STEM CELL LABORATORY (STCL)

DOCUMENT NUMBER: STCL-EQUIP-022 JA2

DOCUMENT TITLE:

Cellometer Program Used for Thawed MSC Products JA2

DOCUMENT NOTES:

Document Information

Revision: 01	**Vault:** STCL-Equipment-rel
Status: Release	**Document Type:** STCL

Date Information

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Effective Date: 04 Mar 2021	**Expiration Date:**

Control Information

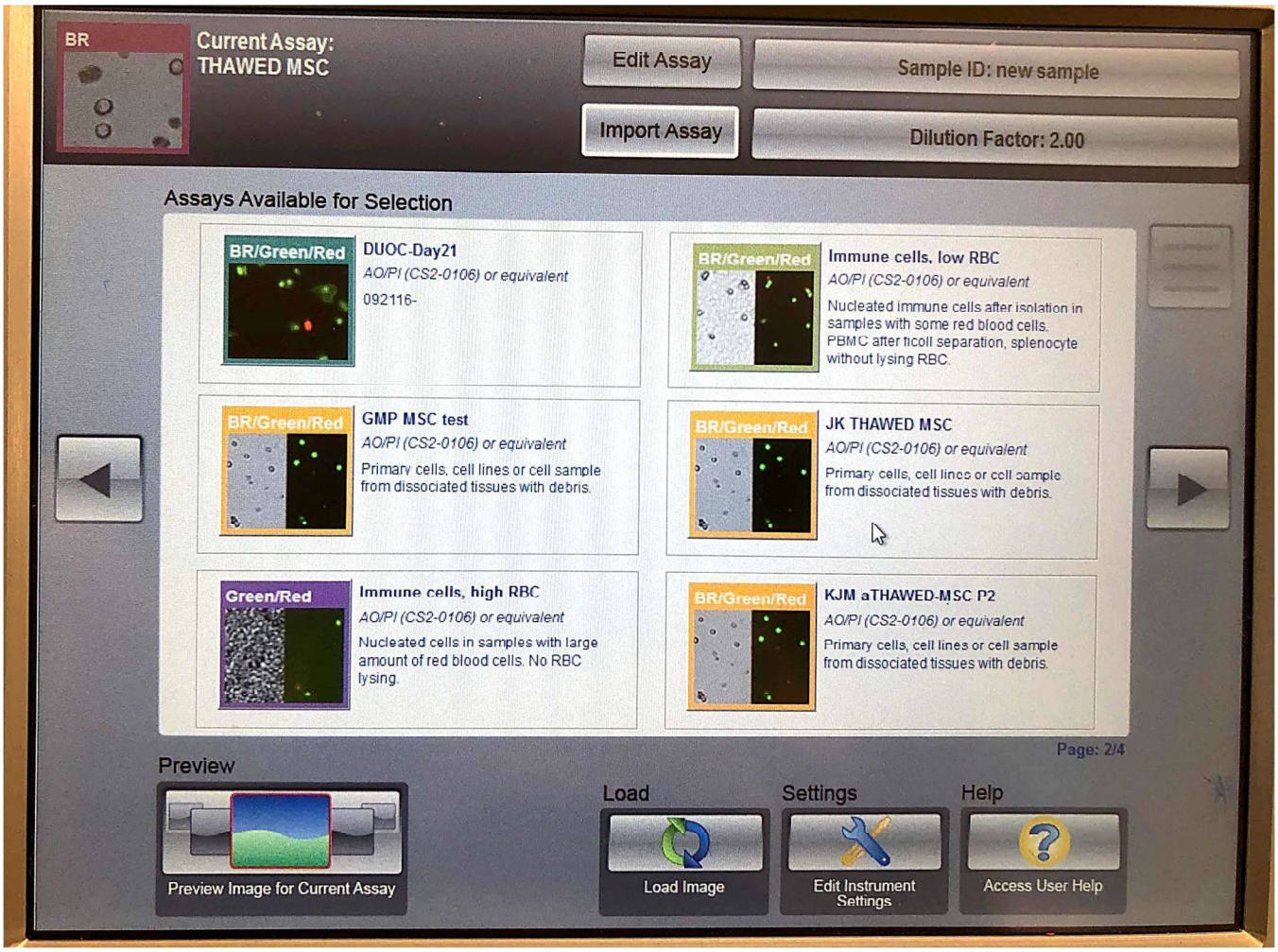
Author: WATER002	**Owner:** WATER002
Previous Number: None	**Change Number:** STCL-CCR-511

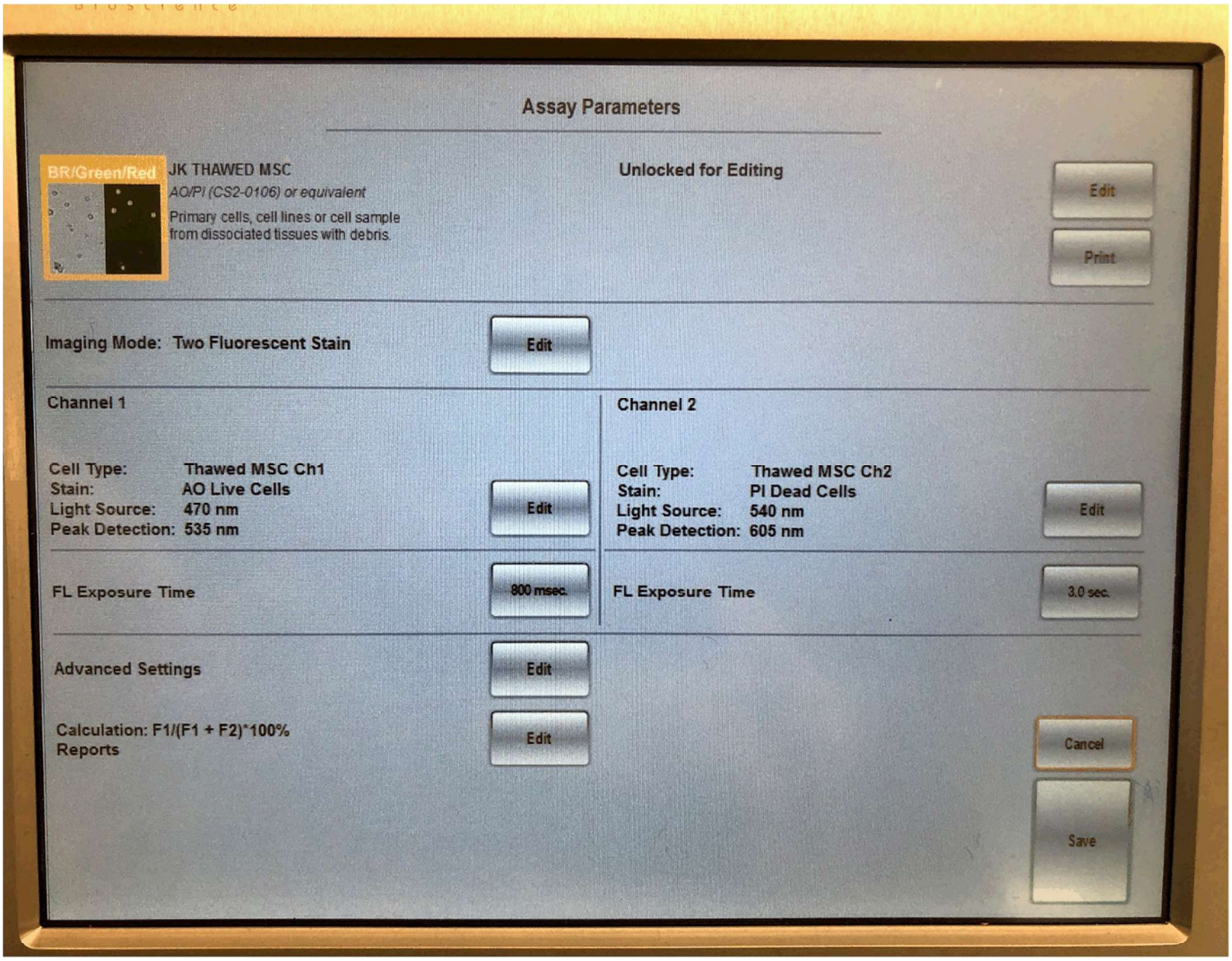
STCL-EQUIP-022 (JA2) Cellometer Program Used for Thawed MSC Products

NOTE: The Cell Type most recently used for Channel 1 was Assay 2 Immune Cells Live and Cell Type most recently used for Channel 2 was Assay 2 Immune Cells Dead. These Cell Types may be used as a starting template for future assay updates; however, parameters within these Cell Types have been modified for both Channel 1 and Channel 2 as summarized below.

Checking the Assay settings: If Cell Type is locked, the settings can only be viewed. Minus a few images, this job aid show images for which **JK Thawed MSC** as an unlocked assay with unlocked Cell Types for the purpose of defining the assay parameters but also includes instructions for viewing/modifying settings in a locked Assay with locked Cell Types.

