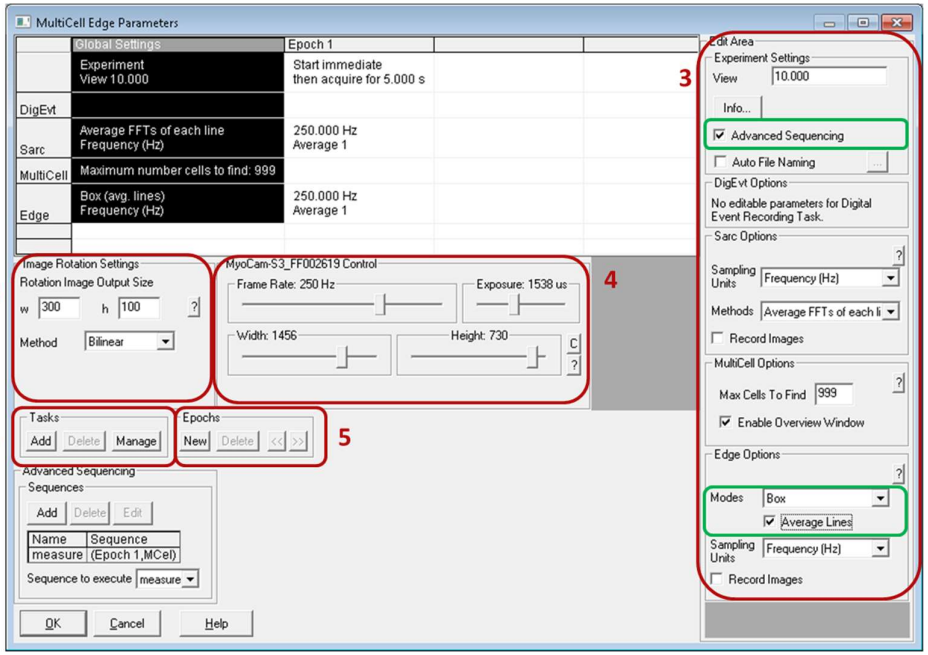
- Select Collect → Task Manager → MultiCell → [Edit] → Fluorescence radius: 50um

Add Image rotation manager

- Select Collect → Acquisition filter → [Add] → Rotation manager → [OK]
- Select Collect → Task manager → Sarcomere length → [Edit] → Camera: Image Rotation Filter
- Select Collect → Task manager → SoftEdge Length Measurement → [Edit] → Camera: Image Rotation Filter

3.2.1.1 *Experiment Manager in IonWizard using MultiCell*

Select Collect → Experiments → [Add] [name e.g. MultiCell] → [Edit]
This opens the following window:



- *Selection 1:* For the **Global Settings** add at least the following tasks by pressing [Add]
  - MultiCell: Max cells to find keep default 999 (*Selection 3*)
  - Event recording: Mark

  Depending on your experiment add:
  - Sarc: Average FFT of each line, frame rate 250 Hz *(Selection 3 and 4)*

    AND/OR
  - Edge: select Modes: Box for automatic edge detection. Select Average lines (*Selection 3)*

    AND/OR
  - Dual Excitation

    AND/OR
  - CytoMotion: Select Pixel Intensity and/or Pixel Correlation. Set lowest expecting beating frequency.
- *Selection 2:*
  - Image Rotation Settings: use Bilinear method
  - Rotation Image Output size (i.e. size of cell selection): default is 300 x 100. Can be adjusted for larger cells.
- *Selection 3:* Select Advanced Sequencing to setup measurements using MultiCell (*green box*).
- *Selection 4:* Set frame rate to 250Hz, adjust Exposure time if image is too bright
- *Selection 5:* Create new Epoch(s). For automated measurements it is recommended to use *start: immediately*

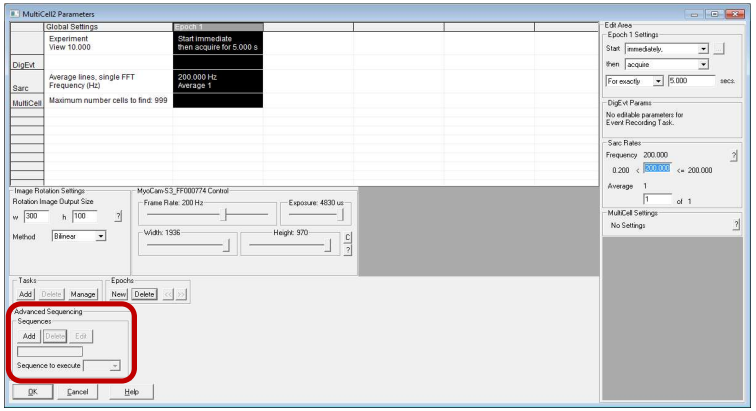
3.2.2 Advanced Sequencing

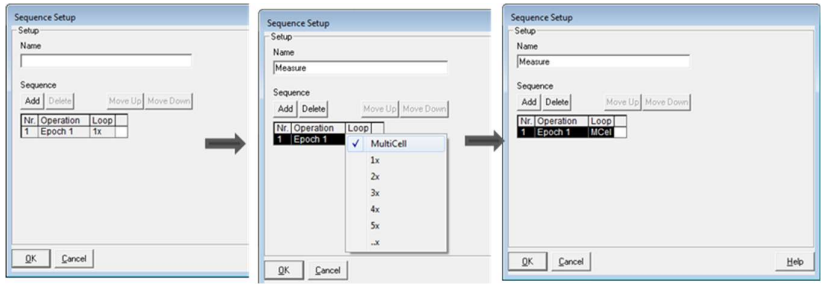
To use the MultiCell system advanced sequencing is necessary!!!

