IN Carta

Image Analysis Software

SINAP User Guide

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MOLECULAR DEVICES

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Contents

Chapter 1: IN Carta Image Analysis Software

The IN Carta® Image Analysis Software provides powerful analytics for advanced phenotypic classification and 3D image analysis. It delivers robust, quantitative results from complex biological images and datasets using advanced AI technology.

Faster Data

- Intuitive design makes complex analysis accessible with minimal training.
- Shorten analysis time with true parallel processing.

Reliable Data

- Sophisticated algorithms generate reliable data with minimal user input.
- Improved segmentation algorithms represent cellular structures more accurately.

Results That Matter

- See real results quickly—from populations to single cells—using integrated data visualization tools.
- User-friendly interface guides you through your discoveries with continual updates that grow with your needs.

Terminology

The following table defines the terms and abbreviations used in this guide.

Term	Definition
Annotation	User-created labeling (target/background) of input data to define areas of interest.
Background	Area of an image that is not of interest.
Base Model	One of the pre-trained neural network models provided with the IN Carta SINAP module for a range of segmentation applications.
Epoch	Single iteration of learning algorithm working through entire training set in an attempt of creating a model.
FOV	Field of view
Ground Truth	User-annotated input data
Input data	Images
Model	Trained deep-learning neural network
ROI	Region of interest
Target	Biological structure that is of interest
Training Set	Set of annotated images used to train a model

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest level of technical service.

Our Support website—www.moleculardevices.com/service-support—describes the support options offered by Molecular Devices, including service plans and professional services. It also has a link to the Molecular Devices Knowledge Base, which contains documentation, technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance, you can submit a request to Molecular Devices Technical Support.

Technical Support

To contact Molecular Devices Technical Support, submit a support request through the Molecular Devices Knowledge Base at support.moleculardevices.com.

You can also submit a support request by phone. For regional support contact information, go to www.moleculardevices.com/contact.

To expedite support, be prepared to provide the software version and your activation ID. To display this information, at the bottom left of the IN Carta window, click  **Settings**.

Documentation

Review the product documentation on the Molecular Devices Knowledge Base at support.moleculardevices.com. In addition, online Help is available within the IN Carta software.

Additional Resources

Web-based microscopy courses:

- www.microscopyu.com
- www.ibiology.org/ibioeducation/taking-courses/ibiology-microscopy-short-course.html

The *Molecular Probes Handbook* offers advice on fluorescent probes and can help you determine if there are better stains available for your analysis:

- www.thermofisher.com/us/en/home/references/molecular-probes-the-handbook.html

The *Assay Guidance Manual* details state-of-the-art approaches to high-content screening (HCS) and discusses challenges specific to HCS. It serves as a good introduction for new HCS practitioners.

- www.ncbi.nlm.nih.gov/books/NBK100913

About This Guide

This guide is intended for the scientist using the IN Carta software. It provides an overview of the IN Carta SINAP module and describes how to use IN Carta SINAP to run your methods.

The information in this guide is valid for IN Carta software version 2.1 and is subject to change without notice. We recommend that you review the guide on the Molecular Devices Knowledge Base at support.moleculardevices.com for the most up-to-date information.

Chapter 2: IN Carta SINAP Module

The IN Carta SINAP module uses machine learning as an aid for creating annotated ground truth training sets for the deep-learning component of the IN Carta software.

Deep learning is an area of AI that uses multi-layered neural networks to mimic the way that the human brain processes information. A network becomes more accurate as it is provided with more ground truth data from which to learn.

With deep learning, there is no requirement to define specific features of interest. A model learns in a similar way to humans; as a result, it can outperform traditional segmentation methods in many applications.

Note:
An additional license is required to use the SINAP module. Many functions are available to all users, including training/applying/saving a model and applying analysis settings for SINAP segmentation to an image. However, the following functions are not available without a valid license:

- Saving a protocol with SINAP segmentation.
- Running a protocol with SINAP segmentation.

If you are running the IN Carta software with a network license, you may be able to reserve a license to access the SINAP module. See Settings for details.

Deep-Learning Segmentation

Segmentation is the foundation of the image analysis pipeline, allowing researchers to identify regions of interest in tissue, whole organisms, individual cells, nuclei, and organelles. Through segmentation, researchers can extract information from images to quantitatively compare differences across diverse concentrations, treatments, time, genetics, and so on.

Segmentation is the foundation for every analysis that follows, so accuracy is crucial. Reliable identification of structures, shapes, and sizes is vital for robust analyses. Errors in segmentation will be propagated and multiplied through the rest of the analysis, effectively lowering the assay robustness (less accurate dose curve, inability to detect small modulations of a phenotype, lower Z' for classification experiments). This leads to false-positives and false-negatives, and it ultimately wastes time, money, and resources.