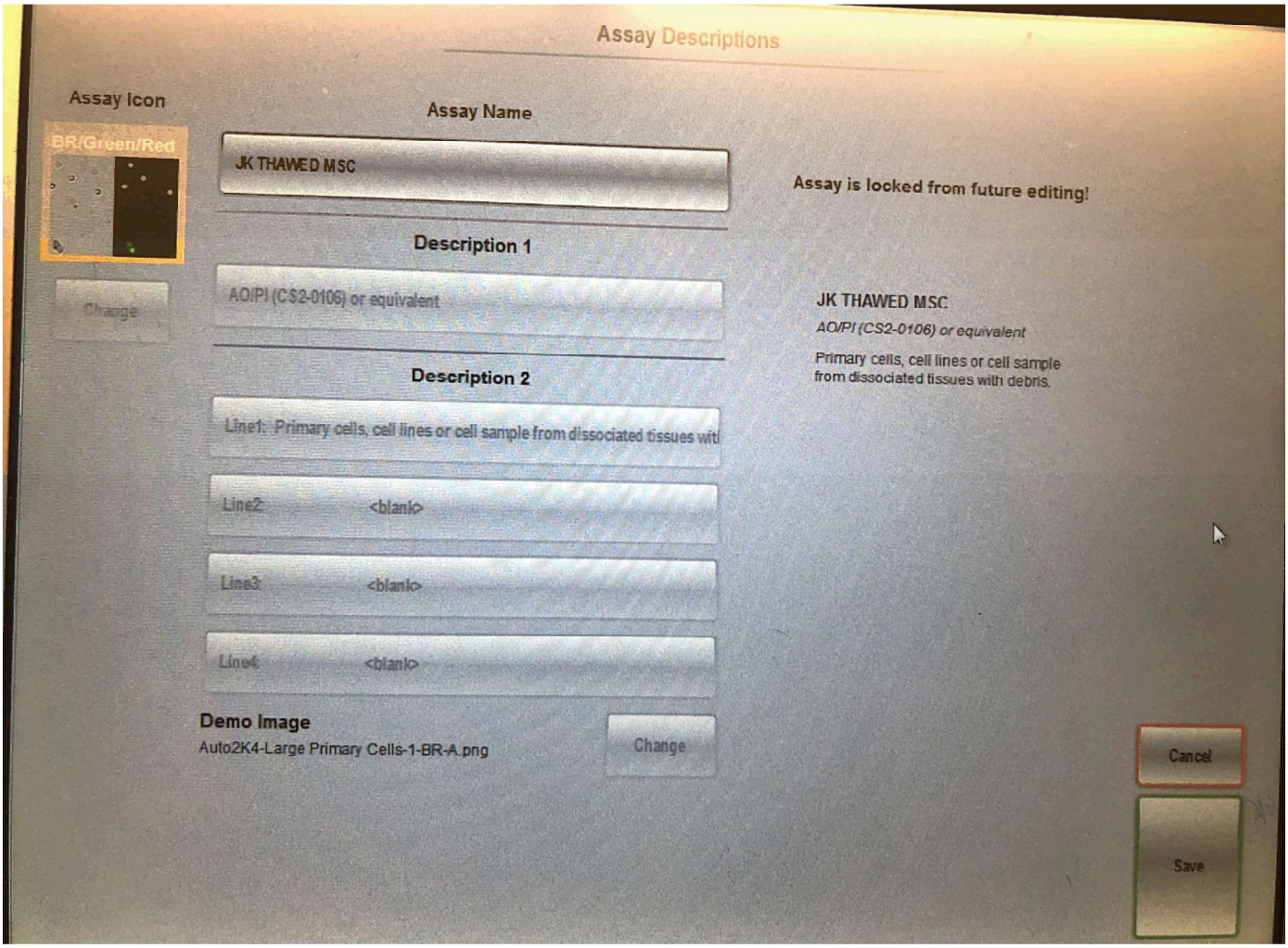
Highlight the correct assay, then click on "Edit Assay"





The user may click on Print to print a summary of the settings. See the attached settings at the end of this Job Aid for the printed summary of **JK Thawed MSC** assay settings.

To modify a locked assay, it must be renamed. If an assay is locked, click on Edit next to "Locked for editing"



Assay Descriptions - Renaming a new copy to update an assay that is locked.

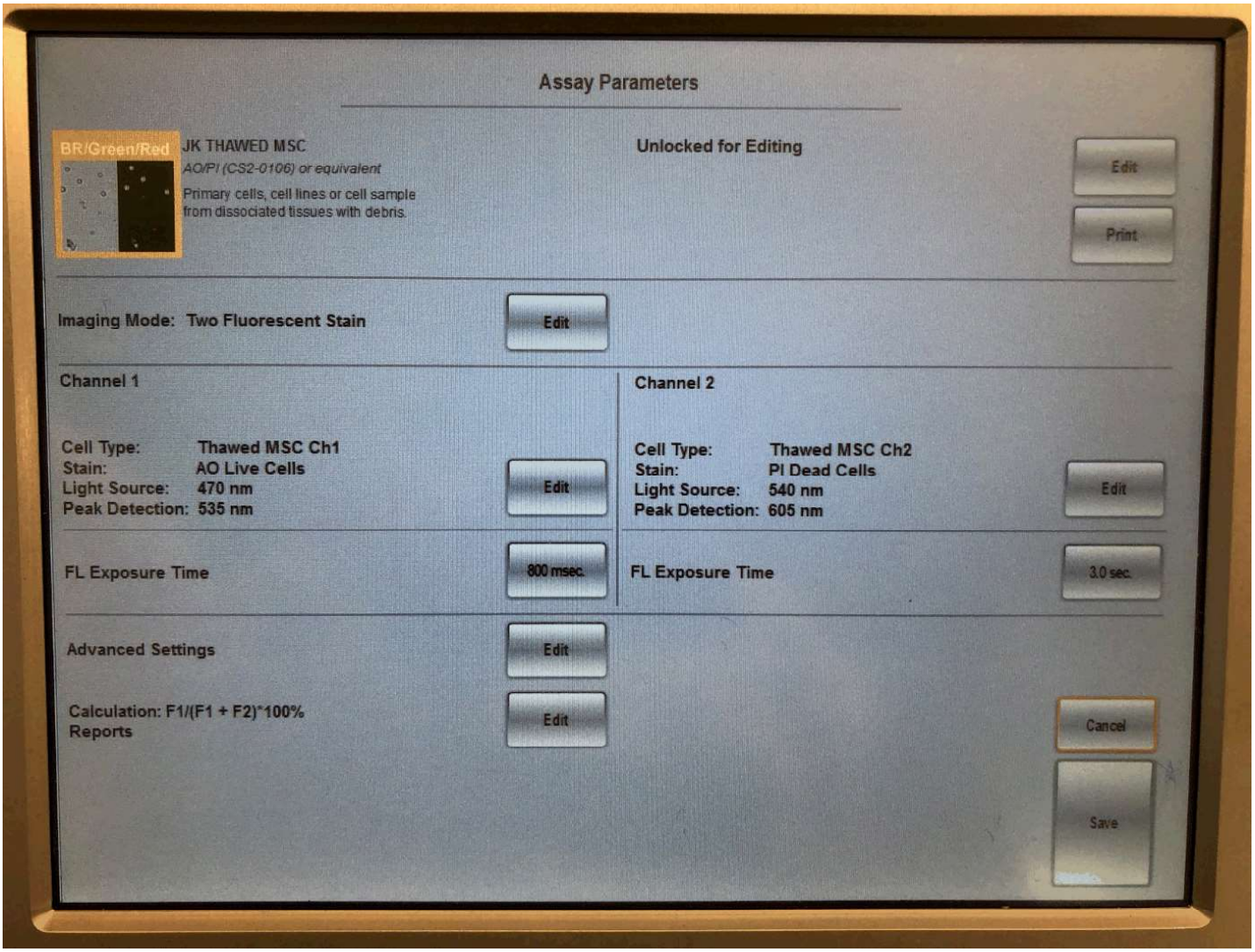
Select the assay title located under Assay Name, and change the name of the assay.

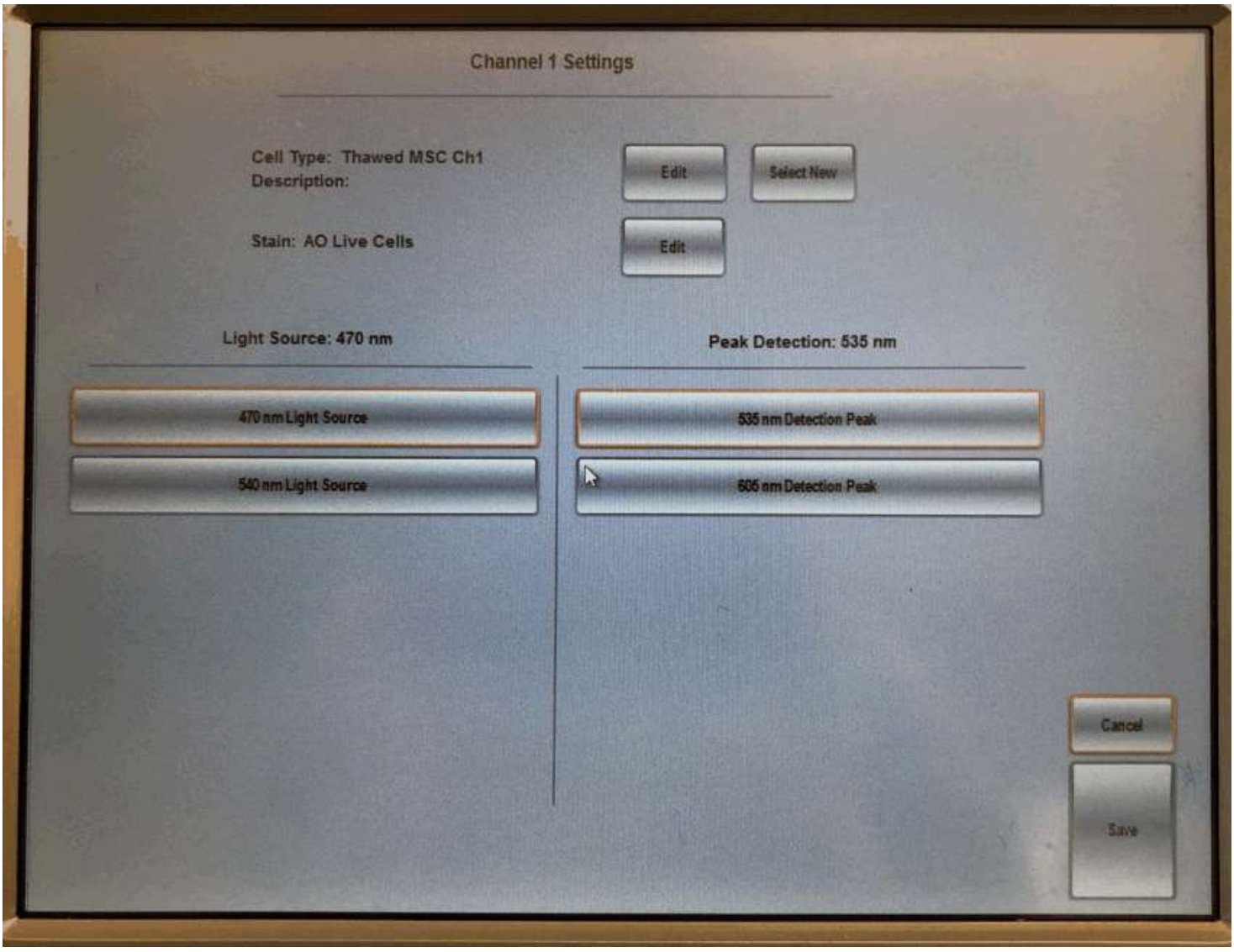
The prompt to “KEEP” or “CHANGE the cell type name to match the new assay name will appear if the assay title has been changed.

