QiTissue Software

Frequently Asked Questions

1. Memory Management

Q. Where do you specify to use external SSD space?
A: In the Preferences file menu, under Memory Management. Select which drive you would like to allocate cache to as the first "primary" drive.

Q. If I would like to analyze some bigger files on my personal laptop, would it be better to plug in my external hard drive?
A: Much, much better. Make sure to point your generated cache files to that external drive, which can be done in Preferences -> Memory Management, by setting it as your primary disk drive. For more info on that refer here.

2. Importing Datasets

Q. What prerequisites are needed to open the dataset? Does it need to be in a specific format?
A: Refer here for file formatting integration with QiTissue.

Q. Can I remove existing datasets from the Project Browser?
A: Yes. You can remove cached datasets from the Project Browser. Some quick changes you can make to a dataset without requiring removal – you can rename the dataset that appears on the list by right-clicking and selecting rename. You can also click the "Track directory changes" checkbox when first importing a dataset, so that any channels added or removed from the folder will automatically be detected.

Q: Does QiTissue write cache into my original image dataset folder? Will my data be affected at all?
A: Nothing writes into the folder containing your image dataset files. QiTissue writes its own cache image data files, metadata, and processing pipelines to your primary cache folder, which it uses to pull from. No original image data files will be affected.

3. Annotations and Gates

Q. Is there a way to measure the pixels? A scale bar or so? To help with the min/max segmentation?
A: There is a scale bar in the Tools palette, where you can place it anywhere along the Image and adjust its length (in px or micron). You may also adjust the line width, font, and other settings in the Annotation tab of the Properties panel (just be sure to have the scale bar selected).

Q. Can you create regions of exclusion around cells within a region of interest so that those cells are not segmented?
A: Yes, you can create regions of exclusion within regions of interest to avoid segmentation. To do so draw a shape with the drawing tool palette, go to the Properties of that annotation and specify a role of "Region to Exclude". Make sure to have

"Use Exclusion Regions" checked in the General Settings of the segmentation window. You may also apply a setting for ROI segmentation to prevent cells from being cut and having the nucleus/cytoplasm delimited by checking "Remove Edge Objects."

4. Clustering Algorithms

Q. How do you decide the number of clusters for the Kmeans clustering?
A: You can input the number of clusters for Kmeans. Typically, you know how many cell types to expect. Based on how many CD markers and others, you would estimate how many cell types. Say you have 20 different cell types; you would expect about 20 clusters. It is often better to over-cluster, meaning specify a larger number of clusters than you expect, because you can always merge them in the UI (feature plot, node editor, etc.).

5. Segmentation and Parameters

Q. Which DAPI channel is used for segmentation?
A: By default, the DAPI channel of the first cycle is used because it should have optimal integrity of all the nuclei. You are however free to choose a different cycle.

Q. How can you control which markers are selected for segmentation?
A: The biomarkers that are selected for segmentation are derived automatically from the biomarker knowledge base, which is already preloaded. If you would like to add other biomarkers as favorites for segmentation, you can do so by checking the check-box in the biomarker reference browser. Note that it is easier to specify that once rather than do it for each image.

Q. What happens if a dataset does not contain the markers it would like to use for the segmentation?
A: QiTissue will run a channel interpretation on the dataset to detect any markers that are useful for segmentation. It will run that against the internal biomarker database and apply those additional settings. If none are detected, then no settings will be applied. To see what that means for your specific image, run the Report menu -> Channel interpretation.

Q. Is there some recommended way to evaluate how good the segmentation was? Or is it just up to the users eye?
A: It is mainly a visual check. You should evaluate it to a golden truth with a human eye. Eventually, we hope to do it in a fully automatic way where you don't have to set any parameters and the algorithm does it all by itself. But a visual check is needed for now.

6. Features and Analysis

Q. Can you extract the intensity of a particular region (spot) on the image over multiple cycles?
A: You can do that, for example with the nucleus, and measure the intensity of DAPI over each staining cycle. Also, a quality metric can be extracted over each staining cycle to potentially point out a problem where tissue may not be adhering properly and use that as a quality assessment parameter.

Q. How is expression defined?
A: *Nuc Exp*: Expression in the nucleus (usually measured as average intensity in that area only – but could also be a spot count or a 75 percentile, or other, just depends on the type of marker).

Cyto Exp: Expression in the cytoplasm (usually measured as average intensity in that area only – but could also be a spot count or a 75 percentile, or other, just depends on the type of marker).

Cell Exp: Smart "conclusion" about the expression; it could be the nuclear or the cytoplasm expression or a combination, depending on the marker, and depending on the 3D situation of the cell.