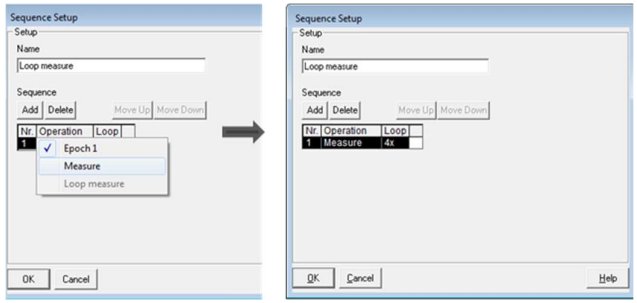
Adding a sequence (*red box*)– only possible after at least one Epoch is created

- **Single measurement per cell**
  - Press `[Add]`, the Sequence Setup window pops up. This will create a new sequence automatically containing Epoch1*1 (this means it will perform Epoch 1, once). By double clicking on this section a drop down menu appears. Select MultiCell. This will redirect IonWizard to MultiCell in which you can automatically or manually find and measure cells. The settings of MultiCell are described in ***section 4.2.***
  - Give your sequence a name (e.g. Measure), easier for creating loops (see below)

- **Repeated measures**
  - Create first sequence as described above (Epoch1 * MCell)
  - Create a 2nd sequence (name e.g. Loop measure) and double click on Epoch1 and select Sequence 1 (i.e. Measure). Change loop to 4 to perform 4 repeated measures.
  - In the advanced sequencing panel, set the *Sequence to execute* to the wanted sequence. Double click on the box to see the drop down menu and select the sequence (i.e. Loop measure).

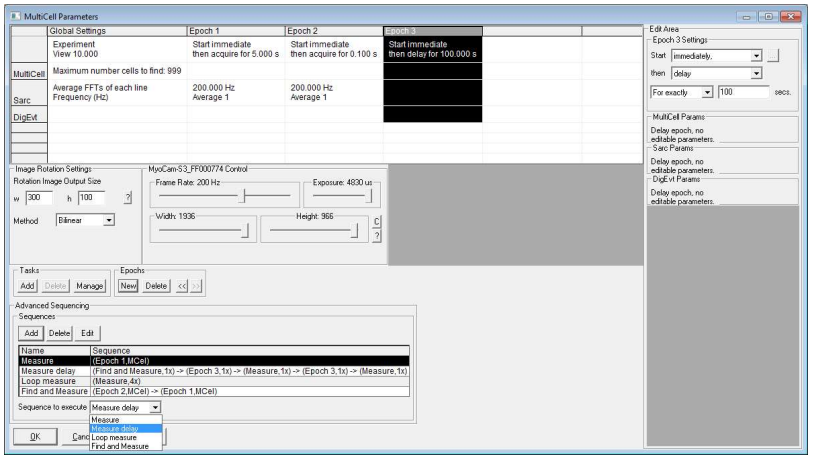






Tip:
If you are not sure how many repeated measures you need. Create a sequence with more than enough loops. Adding an additional loop when an experiment is running is not possible, but it is possible to stop an experiment halfway a sequence.

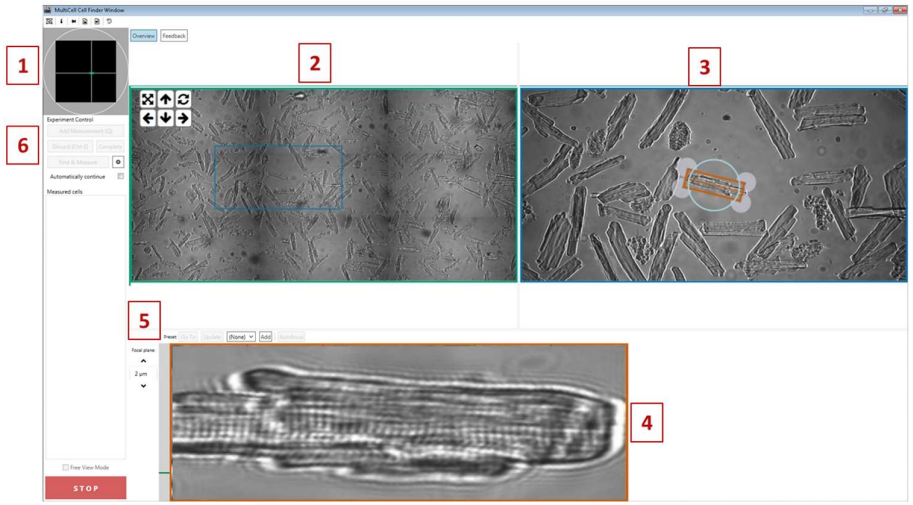
- **Multiple epochs and loops**
  - Create additional epochs e.g. longer measurements or a delay
    (With a delay you can also choose *start: after a key press*, for example start delay after you have added your compound and start your measurements automatically after a defined incubation time)
  - Create an additional step in the sequence by pressing the [Add] in the sequence setup window. Select another Epoch and define what you want to do.
  - Select the desired Sequence in *Sequence to execute.*



4 MultiCell Tool

4.1 Cell Finder Window

Press [`Open Cell Finder`] button in IonWizard. This will open the MultiCell Cell Finder window:



The user interface consists of the following screens. By dragging the corners of a section you can enlarge or reduce the size of the windows to your preferences.

- Return screen settings to default
- Open Help window
- Pin the MultiCell screen on your window
- See and adjust the cell image saving settings
- Record video of live camera view (default duration is 4 seconds)
- Restore cell list (See ***section 4.3.7***)

1. Dish overview: Represents the cell dish and indicates location of the objective relative to your well dish containing the myocytes (*green*). Clicking at a position in the black square will move the objective to that position and take a new *9-panel overview* (2).

2. Overview: An overview of part of the dish. Can be used to find cells quickly. Clicking in the 9-panel image will move the objective to that position (*blue square*) and update the *real-time image* (3).

   Feedback: Default is black, becomes active after pressing [Find & Measure]. Shows feedback on the automatic cell finding algorithm (See ***section 4.3.5***)

3. Live real-time image: Indicates cell selection in *orange*. Clicking in this window will move the objective and the cell selection. Dragging on the grey circles can be used to rotate the cell selection window. These actions can also be performed by the short cut keys as described in the table

4. Cell-selection window: Shows the cell selected in (3). Use this window for fine focus.
   - Includes Edge boxes (red = left, green = right) if edge is in the experiment.

5. Focus Menu: Buttons can be used to adjust focus settings. See ***section 4.1.1***

6. Experimental control: Shows all options to perform measurements and the list of measured cells. Settings of finding cells can be reached by pressing  button see ***section 4.2.*** All other buttons become active after starting an experiment in the IonWizard window.