When prompted, click on “**CHANGE**” so that any changes made to Cell Type won’t affect other assays that share the same Cell Type. Slide the **LOCK** to off so that the Assay can be revised and click on **SAVE**.

Under Channel 1, Cell Type click on “Edit”

NOTE: For this example, Cell Type is unlocked for editing and may not be viewed when Cell Type is locked.



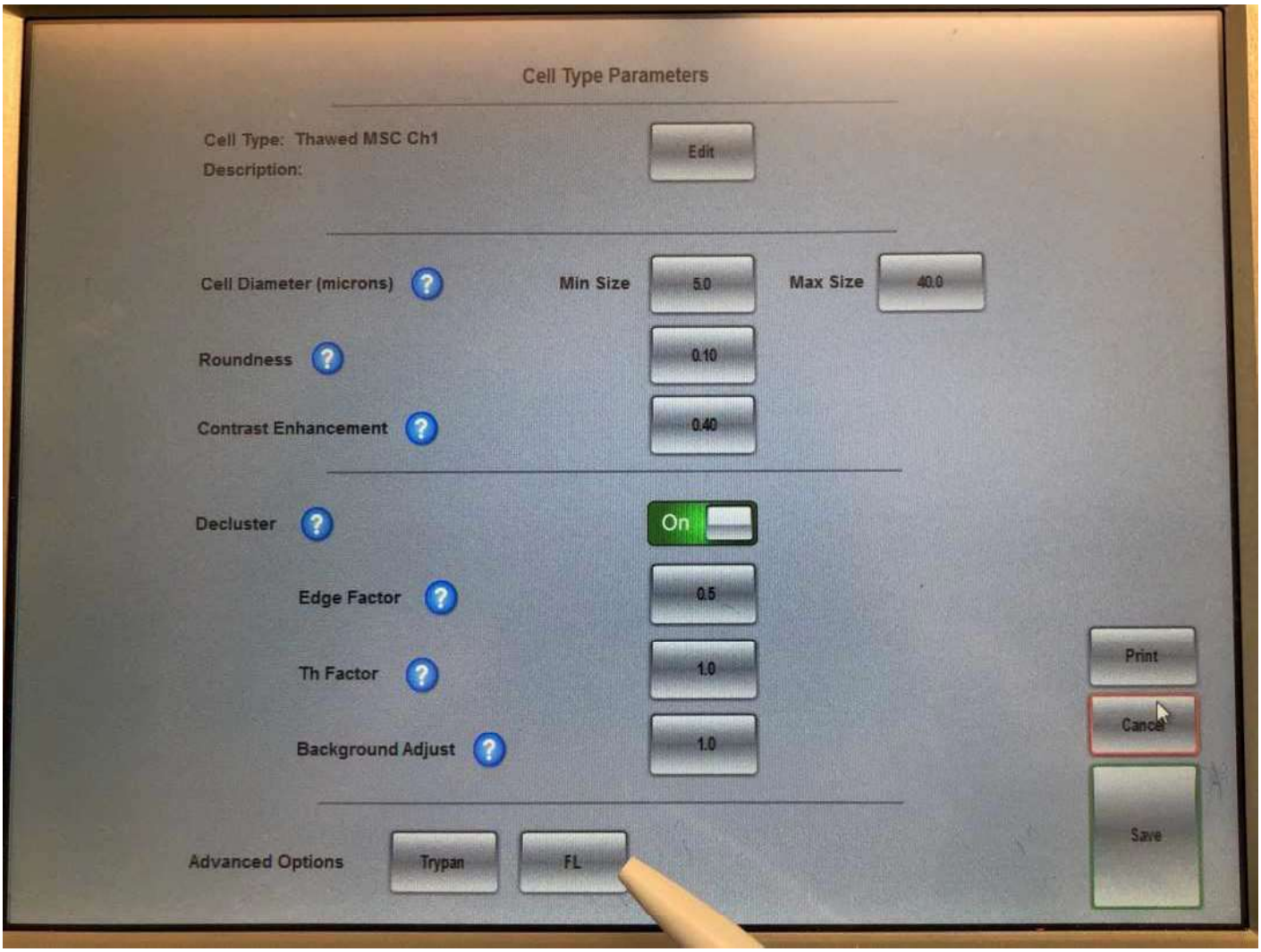


Channel 1 Settings

Confirm the Light Source and Peak Detection settings in this screen match what is pictured above and that the correct Cell Type is listed (*for this assay:* ***Thawed MSC Ch1***). Verify the **STAIN** is set to AO Live Cells.

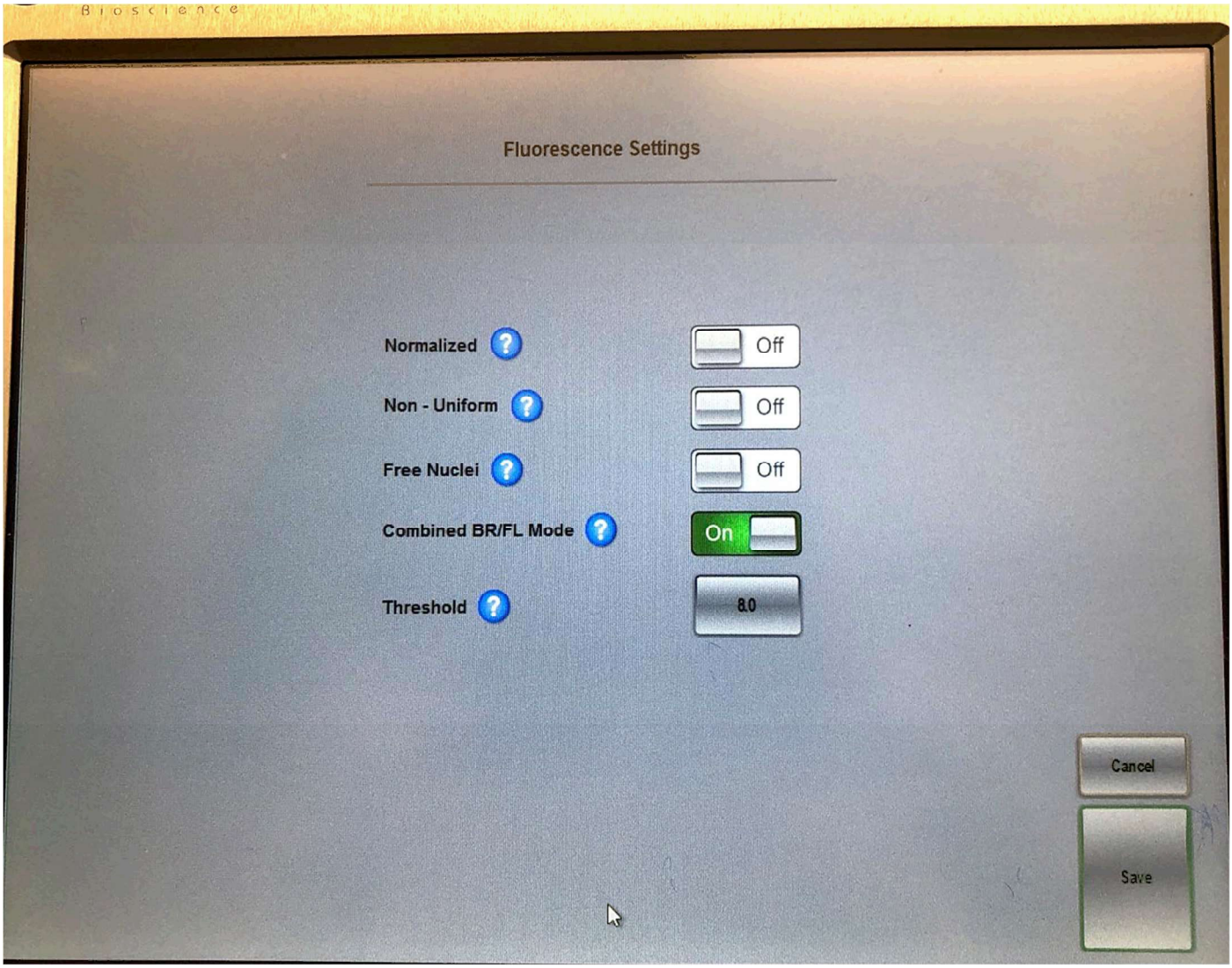
If modifying an assay that has locked Cell Type, click on **SELECT NEW** to choose an unlocked Cell Type to work from. Highlight the desired Cell Type, rename the Cell Type to avoid making inadvertent changes to assays that share the same Cell Type, and click **DONE**

To the Right of Cell Type, **Thawed MSC Ch1**, click on Edit to view Cell Type parameters (this feature will only work when Cell Type is unlocked)