Current segmentation algorithms often struggle to deal with non-optimal images where poor contrast, signal variability, or high complexity of biological structures mean that you must compromise your analyses and work with a solution that accommodates some of their data, but not all. This results in multiple, specialized tools, each suited for an application or dataset, which often require training to be used effectively.

The advent of artificial intelligence (AI) has improved the situation. The development of computer systems able to perform tasks through machine- and deep-learning— tasks such as visual perception, speech recognition, decision-making, and translation between languages— has addressed some of the intractable problems. These solutions still require a large amount of annotated *ground truth* data to create a reliable, accurate training set.

A learning algorithm capable of being taught to detect the biology in which a researcher is interested is required.

The SINAP module is a trainable segmentation module that uses both classic machine learning and deep learning in an intuitive format to produce robust, reliable segmentation across a wide variety of applications.

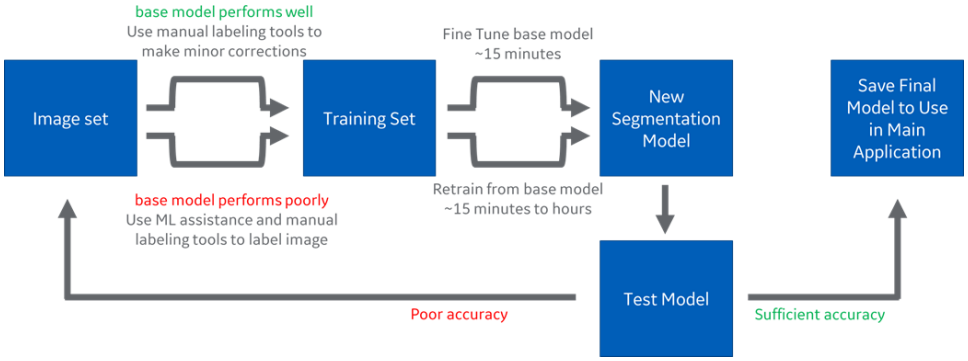
Pre-Trained Models

The SINAP module uses pre-trained *base models* to reduce the requirement for large amounts of annotated ground truth data. This saves time and reduces the amount of data required for a model to be accurate. If a model is a good match for the target of interest, the fine tuning only requires a few annotated images to be added to a training set.

When the structure of interest is dissimilar to the images used to train a model, the performance of the model may not be optimal. In this case, you can re-train an existing model to suit their application.

Re-training a model using the SINAP module adopts an iterative approach, where you add more annotated ground truth data until the model accurately differentiates the target from the background. This takes longer than fine-tuning and requires more data in the training set. Timing and size of the training set is highly variable and is dependent on how different the structures are to the base model and complexity of the problem.

In either case, when you are satisfied with the performance of the model, you can save and apply it to the full dataset. The saved model will also be available as a pre-trained model for subsequent use in other experiments, as shown below.



Chapter 3: Using the SINAP Module

This section contains instructions on how to run your methods using the SINAP module.

Saving Data and Data Storage

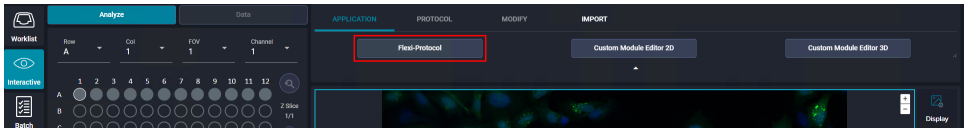
Consider the following when working with the SINAP module:

- After saving a model, you cannot delete or overwrite it.
- Upon editing and saving, a new model is created with a unique identifier.
- Training set data is stored in a temporary folder while the SINAP application is being used.
- Each time the SINAP application is started, all previously stored training-related data is deleted.
- If you close the SINAP application without saving a model, any changes made to the model are lost.

Launching SINAP Application

To launch SINAP:

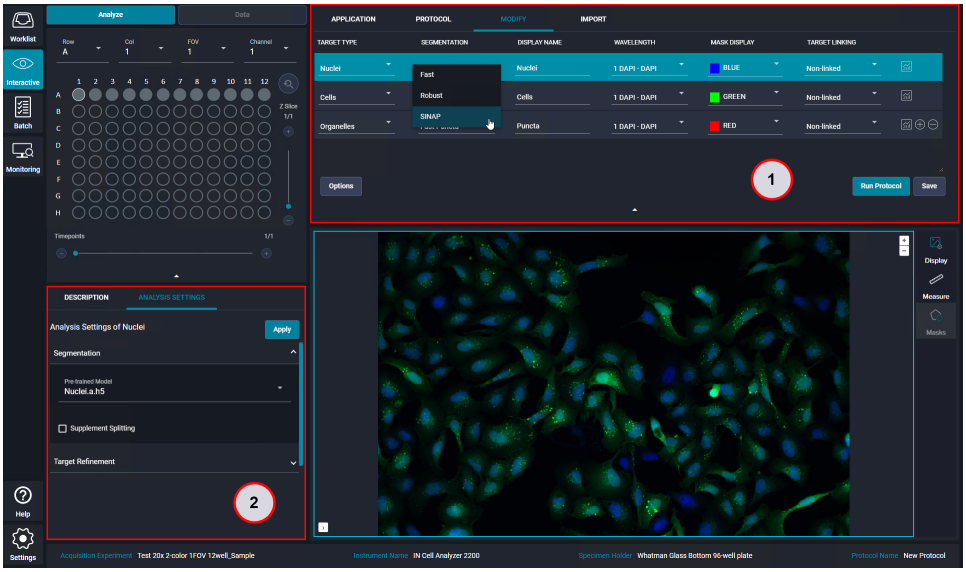
1. From the **Worklist** page, click the experiment you want to open.
2. If results exist for the experiment, click **New**.
   The **Interactive: Analyze** page opens. In the Protocol Editor Table, the **Application** tab displays.
3. On the **Application** tab, click **Flexi-Protocol** to start the segmentation workflow.



To use a pre-trained model with no further input or training, see Creating a New Protocol with Deep-Learning Analysis on page 13.

To refine or create a new model, see Refining a Pre-Trained Model on page 14.

Creating a New Protocol with Deep-Learning Analysis



Part	Name
1	Protocol Editor Table
2	Analysis Settings

To create a new protocol with deep-learning analysis:

1. In the Protocol Editor Table, on the **Protocol** tab, click **New** to create a new protocol.
2. On the **Modify** tab, click **Add a New Target** to add rows for each target, as needed.
3. Assign a **Target Type** for each target (either **Nuclei**, **Cells**, or **Organelles**).
4. In the row for each target for deep-learning analysis, click the **Segmentation** drop-down, and select **SINAP**.
5. In the **Display Name** field, enter a display name for the protocol (for example, nuclei, puncta, mitochondria, other structure, and so on).
6. Click the **Wavelength** drop-down list, and select a channel of interest.
7. In Analysis Settings, click the **Pre-trained Model** drop-down, and select a model.

   Tip: See the *IN Carta Help* for details on other Analysis Settings options.

8. Click **Apply**.
9. If you are satisfied with the segmentation, in the Protocol Editor Table, click **Run Protocol** to analyze the dataset. Otherwise, see Refining a Pre-Trained Model on page 14.

Refining a Pre-Trained Model

Consider the following when generating a training set:

- Different methods of adding images to training set can be used to generate a training set.
- You can use ROI (Region of Interest) to specify a properly segmented region within an active image. When you click **Add to Training Set** with ROI active, the software adds only the ROI to the training set. Click inside the ROI to change its size or position.
- You can add multiple ROIs to a training set for any FOV.
- It is important to consistently label the image. Poor quality ground truth is the biggest obstacle to quickly training a model.

> **Tip:** It is more important to label the right pixels than to label many pixels. You can find the most useful information at boundaries between target and background. Use the  **Brush** tool to precisely annotate these areas.

- You can annotate the same input images differently to train a model to recognize a new target. In this way, you can train models to recognize multiple targets from the same input images.

To refine a pre-trained model:

1. In the Protocol Editor Table, click **SINAP** to open the SINAP window.
2. In the **Image Selection** pane, select the **Channel**, **Row**, **Column**, **Time**, and **Z-slice** from the drop-downs.

Part	Name
1	Image Selection Pane
2	Label Pane
3	Neural Network Segmentation Pane

AI-Assisted Segmentation

When a pre-trained model produces results that are close to expected and require minor corrections, you only need to fine-tune the model to correct areas that have been mislabeled as **Target** or **Background**. See Workflow 1: Minor Corrections on this page for details.

In some cases, a model may produce suboptimal segmentation and requires training. See Workflow 2: Major Corrections on page 16 for details.

Workflow 1: Minor Corrections

Use this workflow if a model produces optimal segmentation and requires minor corrections.

To make minor corrections:

1. In the **Image Selection** pane, click **Random FOV** to select a field of view.
2. On the right side of the screen, click **Adjust** and use the sliders to modify the brightness and contrast.
3. (Optional) To make image segmentation faster, define a region of interest by clicking **ROI**.
4. In the **Neural Network Segmentation** pane, click the **Model** drop-down, and select the model.
5. Click **Apply**.
6. In the **Overlays** pane, use the **Annotations** and **Segmentation** sliders to adjust the opacity of the masks.
7. In the **Labels** pane, do one of the following:
   - Select **Background** to train the model to remove false-positive regions.
   - Select **Target** to train the model to remove false-negative regions.
8. Use the labeling tools to annotate example regions for **Target** and **Background**.