The latter deserves a little more explanation via examples:

1) Ki67 marker that should be expression only in the nucleus > Cell Exp is always taking Nuc Exp.
2) Any Cytoplasm bound marker that should be expressing only in the cytoplasm > Cell Exp is always taking Cyto Exp.
3) Any Cell Surface bound marker that should be expressing on the cell membrane > this depends on whether the cell is in focus or not. If it is in focus, use Cyto Exp. If it is out of focus we are looking at the top/bottom of the cell membrane, so the expression can be seen in the nuclear are just as well, or even more so than in the cyto area. > Cell Exp is now taking highest one: Cyto Exp or Nuc Exp. We get a little "in focus feature" for free as well here.
4) More logic can be added later too, like what if the "expression" is consider the translation from nuc to cyto, so there > Cell Exp feature should be the Nuc/Cyto expression ratio.

   In summary, you can simply select Cell Exp for each biomarker and automatically get the correct value.

Q. Can you save segmentation settings and apply it to different datasets?
A: We will eventually have a "Favorites" icon in the segmentation window for saving those parameters, which you can recall for different datasets at any time and share with others.

Q. Can you analyze any similarities between different biomarkers?
A: You can utilize the Biomarker Correlation Matrix to view co-localization of different biomarkers.

Q. Is the analysis available at the image, field, single cell level?
A: Yes. Browse through the feature reference browser to see all of the cell and image feature data. You can extract a portion of those for now, and more will be available in the future.

Q. Can you save the analysis template and apply it to other samples?
A: There will be a favorites "heart" menu in the workflow editor window, where you will be able to apply saved gating strategies and parameter settings to other datasets.

Q. Will the software have pre-configured panels for certain tissue types?
A: We want to get the heart menus knowledgeable of tissue types, but that is not the case yet. Biomarkers and cell types in our databases help to inform it, and tissue types will come next. Only labelling panels and their pre-configurations can be added, these in a way may be specific to tissue types already.

Q: Can an analysis module that is independently developed run with QiTissue modules?
A: We could rapidly prototype solutions in QiTissue because it has a large library of image processing and visualization functions. If it is deemed useful, we could derive a small subset of functionality in a library for particular purposes. Note that QiTissue is all written in C++, without additional license fees.

Q: Will QiTissue use the data that is stored on the cloud to analyze between datasets (e.g. comparison between patient data)?
A: Yes there will be, we are setting up infrastructure for data lake, and inputs/ outputs, but no dashboard exists yet.

Q: There are some cells at the edges of the ROIs, are these cells included in the dataset?
A: These cells are not included in the generated data.

Q: Can you measure if T-cells are close to cells expressing checkpoint ABC?
A: You can gate the cell population that is expressing the checkpoint, then phenotype the T cells and measure distances between them by calculating distance maps and features.

Q: Is there a way to define a cell population and then do a heat map on the second marker and give it different colors on the image?
A: Yes this can all be constructed in the workflow editor.

Q: Can you measure the distance on the image?
A: Yes, distance is in pixels, but it can be recalibrated to microns.

Q: Can you translate the pixels into micrometers?
A: Yes.

7. Exporting and Sharing

Q. Can you export a certain image view?
A: There are many ways to export images, data, and graphs. You can export all the channels in your image by going to File menu -> Export -> Export Select Channels as New Image tifs. You can export a screenshot of what is in your current image viewer by going to the File menu -> Save Image As. You can also do that by creating perspectives as scenes in the sequencer and exporting the scenes as tif images or as a movie through the sequencer widget. Specific settings for segmentation analysis will eventually be shareable using the favorites “heart” icon, along with other analyses or gating strategies constructible in QiTissue.

Q: Can you export the result of the analysis?
A: Yes. You can export the results of single-cell feature analysis as CSV files or as FCS files. You can also export the result of segmentation as a labeled image. Finally, you can export all the results of the analysis in a convenient report format.

Q: What are the export options?
A: You can export views (perspectives) through the sequencer as tif images or as an entire movie (mp4 or avi). Data from the feature and metadata tables can be exported as a fcs or csv. The feature plot can be exported as a png, tif, or jpg. Simply find the export icon (upward arrow) in the top right of each widget. There are also additional export options in the File menu under Export.

Q. Can you share the sequencer as a video? Can you also share it as a script so others can reload it and also look at it?
A: You can export and share the sequencer video as a .avi or .mp4. Currently, there isn’t a way to load a sequencer video as a script or import file into QiTissue. However, within the favorites section in the sequencer widget, in the next version you will be able to recall previous sequencer timelines, as well as share them to QiCloud for others to access.

Q. Is there a way to select only 1 of the scenes in the sequencer for exporting?
A: This will be available in a release soon.

Q. Are there other ways to demonstrate the data besides 1 picture for a few seconds each?
A: Using the sequencer, you can create a slideshow, a movie with custom duration, and a complete dataset report as html on QiCloud, or as a pdf.

8. Dataset Preprocessing

Q. How do you properly overlap images with different channel markers?
A: Using the registration widget. You can register the individual cycles based on the DAPI channel that is included in every cycle. It can do a fine adjustment and a coarse adjustment. Most MACSima data comes preprocessed entirely, so it should come registered already.