Channel 1 Settings – Cell Type Parameters

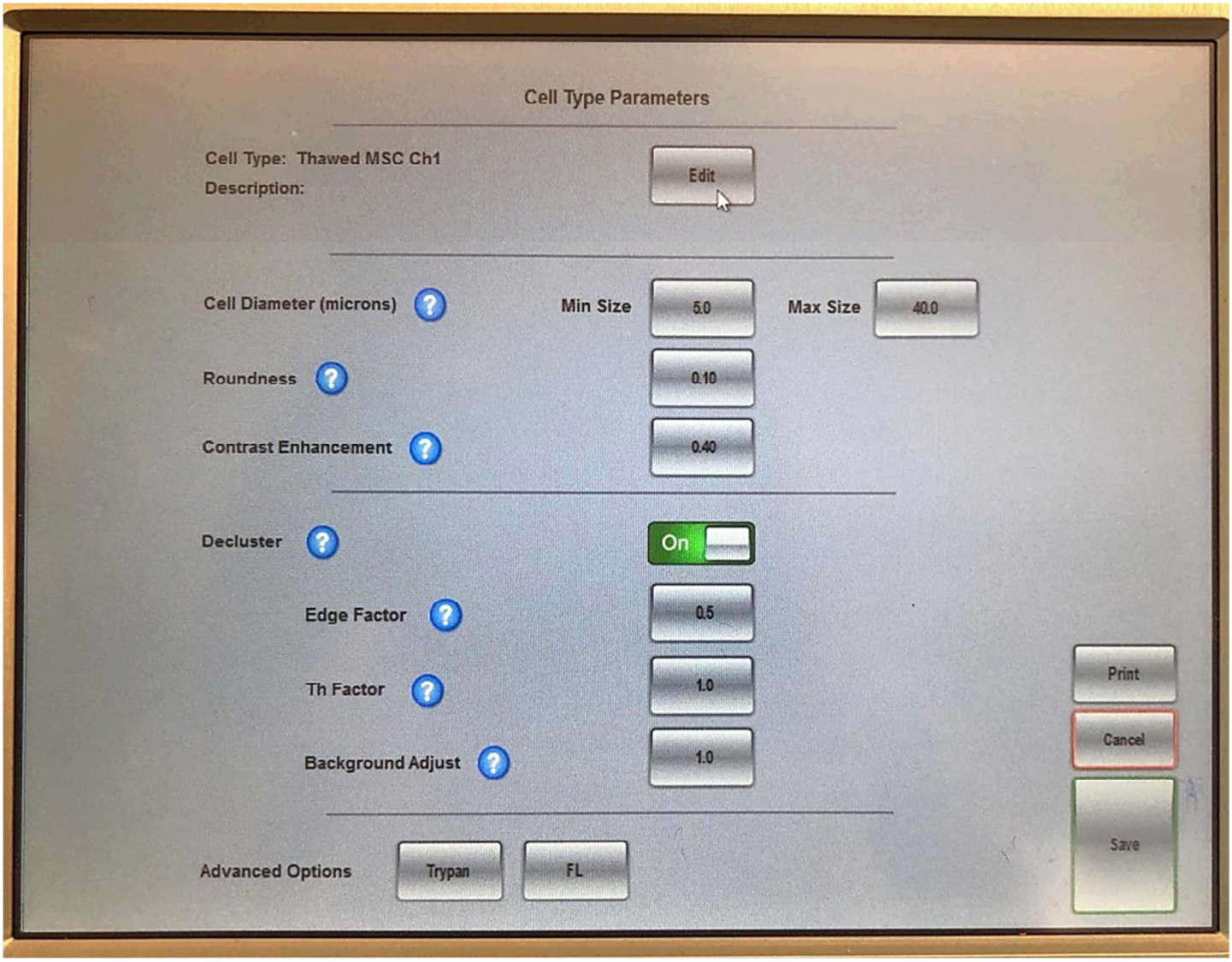
Click on **Advanced Options** for "**FL**"



Channel 1 Settings –Fluorescence Settings

Confirm the settings match those listed above:

- **Threshold** should equal 8.0 **for Channel 1**
- **Combined BR/FL Mode** is ON
- Click on SAVE to save changes or CANCEL if no changes were made.



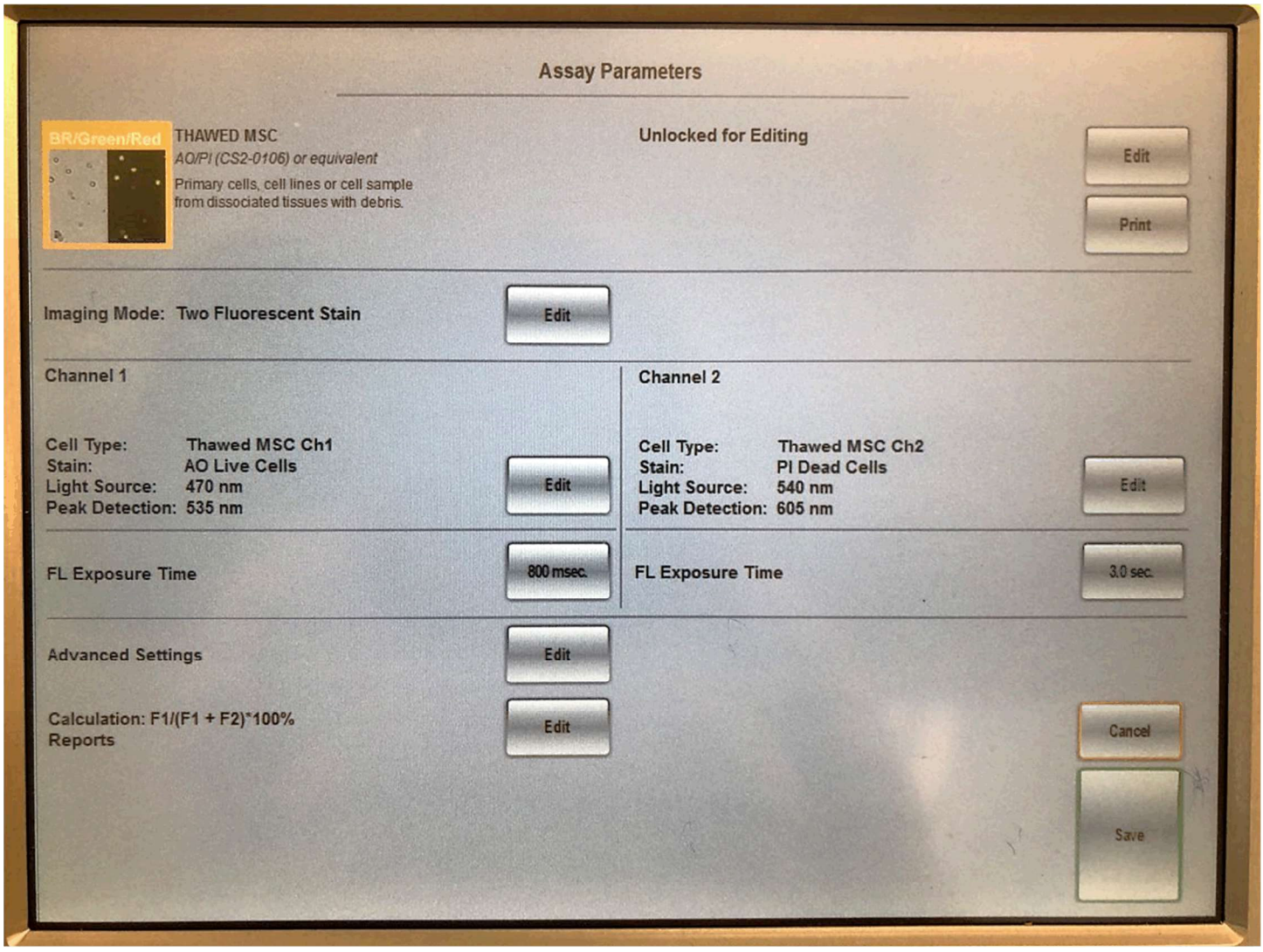
Channel 1 Settings – Cell Type Parameters

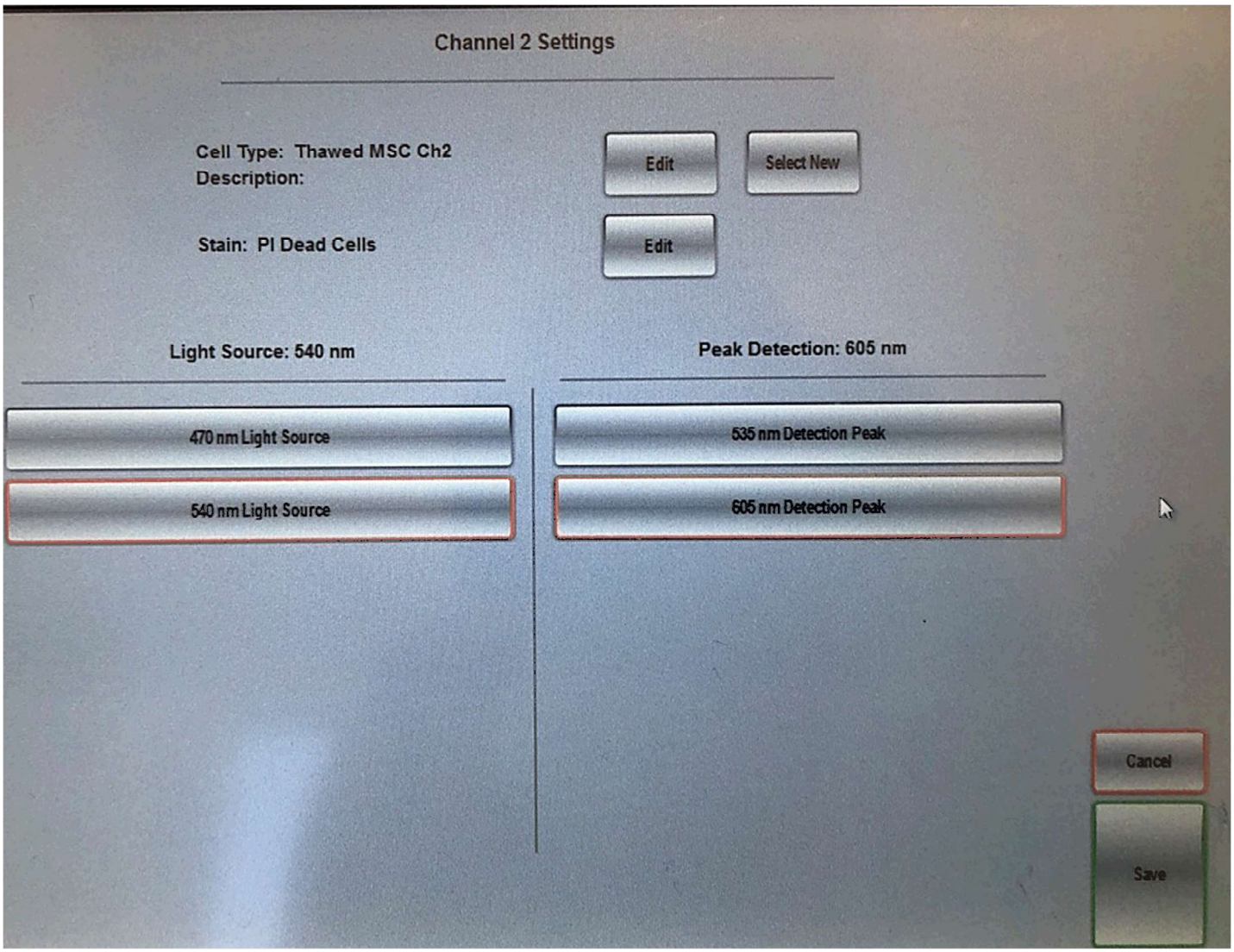
Confirm the settings match those pictured above. **Minimum cell diameter**: 5.0 and **Max Cell Diameter**: 40.0.