Key	Action
W	Move objective up by current Step size
S	Move objective down by current Step size
A	Rotate *cell-selection box* in counter clock wise direction
D	Rotate *cell-selection box* in clock wise direction
Shift + W	Move the *cell-selection box* up
Shift + S	Move the *cell-selection box* down
Shift + A	Move the *cell-selection box* left
Shift + D	Move the *cell-selection box* right
Q	Add measurement or Update
Z	Accept measurement
X	Reject measurement
Ctrl + E	Discard cell

4.1.1 Focus settings

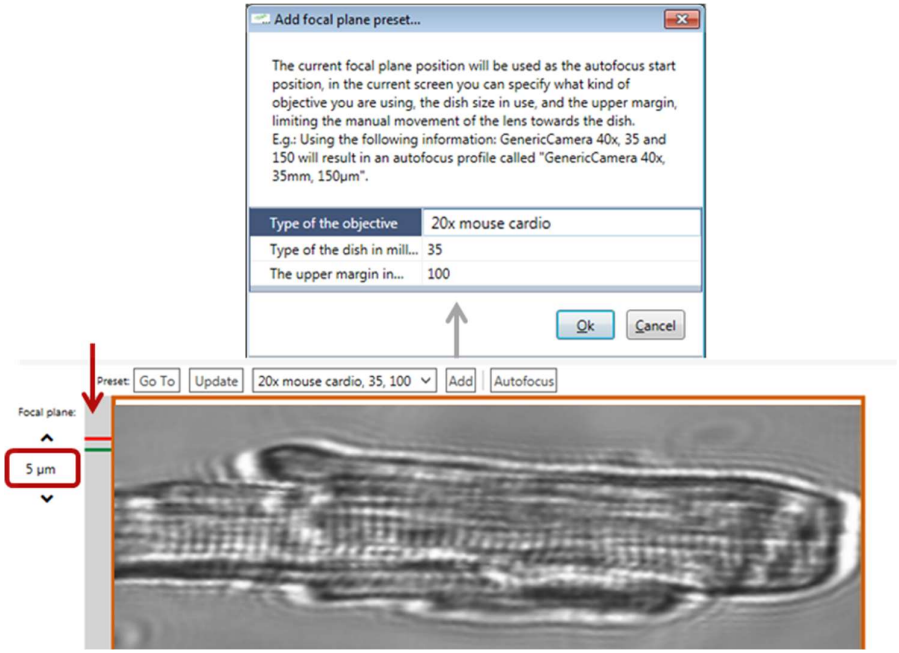
To get the cells into focus, you need to adjust the focal plane. This can be controlled on the left side of the cell selection window. The grey bar (*red arrow)* indicates the range of the objective.

- **Coarse focus:** Click on the grey bar (*red arrow*) next to the cell selection window. Resulting in the objective to go the indicated height.
- **Fine focus:** Adjust step size by clicking on the number (*red box)*

  ^ Move objective up by selected step size

  v Move objective down by selected step size

- **Save focus settings and use this for Autofocus**
  - Adjust Focal plane to focus a cell
  - Press `[Add]` and fill in the details:
    - Objective type: free field
    - Dish size: e.g. 35mm
    - Upper margin in um: e.g. 100 (i.e. this is to prevent the objective to push against your dish when performing autofocus)



Green bar indicates the pre-set value, red bar indicates upper margin.

Next time, select your focus setting and press `[Go to]` or `[Autofocus]`. Objective will go to specified focal plane and will move up and down and determine focus on edge of the cell for Autofocus.

`[Update]` can be used to temporarily update your focus settings for the current Experiment.

4.2 Experimental control

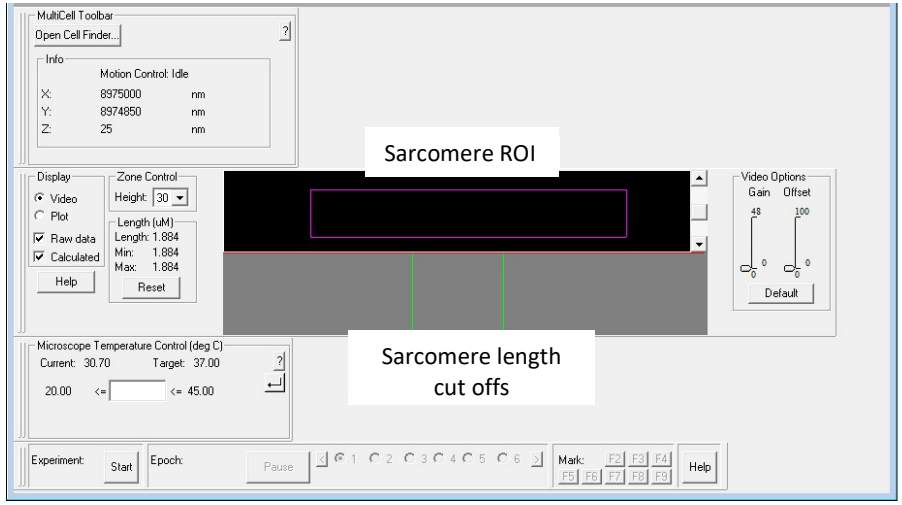
Performing measurements with MultiCell consists of the following steps:

1. press start in IonWizard
2. Bring your cells into focus
3. Adjust Cell finding settings
4. Press [`Find & Measure`] or [`Add measurement`]
5. Press [`Complete`]
6. (Press [`Automatic measure`])
7. Save file in IonWizard

The steps are described in more detail below.

4.2.1 Start

In the IonWizard screen, check the size of the Sarcomere ROI (*purple box*) and place it in the middle of the window. Additionally, check sarcomere length cut off values (*green bars*). Start an Experiment in IonWizard by pressing the [`Start`] button. The first step of your sequence is now activated, this should contain MultiCell and automatically you will be redirected to the MultiCell window. Now, all others buttons below Experiment Control are active.