Q. Can you register images that were acquired with different objectives under different magnification?
A: Not at the moment but in a future update yes.

Q. How small could the registration signal be to still work with your other channels?
A: The DAPI image can be quite dim and registration would still work. However, DAPI in general is a very strong stain so you will unlikely run into this concern.

Q. Will there be a tiling/ stitching function?
A: The MACSima is fully processing the images, not in QiTissue. But this is being changed so that it will be done in QiTissue, as well as run the same code within the MACSima machine on the fly when it is acquiring images – stitching and tiling would be a part of both.

Q: Does the current version of QiTissue have a tool to stitch images that overlay in the current MACSima version?
A: Yes, stitching is supported in the current release under the MICS file menu.

Q: Is there any way to take out artifacts or bias from the image to better see the biology?
A: Yes, you can subtract background, subtract autofluorescence, and autodetect debris in the File menu.

Q: Where can I find "Reset Registration to Zero"?
A: It is located in the CyclicIF File menu.

Q: Can QiTissue currently handle primary data containing line artifacts used on an older software version of the MACSima?
A: Yes it can. There is a Remove All Line Artifacts as well as a Restitch function in the MICS file menu.

9. Visualization and Display Settings

Q. Can you have 3D images?
A: You can visualize 2D images in 3D (by turning the pixel intensity into a 3D “heightmap” representation). There will be an update to QiTissue soon to include full 3D imaging as well.

Q. Is there a way of adjusting multiple Histogram channels at once?
A: You cannot open multiple Histograms at once, but you can apply display settings adjustments to all of the channels using the contrast icon at the top of the Channels list.

Q: How can you see your selection on the image when you create a merge node?
A: Click the eyeball in the upper right-hand corner of the merge node.

Q: If you copy and paste display setting changes that you've made on all channels onto a new dataset where the number of biomarker channels does not exactly match, will it still work?
A: Yes it will work. Functions such as copy/paste and favorites between datasets will accurately identify matching markers.

10. Python API Documentation

Q: Do the widgets need a framework to work together? Is it difficult to take a

single widget and reuse it separately without the surroundings?
A: Widgets and underlying data models are well organized, and code is written with minimal cross-dependencies, but it would be better to adopt a bundle of infrastructure as opposed to the single widget. For example, it would make more sense to take Histogram, Image displays, and annotations together as opposed to just one isolated module.

Q: What is Qt?
A: A cross-platform development environment that helps us release QiTissue on Mac. Windows and Linux. (https://www.qt.io)

Q: Can you add widgets into QiTissue through the Python interface?
A: Yes, refer to the Python Documentation that can be found within the Reference Browser Window. Simply choose "QiTissue Python Reference Manuel" in the dropdown list.

11. Miscellaneous Topics

Q. Can you have multiple channels per cycle?
A: Yes. In many cases, CyclicIF data has 3-5 channels per cycle.

Q. Can you have multiple cycles of multiple channels?
A: Yes you can expand a dataset to hundreds of channels over many cycles.

Q. Does the updated version require a completely new download or can it be updated in the program itself?
A: Yes, you will need a new download. Simply reinstall like you did the first version. You can replace the previous version with the new version when it is installed. This is done through the installation steps. There is no way to update QiTissue within the program itself.

Q. If you save the project, are the settings also saved?
A: All actions and status are saved automatically, all the time and when exiting QiTissue. Whenever the program is opened, all widgets, settings, and datasets reappear exactly as you left them.

Q. When could we have access to the cloud base solution? Wouldn't that take care of the laptop hardware limitation?
A: QiCloud is not yet ready, but we hope to have it released in the first half of 2021 as a beta.

Q. Will saved routines be utilized to strengthen machine learning?
A: Yes, almost everything informs machine learning.

Q: Does QiTissue plan on extending its capability to recommending therapy points in a medical environment?
A: QiTissue is sold for research only, but we do have future plans for clinical applications. We're currently involved in a number of clinical studies.

Q: What hardware does Miltenyi need to provide to realize QiTissue plans?
A: Refer here.

Q: Is there a possibility to store the data on the cloud?
A: Yes, QiTissue has a connection to QiCloud. You can upload and share with other users, or see it on a web-based viewer. It is not released yet, but will be available during 2021.

Q: Does QiTissue run on multiple operating systems?
A: Because of Qt cross platform environment, we maintain the same codebase that compiles on 3 different platforms.

Q: Do I have to feed the software any information on markers? Or does it automatically do this?
A: The software has a built-in biomarker library which it reads from. Information on biomarkers can be edited locally through the Biomarker Reference window.

Q: Can the current version of MACSima support a larger study, or should we wait for an update?
A: We highly recommend using the new and improved pre-processing pipeline for new large studies.

Q: How long is it going to take the new QiTissue version to be released?
A: We periodically (every few months or so) update the installation build available. You can download the latest version here.

Q: Where is the new version available to download from?
A: At our download website, available here.