If modifying an unlocked Cell Type, it is recommended to change the cell type name in this window by clicking on **EDIT** to the right of Cell Type and enter a new Cell Type Name below Cell Name. To Lock the Cell type, in the same edit Cell Type window, slide the option to "**Lock Cell Type from Future Editing**" to ON. Once prompted, click on **LOCK**. Click on **SAVE**. This step will need to be completed for both Channel 1 and Channel 2 settings if locking Cell Type.

Click on Save if any changes were made, or cancel if no changes were made.

Underneath **Channel 2**, click on "**Edit**" to the right of **Cell Type**.



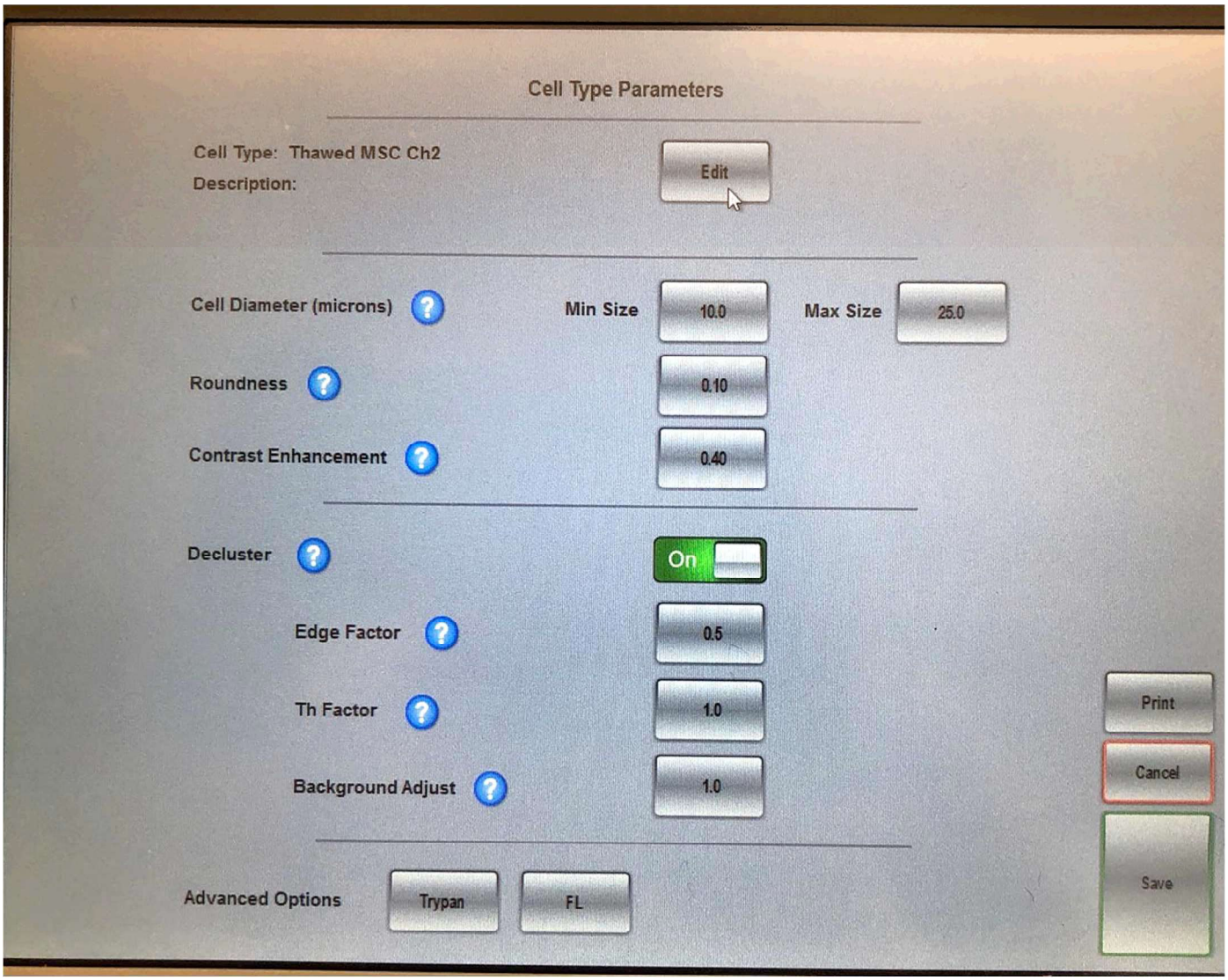


Channel 2 Settings

Confirm the Light Source and Peak Detection settings in this screen match what is pictured above and that the correct Cell Type is listed (for this assay: Thawed MSC Ch2). Verify the **STAIN** is set to PI Dead Cells.

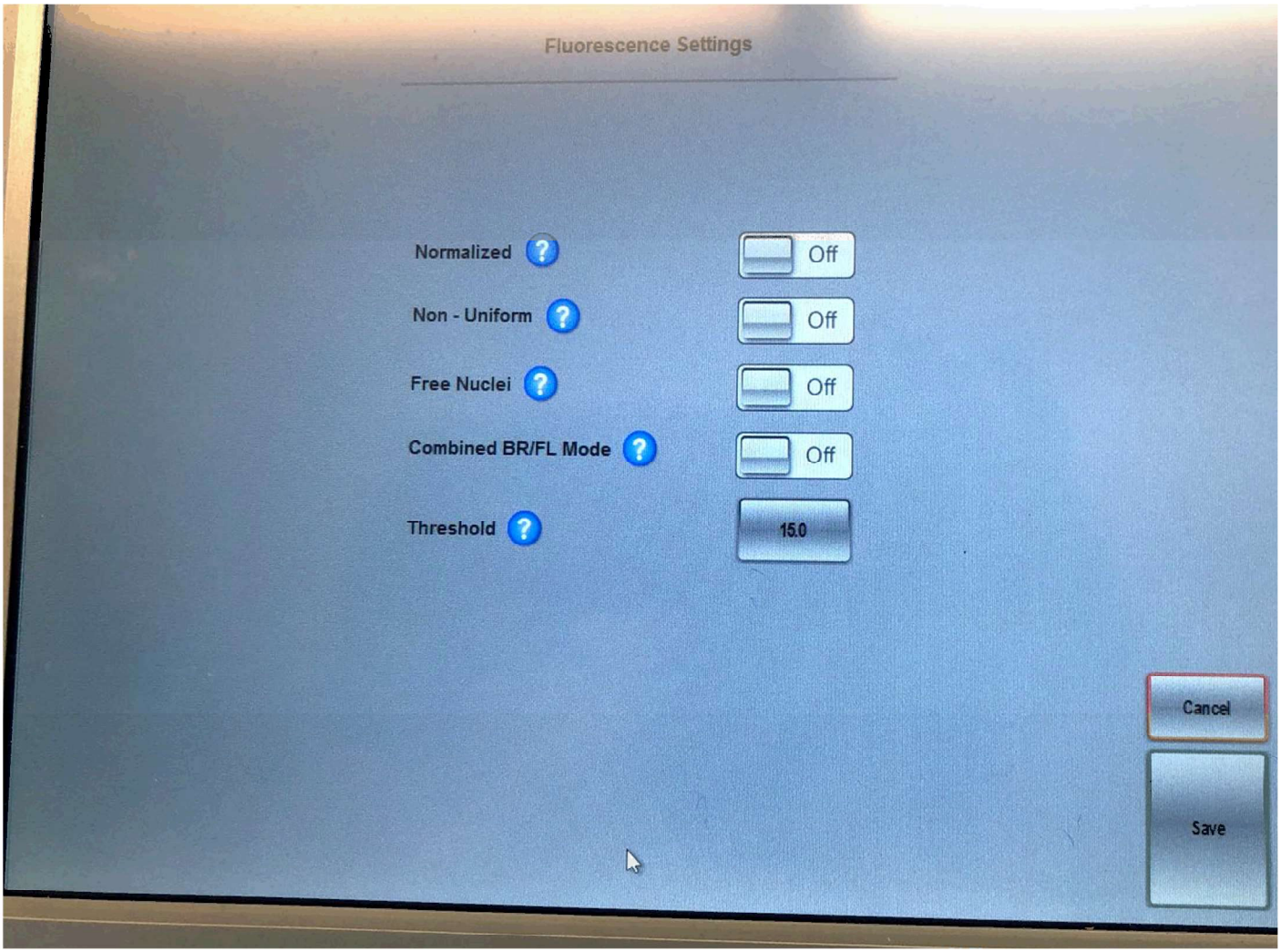
If modifying an assay that has locked Cell Type, click on **SELECT NEW** to choose an unlocked Cell Type to work from. Highlight the desired Cell Type, rename the Cell Type to avoid making inadvertent changes to assays that share the same Cell Type, and click **DONE**

To the Right of Cell Type, **Thawed MSC Ch2**, click on Edit to view Cell Type parameters (*this feature will only work when Cell Type is unlocked*)