You have 2 options:

1. Manually find and measure cells
2. Automatically find and measure cells

4.2.2 Manually find and measure cells

- Click or use short cut keys to find a good cell and bring it into focus.
- Press `[Add Measurement]` in Experimental Control and the cell will be measured and added to the list below.
- A pop up will appear and ask whether you want to `Accept` or `Reject` the measurement.
    - `Accept`: Cell is added to the list. A green-box will appear around the cell in 9-panel and live-real-time image.
    - `Reject`: Cell is not added to the list yet. You can update the focus or location and try again OR move to a new cell
    - *When moved to a next cell, start a new measurement and the cell will be automatically discarded. Discarded cells are indicated by a red cross in the cell list and by a red-box around the cell in the images.*

4.2.3 Automatically find and measure cells

- Check and/or adjust your cell finding settings by pressing .  An extensive explanation of these settings can be found in ***section 4.3 on page 19.***
- Press `[Find & Measure]` and MultiCell will start looking for the number of cells as defined by your settings.
- Found and measured cells are added to the list below. In addition, measured cells have green boxes around them in all windows.

MultiCell will stop when it reached the desired number of cells OR when you press the `[stop]` button OR when it can not find any more cells.

Tip:

`[Find & Measure]` can be repeated multiple times, cell finding settings can be adjusted each time.

You can switch back and forth between manually and automatically finding and measuring cells.

4.2.4 Free view mode

Cells that were found and measured can be checked using the Free View Mode.

- Click on the check box in front of `Free View Mode`.
- Navigate through the cells in the list by clicking or using the arrow keys on your keyboard. The active cell is indicated in green
- You can discard a cell by clicking [`Discard`]. A red cross will appear on the cell in the list.
- Adjust focus or manipulate location/orientation of the cell and press [`Update`] to apply your changes. The thumbnail will also be updated.
- To continue uncheck the `Free View Mode`

4.2.5 Complete

Press [`Complete`] once you are finished finding cells. After this action it is not possible to add more cells to your list.

Next, two things can happen depending on your sequence:

- If you have run the last step of your sequence, the MultiCell window will close and the experiment will be completed. → Save your file in IonWizard. File → save file
- Window will automatically switch back to the first cell of the list
  - Press [`Automatic Measure`]: all cells in your list will be automatically measured as defined by the next step of your sequence.
  - Press [`Add Measurement`] to manually measure the active cell.
  - Action can be repeated by the number of steps of your sequence. Thus, if you have defined 4 repeated loops, or 4

different epochs this action can be repeated 3 times i.e. first epoch or loop is “used” for find and measure.

N.B. The MultiCell window will automatically close once the last step of your sequence is finished. Save your file in IonWizard.

4.2.6 Automatically continue

It is also possible to automatically continue through all steps of your sequence by checking the `Automatically continue` check box. This action can be performed at the start or between steps of your sequence.

4.3 Cell finding settings

Start by opening the settings window to find cells by clicking  button.

Top of the window: Profiles can be created to save your cell finding workflow.

[+] Create a new profile

[-] Delete a profile

[ ] Duplicate current profile

Press [Apply] to save your profile.

The columns on the left show the structure of the settings.

4.3.1 Generic configuration

- **Area** – Range of the cell size (length x width) in µm^2
  - **Maximum Area (µm^2) –** Maximum size of the cells. For example, to filter out clumps of cells.
  - **Minimum Area (µm^2)** – Minimum size of the cells. For example, to filter out small debris.

- **Aspect Ratio** - Aspect Ratio represents the width/length ratio of the cells
  - **Maximum Ratio**. A ratio close to 1: shape of the cell close to a square or circle. For example, to filter out round objects like dead cells.
  - **Minimum Ratio** - A ratio close to 0: shape of the cell close to a tall narrow rectangle. For example, to filter out debris

- **Misc**
  - **Cell overlap parameter (µm)**: This parameter is a threshold of distance between two cells. If the distance between the center of two found cells is

Tip:

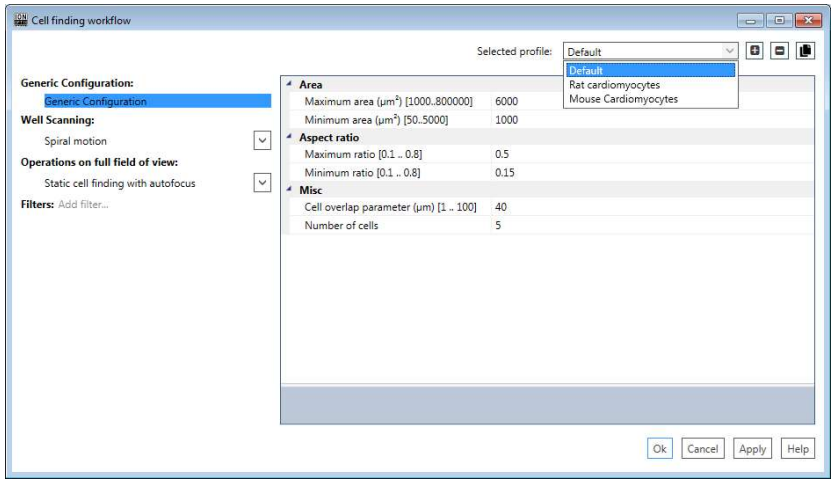
For first time use of automatic cell finding start with only basic settings and increase the complexity once cells are found.

Basic settings example:

- number of cells: 10
- well scanning: no scan or spiral motion
- Operations on full field of view: static cell finding
- Filters: no filters

If no cells are found check the generic settings.

Once cells are found increase complexity by adding filters and add motion detection in Operations of full field of view



smaller than the parameter, it is treated as one single cell. If the distance is bigger than the parameter, it is treated as two different cells.

- **Number of cells:** Defines the number of cells you want to find in one goal.

4.3.2 Well Scanning

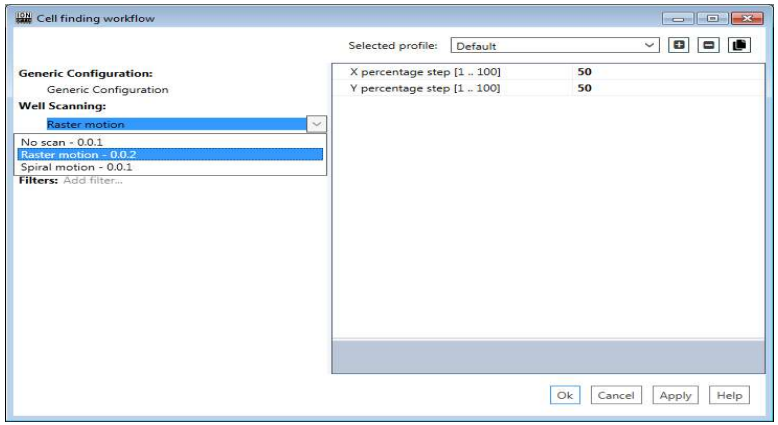
Defines the motion pattern throughout the well. Three options are available in the drop down menu:

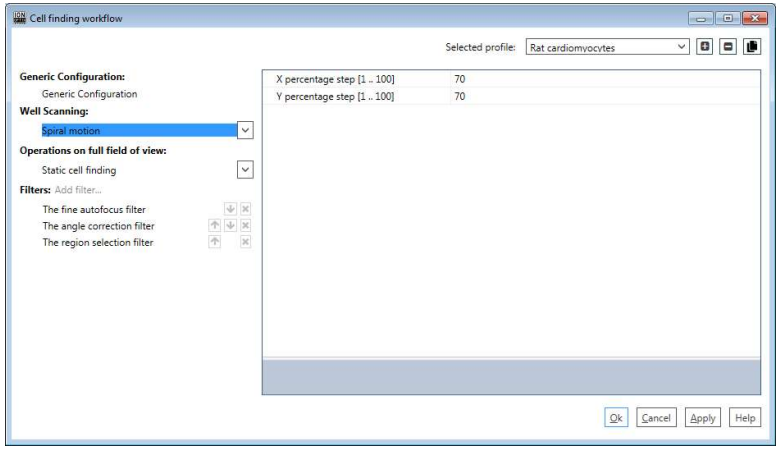
- **No Scan -** System does not move the objective but only finds cells in the current field of view (FOV).

- **Raster Motion** - System moves the objective in a raster pattern. The objective sweeps horizontally line by line. It starts from the current point and goes left. After finishing the line, it continues by going one *YStep* higher and reversing the direction of the X-axis.

- **Spiral Motion** - System moves the objective in a spiral pattern.

  - **X percentage step [1 – 100]** - Step size in the *x*-direction as a percentage of the FOV. A certain degree of overlap in FOVs is desired to find cells on the edges of the FOVs. Step size close to 100: no overlap between FOVs. Step size close to 1: large overlap between FOVs. 70% is a reasonable value.



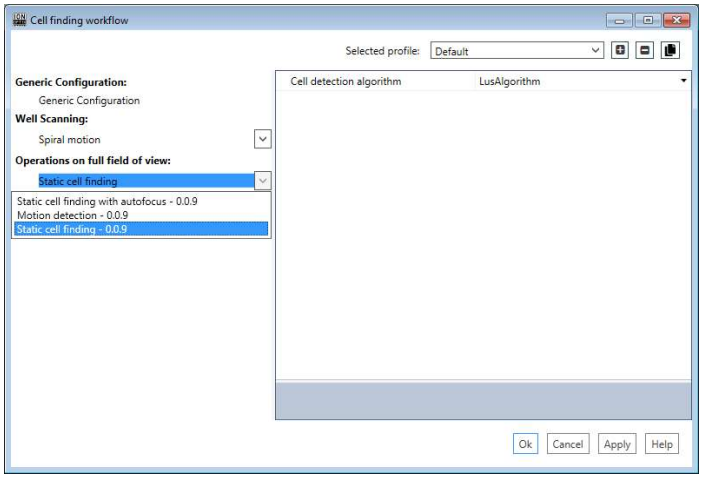


- **Y percentage step [1 – 100] -** Step size in the *y*-direction as a percentage of the FOV. A certain degree of overlap in FOVs is desired to find cells on the edges of the FOVs. Step size close to 100: no overlap between FOVs. Step size close to 1: large overlap between FOVs. 70% is a reasonable value

Tip:
It is recommended to use Spiral Motion rather than Raster Motion. In Raster Motion, the focal plane could be influenced by the tilt of the dish. That means the objective attempts to go out of focus when it is moving from the centre of the dish to the border of the dish where there are less cells or even no cells captured by the software. However, Spiral Motion is circling round the starting point. If a proper starting point is chosen with good cell density, the focal plane can be easily updated with the newly found cells and the objective has a higher chance to stay in focus.

4.3.3 Operations on full field of view

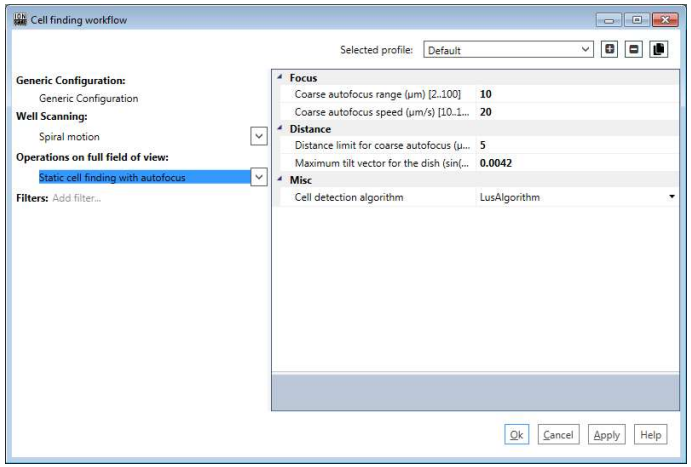
Defines the cell finding method. We have three methods included.



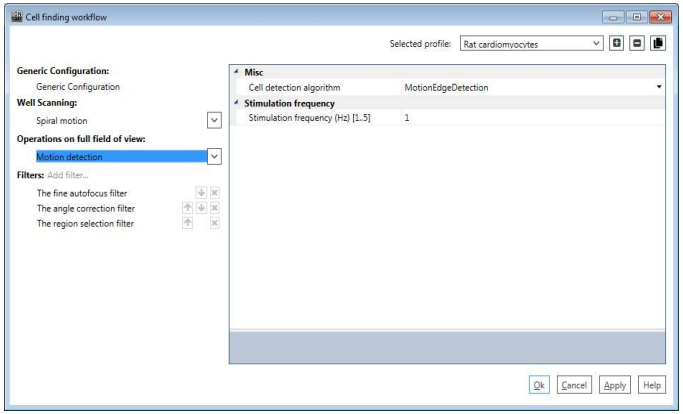
- **Static cell finding** - System detects single cells with a proper size and width/length ratio for each field of view.
  - **Misc**
    - **Cell detection algorithm –** Currently, only 2 options available (EdgeDetection and EnFCMEdgeDetection) which are tested on rat and mouse myocytes. In future, personal algorithms can be developed.