Channel 2 Settings – Cell Type Parameters

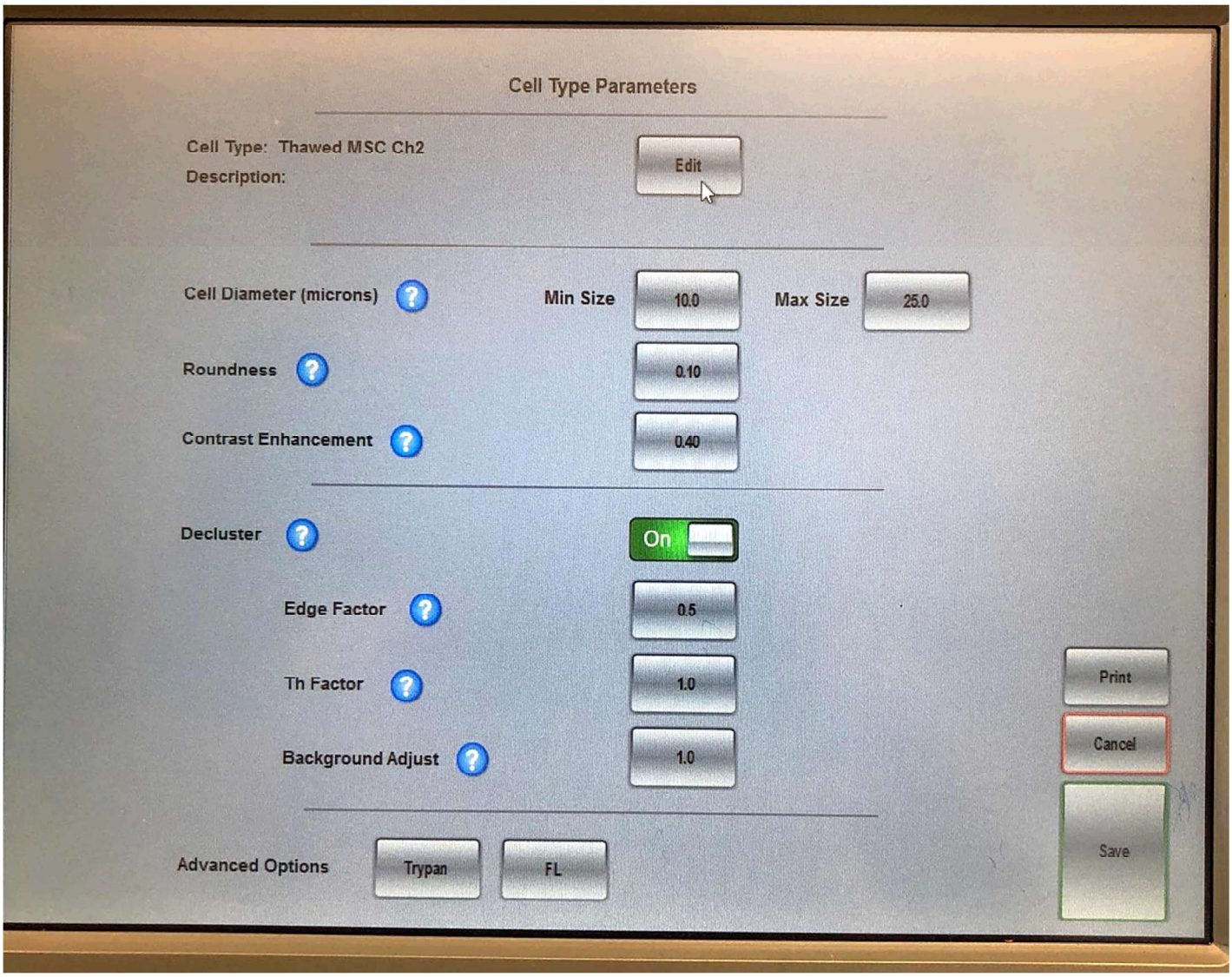
Click on "**FL**" to the right of **Advanced Options**.



Channel 2 Settings – Fluorescence Settings

Confirm the settings match those above. **Threshold** should equal 15.0 for Channel 2, and **Combined BR/FL Mode** is OFF.

Click on SAVE if changes were made or CANCEL.



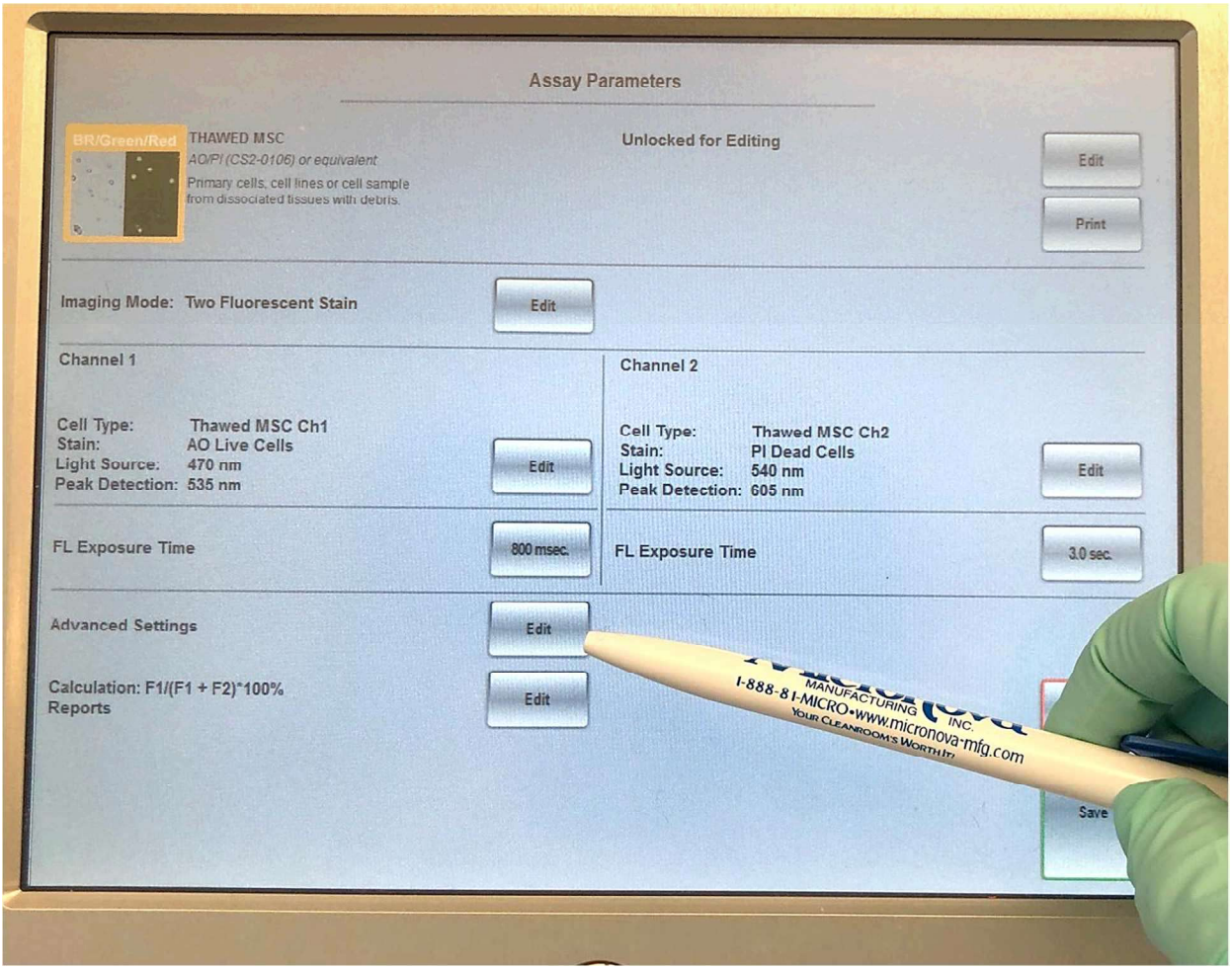
Channel 2 Settings – Cell Type Parameters

Confirm the settings match those pictured above and that the correct Cell Type is listed (for this assay: Thawed MSC Ch2)

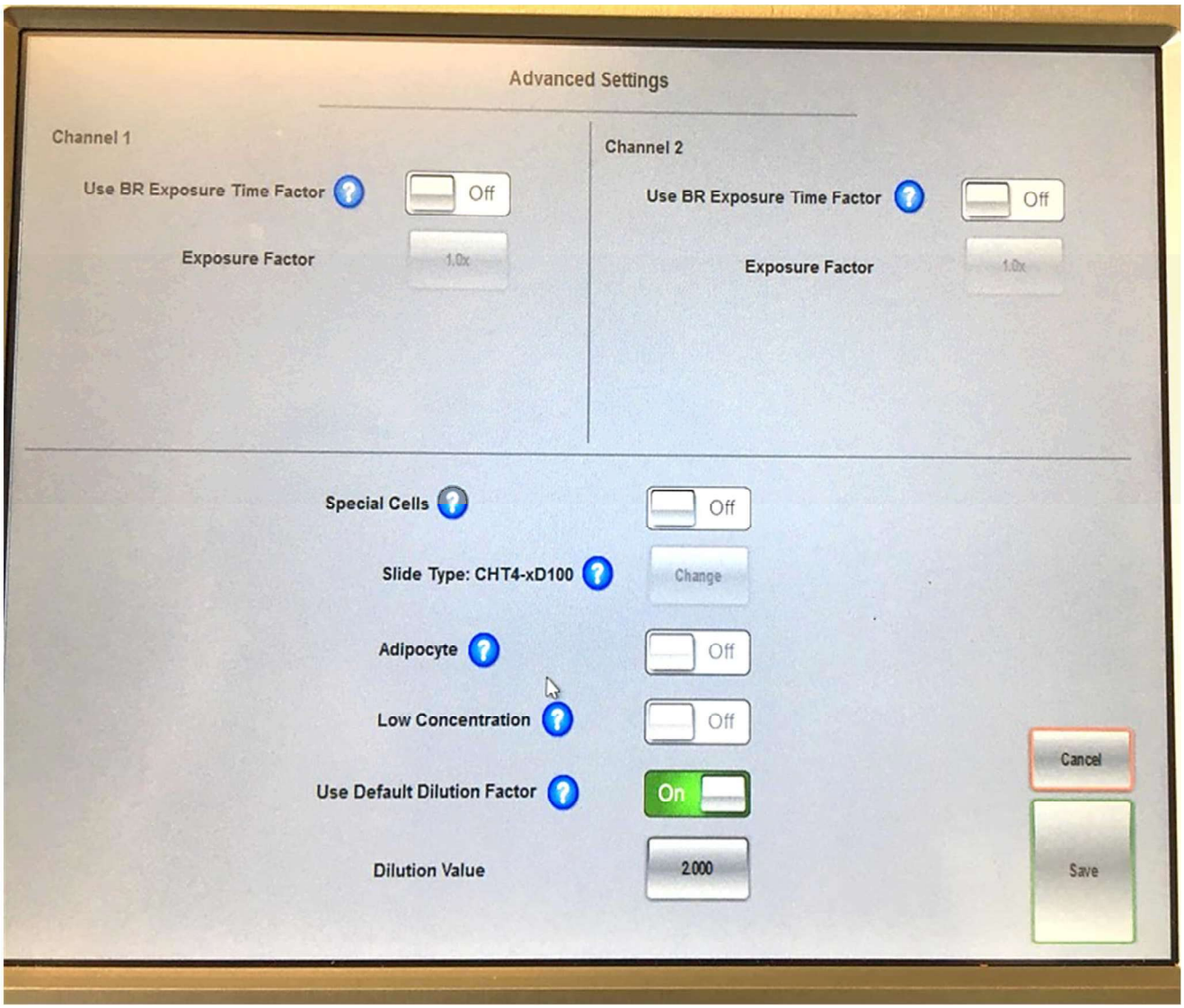
Min Cell Diameter should equal 10.0 and **Max Cell Diameter** should equal 25.0

If modifying an unlocked Cell Type, it is recommended to change the cell type name in this window by clicking on **Edit** to the right of Cell Type and enter a new Cell Type Name below Cell Name.