- **Static cell finding with autofocus** - System detects single cells with a proper size and width/length ratio for each field of view and additionally will perform coarse auto focus
  - **Focus**
    - **Coarse Autofocus Range (µm) -** Defines the range that the objective sweeps over in the focal plane.

- **Coarse Autofocus Speed (µm/s) -** It defines the speed of the objective movement in the focal plane..
- **Distance**
  - **Distance limit for Coarse Autofocus (µm) -** It defines a range in focal plane. If it is in the range, cell detection algorithm can find cells. If it is out of the range, cell detection algorithm can not find the cells because it is out of focus. That means we need to perform coarse auto focus to put the objective on focus.
- **Misc**
  - **Cell detection algorithm -** Currently, only 2 options available (EdgeDetection and EnFCMEdgeDetection) which are tested on rat and mouse myocytes. In future, personal algorithms can be developed.

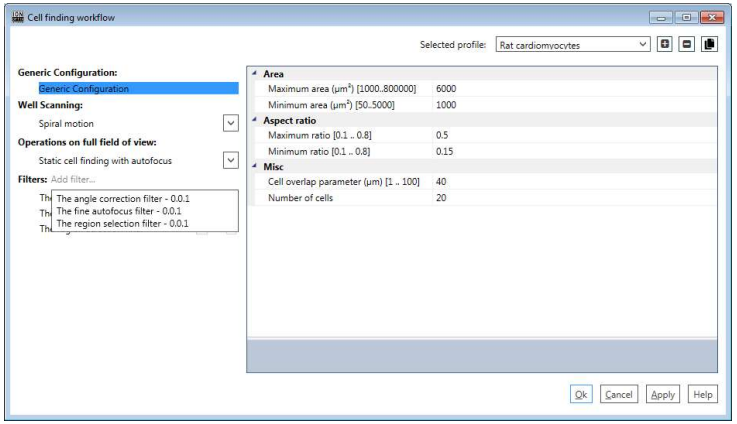


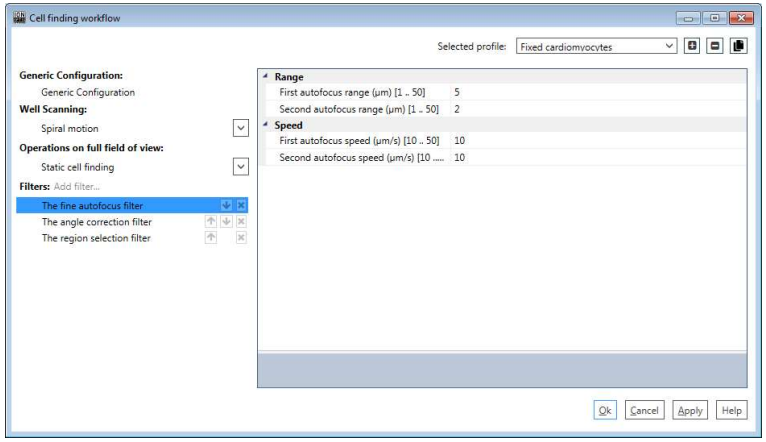
- **Motion detection -** System additionally detects whether the found objects are beating.
  - **Pacing Frequency (Hz) –** Myopacer pacing frequency of the cells.
  - **Misc**
    - **Cell detection algorithm –** Currently, only 2 options available (MotionEdgeDetection and MotionEnFCMEdgeDetection) which are tested on rat and mouse myocytes. In future, personal algorithms can be developed.



4.3.4 Filters

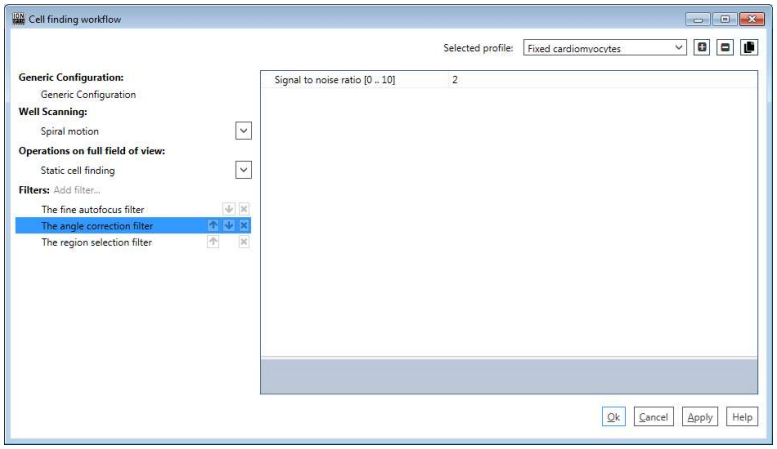
Filters are necessary to optimize cell detection and measurement in each single cell. Filter operate on the cell found and not on the full field of view. You can construct your own pipeline of filters. Currently, three filters are available:





- **Fine autofocus filter -** This filter is used to focus the focal-plane on the sarcomere structure rather than cell edge. It contains two sweeps, checking both the peak and signal to noise ratio in IDFFT frequency space.
  - **First Auto Focus Range (µm) -** It defines the range that the objective sweeps over in the focal plane for the first sweep.
  - **Second Auto Focus Range (µm) -** It defines the range that the objective sweeps over in the focal plane for the second sweep.
  - **First Auto Focus Speed (µm/s) -** It defines the speed of the objective movement in the focal plane for the first sweep.
  - **Second Auto Focus Speed (µm/s) -** It defines the speed of the objective movement in the focal plane for the second sweep.
- **Angle correction filter -** This filter corrects the orientation of the sarcomere structure if there is a mismatch between the orientation of the cell and the sarcomere structure.
  - **Signal to noise ratio –** It additionally checks the Signal to Noise ratio of the sarcomere FFT signal. High SNR selects only cells with a good sarcomere signal.
- **Region selection filter -** This filter selects the best region of sarcomere structure in the cell based on SNR.

Add filters by choosing the name in the drop down menu. Delete filters by pressing `[x]` next to the filter name. Move filters up and down in the list by pressing the arrows.