To Lock the Cell type, in the same edit Cell Type window, slide the option to "Lock Cell Type from Future Editing" to ON. Once prompted, click on **LOCK**. Click on **SAVE**. This step will need to be completed for both Channel 1 and Channel 2 settings.



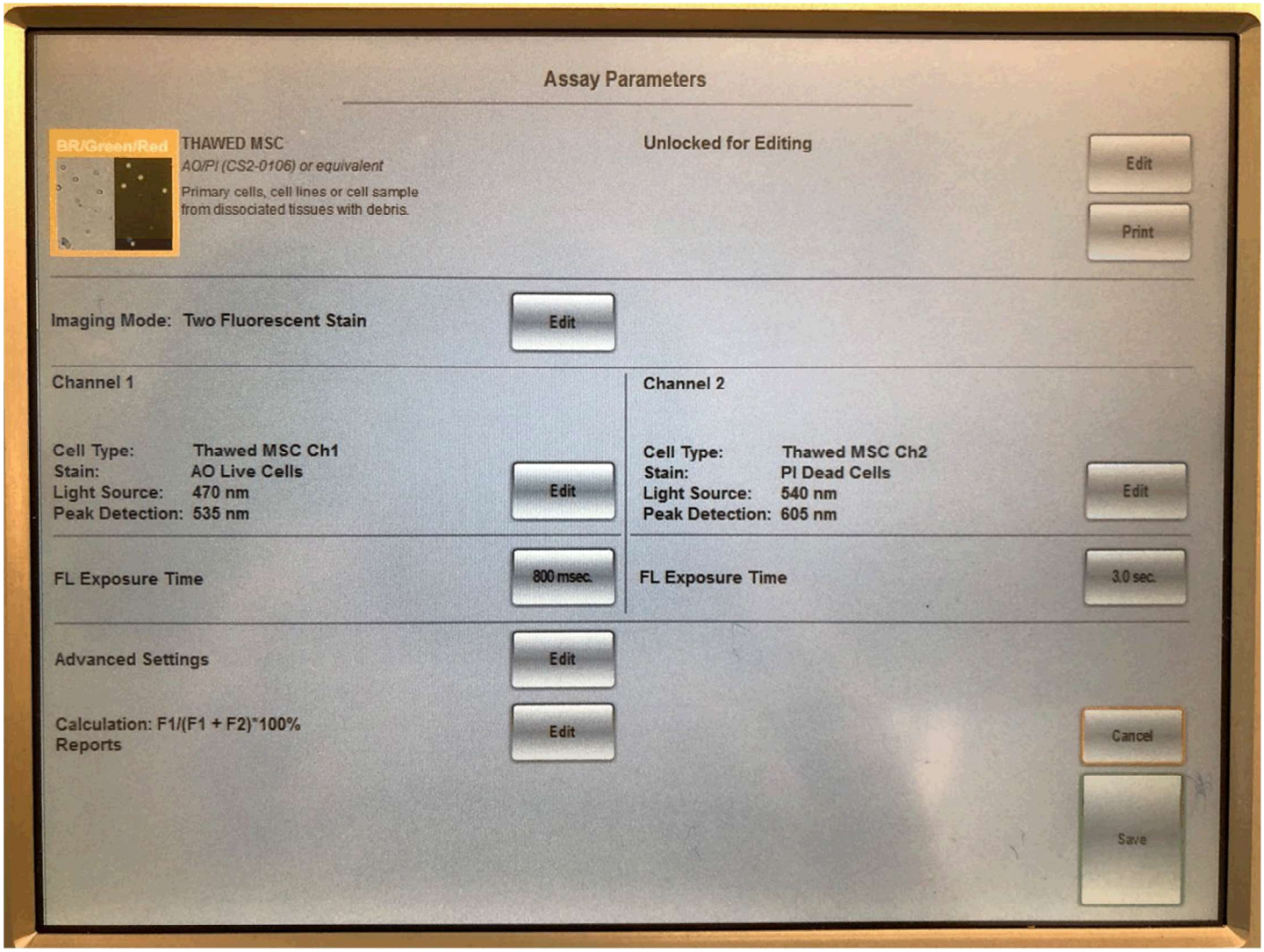
Click on **Advanced Settings**

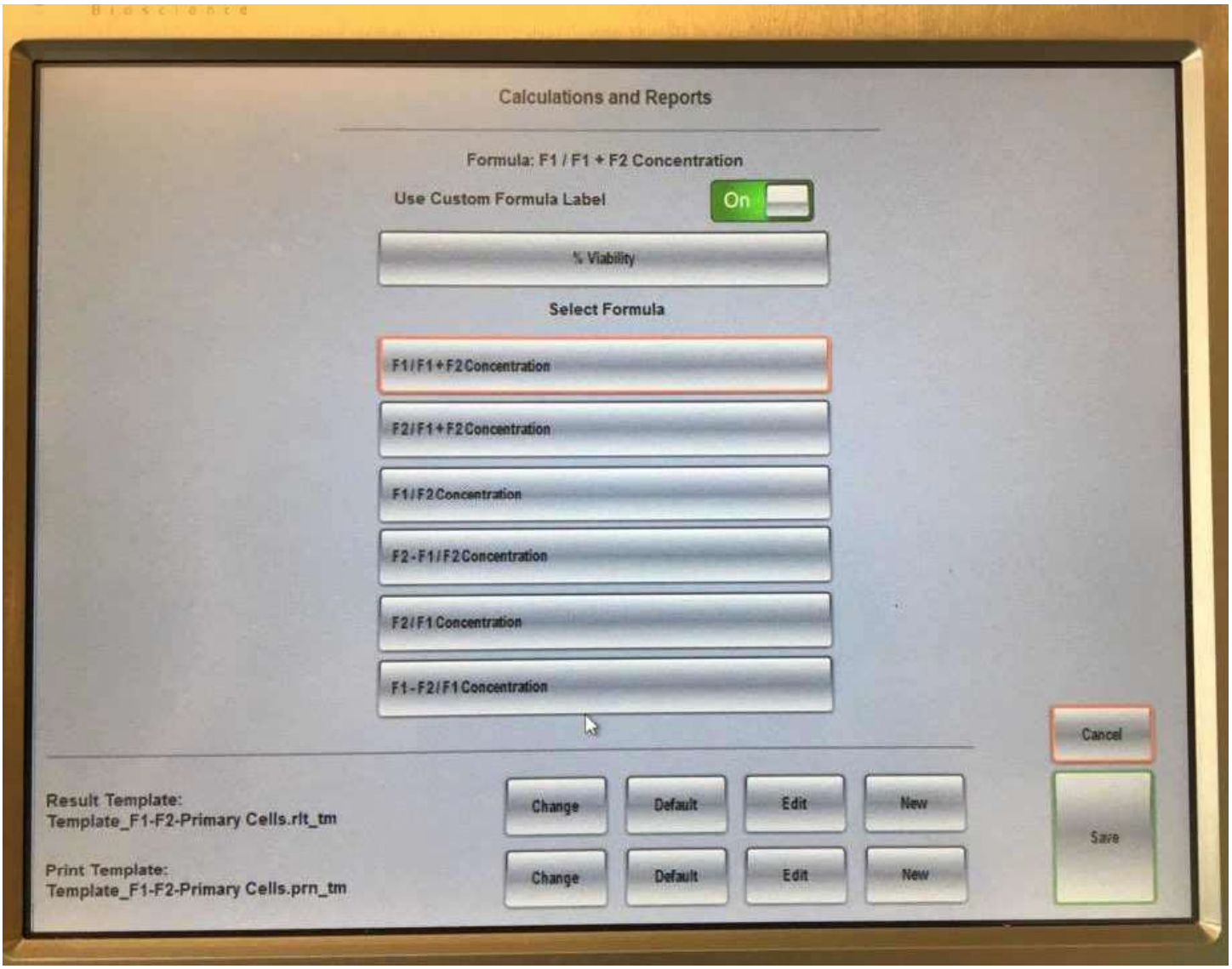


Advanced Settings

Confirm the settings listed match those pictured above. Click CANCEL or SAVE if changes are made.