Tip:

Our suggested order of filters are:

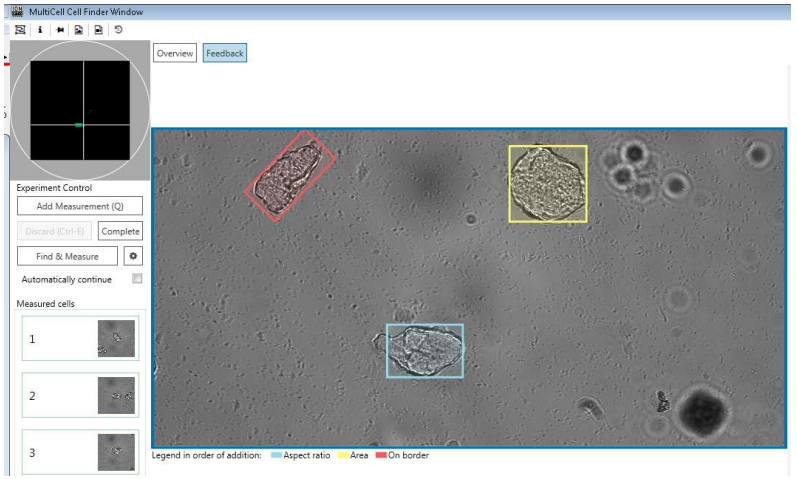
1. The fine autofocus filter
2. The angle correction filter
3. The region selection filter

Press `[OK]` to save the settings and close the window.

4.3.5 Cell finding feedback window

To see the result of the chosen cell finding configuration choose the `[feedback]` window instead of the overview 3x3 window.

Press `[Find and Measure]` to activate the feedback window. This window will indicate which cells have been rejected by the configuration and why. Accepted and measured cells are not labelled.

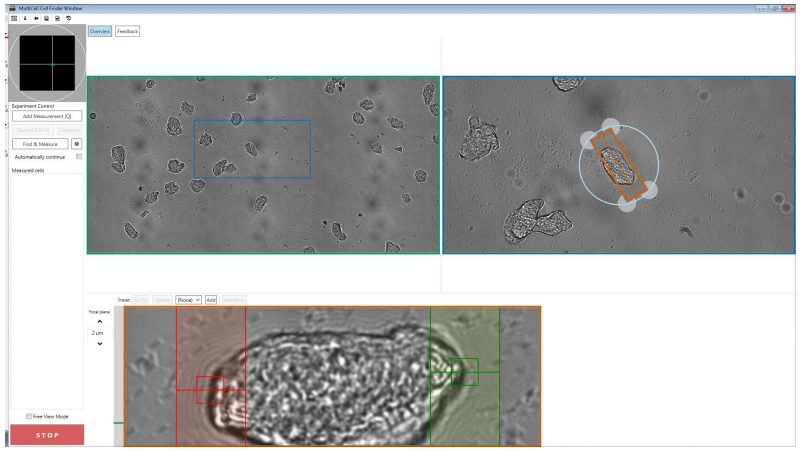


Legend:

- Aspect ratio
- Area
- Cell on border
- Two cells
- Cell not beating

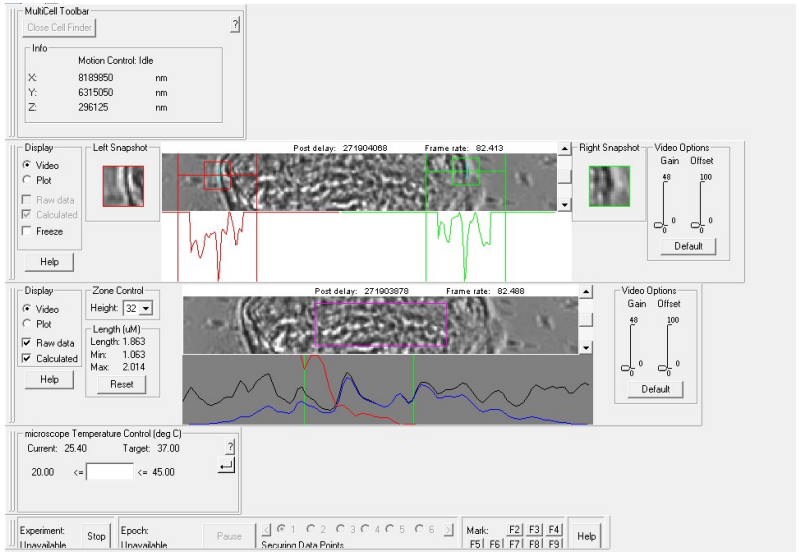
4.3.6 Edge box measurements using MultiCell

The cell length recording task analyzes video images to find the left and right edges of an object. The task produces two raw data traces: left edge position and right edge position, relative to the left side of the image, in pixels. In addition to the raw data display, the task provides the ability to view the data in calibrated units using a user-supplied scaling factor and to display the difference between the edges, which is the cell length. These measurements can be combined with sarcomere length measurements, but also works on its own.



4.3.6.1 Automatic measurements with edge detection

1. Start by opening the settings window to find cells by clicking ⚙ button.
2. Adjust **General configuration, Well Scanning** and **Operations of full field of view** as indicated in ***section 4.3***
3. Remove the angle correction filter and region selection filter by pressing the [x] if you don't combine this measurement with sarcomere length.
4. Add the edge detection filter

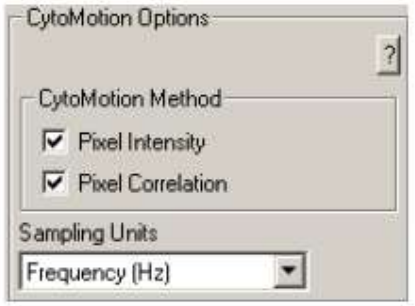


4.3.6.2 Manual edge detection measurements

1. Find cell and adjust rotation
2. Drag the left box (red) and right box (green) to the edge of the cell
3. Check signal of edge in IonWizard, sharp negative peak → adjust box locations if necessary
4. Press `[add measurement]`

4.3.7 CytoMotion measurements using MultiCell

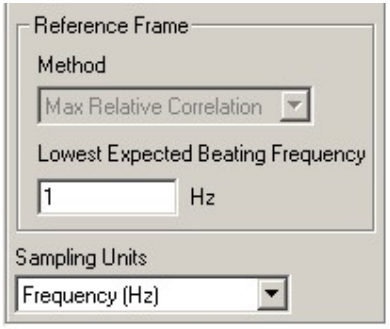
The CytoMotion recording task can be used to measure contractility in for example Induced Pluripotent Stem Cell derived Cardiomyocytes (IPSCs). Initially a reference frame (image) is captured while the myocyte is at rest, then changes in pixel intensity for each frame are compared to the reference for each subsequent image.