Next to **Calculations**, click on "EDIT"

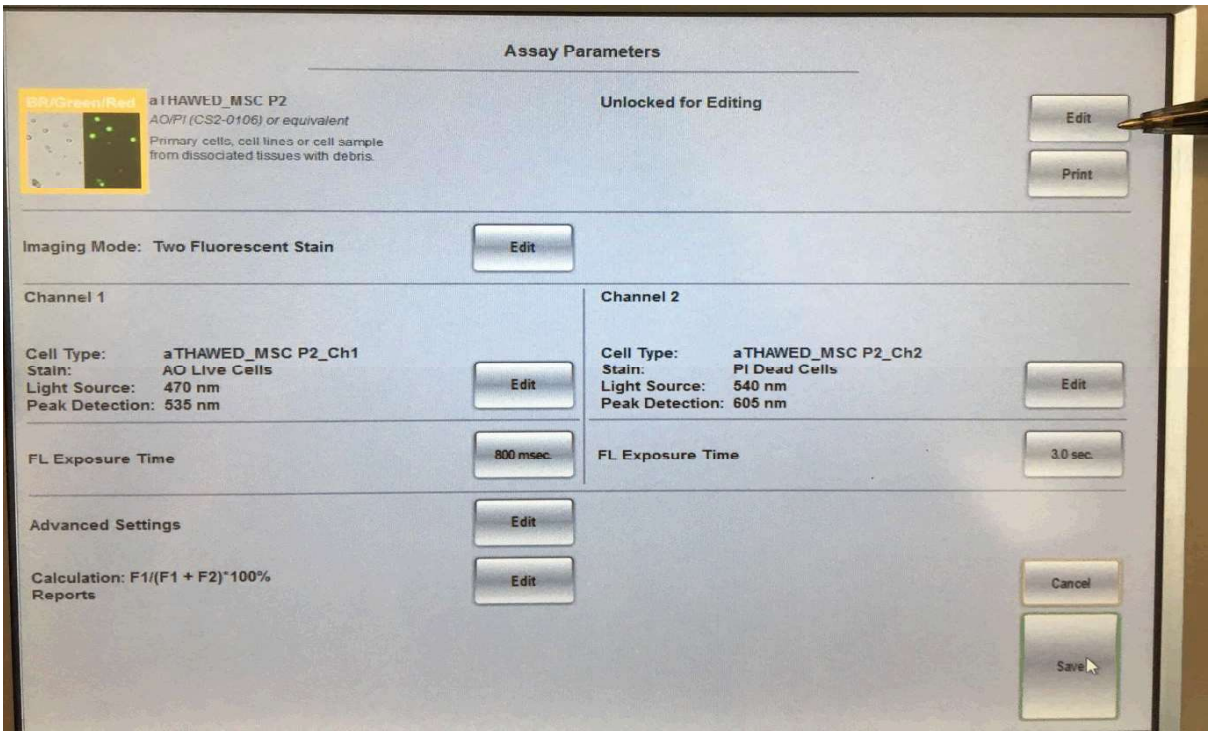




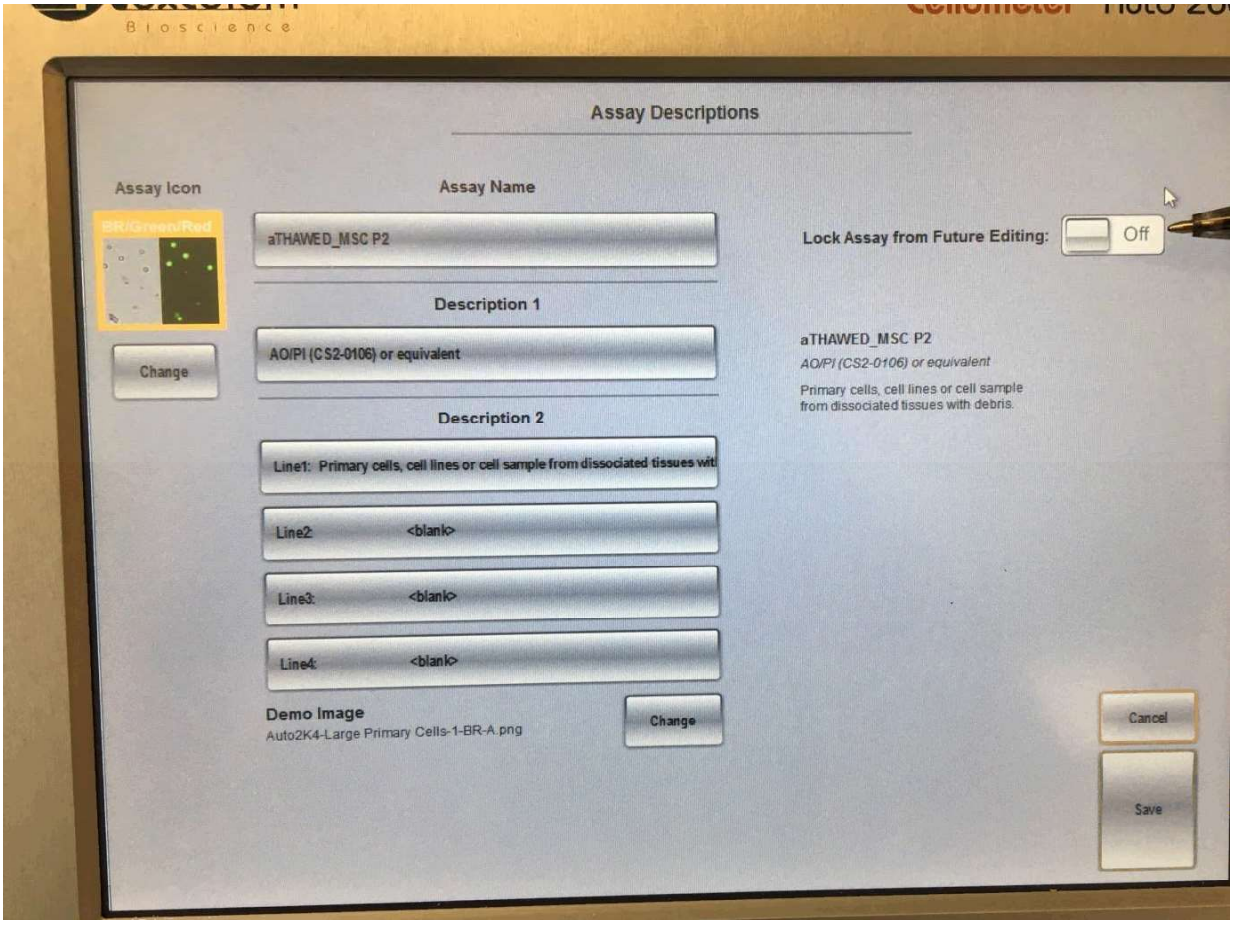
Calculations and Reports Settings

Confirm the settings match those pictured above.

Click CANCEL or SAVE if changes were made.



If revising an assay, once all changes have been made, next to “Unlocked for Editing”, click on “EDIT”



Select to **LOCK** the Assay and click “SAVE”

The **printed settings** for **JK THAWED MSC Assay** are as follows:

Assay Type: JK THAWED MSC

Description: Primary cells, cell lines or cell sample from dissociated tissues with debris.
11/16/2020 16:31:06

Imaging Mode: Fluorescent 1 and Fluorescent 2
Single Chamber Assay
Multimode FL Counting not checked
Slide Type: CHT4-xD100
F1 Cell Type: Thawed MSC Ch1
F1
F1 Filterset: AEM-535-001
F1 Lamp: AEX-470-001
F1 Fluorophore: AO Live Cells
F1 Fluorescent Exposure Time: 800.0 msec
F2 Cell Type: Thawed MSC Ch2
F2
F2 Filterset: AEM-605-001
F2 Lamp: AEX-540-001
F2 Fluorophore: PI Dead Cells
F2 Fluorescent Exposure Time: 3000.0 msec

Count Report Format

Formula = F1 / (F1 + F2) * 100%
Use Custom label: Y
Set Dilution Factor for Assay to 2.000
Acquire Brightfield Image checked

Cell Type: Thawed MSC Ch1

Description:
11/16/2020 16:31:06

Brightfield Parameters

Min Cell Diameter: 5.0 microns
Max Cell Diameter: 40.0 microns
Cell Roundness: 0.10 *default 0.10, range: 0 - 1.0; 1.0 for perfect circle
Contrast Enhancement: 0.40 *default 0.40, range: 0 - 0.5; high values for light cells
Decluster Cells
Decluster Edge Factor: 0.5 *default 0.50, range: 0 - 1.0; high values for more edge enhancement
Decluster Th Factor: 1.0 *default 1.0, range: 0 - 1.0; high values for more sensitivity
Background Adjustment: 1.0 *default 1.0, range: 0 - 1.0; lower value to pick up dim cells

Trypan Blue Parameters

Min Cell Diameter: 5.0 microns
Max Cell Diameter: 40.0 microns
Sensitivity: 1.0 *default 1.0, range: 0 - 2.0; higher value to pick up dead cells
Uniformity: 150 *default 150, range: 100 - 255; higher value for non-uniform dead cells
Very Dim Dead Cells not Checked

Fluorescent Parameters

Min Cell Diameter: 5.0 microns
Max Cell Diameter: 40.0 microns
Cell Roundness: 0.10 *default 0.10, range: 0 - 1.0; 1.0 for perfect circle
Decluster Th Factor: 1.00 *default 1.0, range: 0 - 1.0; high values for more sensitivity
Manual Threshold (Based upon sensor intensity range)
Threshold Adjustment: 8.0% *default 80, range: 1 - 100%; lower value to pick up dimmer cells
Sum the cell intensity over the entire cell
Non-uniform cell fluorescence not checked
Use a combined BR and FL image for counting

Cell Type: Thawed MSC Ch2

Description:
11/16/2020 16:31:06

Brightfield Parameters

Min Cell Diameter: 10.0 microns
Max Cell Diameter: 25.0 microns
Cell Roundness: 0.10 *default 0.10, range: 0 - 1.0; 1.0 for perfect circle
Contrast Enhancement: 0.40 *default 0.40, range: 0 - 0.5; high values for light cells
Decluster Cells
Decluster Edge Factor: 0.5 *default 0.50, range: 0 - 1.0; high values for more edge enhancement
Decluster Th Factor: 1.0 *default 1.0, range: 0 - 1.0; high values for more sensitivity
Background Adjustment: 1.0 *default 1.0, range: 0 - 1.0; lower value to pick up dim cells

Trypan Blue Parameters

Min Cell Diameter: 10.0 microns
Max Cell Diameter: 25.0 microns
Sensitivity: 1.0 *default 1.0, range: 0 - 2.0; higher value to pick up dead cells
Uniformity: 150 *default 150, range: 100 - 255; higher value for non-uniform dead cells
Very Dim Dead Cells not Checked

Fluorescent Parameters

Min Cell Diameter: 10.0 microns
Max Cell Diameter: 25.0 microns
Cell Roundness: 0.10 *default 0.10, range: 0 - 1.0; 1.0 for perfect circle
Decluster Th Factor: 1.00 *default 1.0, range: 0 - 1.0; high values for more sensitivity
Manual Threshold (Based upon sensor intensity range)
Threshold Adjustment: 15.0% *default 80, range: 1 - 100%; lower value to pick up dimmer cells
Sum the cell intensity over the entire cell
Non-uniform cell fluorescence not checked
Use the FL image for counting

Signature Manifest

Document Number: STCL-EQUIP-022 JA2 **Revision:** 01
Title: Cellometer Program Used for Thawed MSC Products JA2
Effective Date: 04 Mar 2021

All dates and times are in Eastern Time.

STCL-EQUIP-022 JA2 Cellometer Program Used for Thawed MSC Products JA2

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Document Release

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