4.3.7.1 Setting up the task:

There are 2 options:

- Pixel Intensity: Average of the difference between all pixels of the captured reference frame against the current live image
- Pixel Correlation: Average of the correlation of each pixel between the captured reference frame against the current live image.



Reference frame:

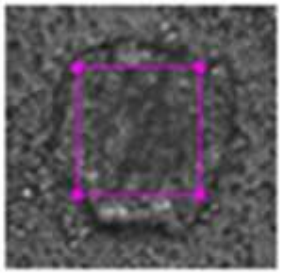
In MultiCell the reference frame is automatically captured. For automatic detection of the reference frame at diastole you can adjust the **lowest expected beating frequency** in the Experiment Manager. This will determine the time it takes to find the reference frame. E.g. for an expected beat frequency of 1Hz this will take ~1seconds, for an expected beat frequency of 0.2Hz this will take ~5 seconds.

Region of interest (ROI) size:

The size of the region in which CytoMotion is measured can be adjusted depending on magnification, type of construct and type of experiment.

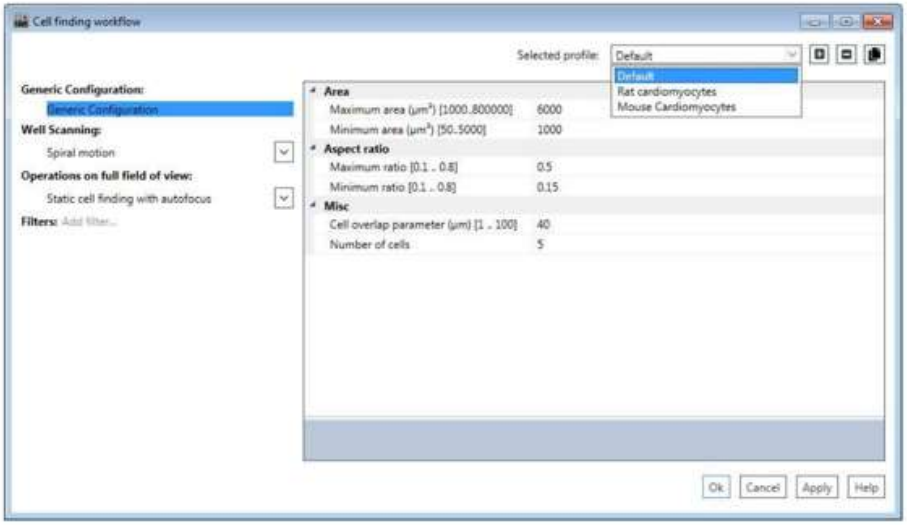
Adjusting ROI with MultiCell:

- Adjust image rotation size in Experiment Manager. Maximum is 500 x 500.



Additionally you can reduce the ROI in IonWizard window at the start of an experiment by dragging the purple/pink box to the desired size

4.3.7.2 Data acquisition with CytoMotion task



Manual measurements: Adjust focus manually. Navigate to a desired location. Press [Add Measurement]. First the reference frame will be determined, this can take 1 -5 sec depending on the set expected beating frequency.

Automated measurements: Beta-version, center of contraction detection. Open Cell finding window by pressing  in the MultiCell window. It is advised to create a separate IPSC profile.

Generic configuration:

- Max area: 5 000
- Min area: 1000
- Max ratio: 0.95
- Min ratio: 0.05
- Cell overlap parameter: 100
- Number of cells: Adjust to the number of locations you want to measure in one well.

Well scanning:

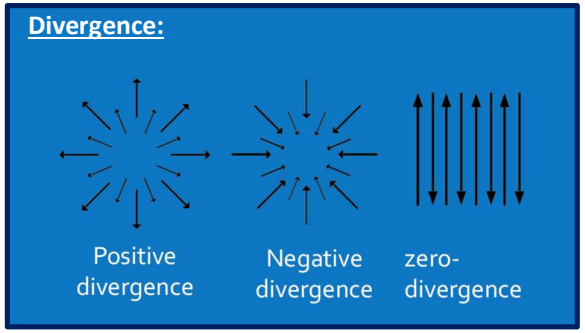
- Spiral motion

Operations on full field of view

- Stem Cell Contraction Detection
  - Binary mask threshold: 180 (absolute divergence threshold)

Filters:

Remove all filters



4.4 Restore cell list

To restore the cell list after an experiment was ended untimely or when the multicell window got closed:

- Open new file in IonWizard
- Open MultiCell window
- Press Start in IonWizard
- Press  (top of the multicell window)
- A list of the cells will appear, but without thumbnails. You can go through the list in “Free view mode”. Thumbnails will be created again when you start measuring.

DO NOT ADD MEASUREMENTS BEFORE YOU HAVE IIMPORTED THE LIST. AS SOON AS YOU START A NEW MEASUREMENT THE PREVIOUS LIST IS CLEARED

For consideration:

- Measurements will continue starting in the run in which the experiment was stopped (starting from cell 1). I.e. if the experiment stopped in the 3rd run from a total of 5 runs. You will be able to measure another 3 runs (3, 4 and 5).
- If the experiment was stopped in the first run (i.e. finding cells). You can still add cells to the list manually and automatically and then continue as usual.
- If you want to use the same list of cells after an experiment was completed you need to make changes to the experiment manager. Increase the number of runs above the number of runs in which these cells were already used.
    - Examples:
        - Your first experiment consisted of only 1 run and the multicell window closed after 1 run. For your next experiment: change experiment manager to consist of at

least 2 runs or more as needed. Then start experiment in IonWizard and import the cell list.

- Your first experiment consisted of 4 runs. Multicell closed at the end of the 4th run. Change experiment manager to have at least 5 runs or more. Then start experiment in IonWizard and import the cell list.