    **Brush**: Click to activate and then drag to draw a free-hand line. Displays in blue when active. Use the slider under the button to adjust line width.

    **Segmented Line**: Click to activate and then drag to create a line with segments. Release to end the segment. Double-click to end the line. Displays in blue when active. Use the slider under the button to adjust line width.

    **Polygon**: Click to activate and then drag to create a filled polygon for the selected label type. This tool is useful to quickly annotate large regions in an image. Each click adds a node or corner to the polygon. Double-click to end the polygon. Displays in blue when active.

    **Connected Components**: Click to automatically create an annotation by tracing an image region with intensities similar to selected pixel. Use the slider to control the tolerance for intensity similarity. Lower tolerance results in smaller annotated regions; at higher tolerance, regions are larger. Displays in blue when active.
   - Select the **Fill Holes** check box to fill holes in the annotated area.
   - Click within a biological object of interest. The calculated annotation mask displays within red box. At this point, the mask is not yet added as an annotation.
   - As needed, add more annotations to correct the segmentation. Hold **SHIFT** and click anywhere in the Image Viewer to add a mask as an annotation.
9. Click **Correct** to apply the annotations to the segmented image.
10. When you are satisfied with the segmentation of the image, click **Add to Training Set** to add the FOV or ROI to the training set.

Workflow 2: Major Corrections

Use this workflow if a model produces sub-optimal segmentation and requires more ground truth images to be added for training.

To make major corrections:

1. In the **Image Selection** pane, click **Random FOV** to select a field of view.
2. On the right side of the screen, click **Adjust** and use the sliders to modify the brightness and contrast.
3. (Optional) To make image segmentation faster, define a region of interest by clicking **ROI**.
4. Use the labeling tools to annotate example regions for **Target** and **Background**.

    **Brush**: Click to activate and then drag to draw a free-hand line. Displays in blue when active. Use the slider under the button to adjust line width.

    **Segmented Line**: Click to activate and then drag to create a line with segments. Release to end the segment. Double-click to end the line. Displays in blue when active. Use the slider under the button to adjust line width.

    **Polygon**: Click to activate and then drag to create a filled polygon for the selected label type. This tool is useful to quickly annotate large regions in an image. Each click adds a node or corner to the polygon. Double-click to end the polygon. Displays in blue when active.

    **Connected Components**: Click to automatically create an annotation by tracing an image region with intensities similar to selected pixel. Use the slider to control the tolerance for intensity similarity. Lower tolerance results in smaller annotated regions; at higher tolerance, regions are larger. Displays in blue when active.
   - Select the **Fill Holes** check box to fill holes in the annotated area.
   - Click within a biological object of interest. The calculated annotation mask displays within red box. At this point, the mask is not yet added as an annotation.
   - As needed, add more annotations to correct the segmentation. Hold **SHIFT** and click anywhere in the Image Viewer to add a mask as an annotation.
5. Click **Segment** pane to segment the current FOV.
   The software uses a machine learning model to predict all unlabeled pixels in an image, assigning them as either **Target** or **Background**.

    **Note:** You can re-use this model for any new FOV that is loaded or when the ROI is moved to a new position.

6. If the resulting segmentation mask is not satisfactory, add more annotations, and segment again.

    **Note:** If the results that are close to expected, consider fine-tuning the model to correct areas that have been mislabeled. See Workflow 1: Minor Corrections on page 15 for details.

7. When you are satisfied with the segmentation of the image, click **Add to Training Set** to add the FOV or ROI to the training set.

Manual Segmentation

When applying a segmentation model or clicking **Segment** produces unsatisfactory results, you may want to train a model by annotating only the target structures in single or multiple fields of view. In this case, all unlabeled pixels are assigned as background.

To perform manual segmentation:

1. In the **Image Selection** pane, click **Random FOV** to select a field of view.
2. On the right side of the screen, click **Adjust** and use the sliders to modify the brightness and contrast.
3. Use the labeling tools to annotate the structures of interest as **Target**. All unlabeled pixels will be assigned as **Background**.

    **Brush**: Click to activate and then drag to draw a free-hand line. Displays in blue when active. Use the slider under the button to adjust line width.

    **Segmented Line**: Click to activate and then drag to create a line with segments. Release to end the segment. Double-click to end the line. Displays in blue when active. Use the slider under the button to adjust line width.

    **Polygon**: Click to activate and then drag to create a filled polygon for the selected label type. This tool is useful to quickly annotate large regions in an image. Each click adds a node or corner to the polygon. Double-click to end the polygon. Displays in blue when active.

    **Connected Components**: Click to automatically create an annotation by tracing an image region with intensities similar to selected pixel. Use the slider to control the tolerance for intensity similarity. Lower tolerance results in smaller annotated regions; at higher tolerance, regions are larger. Displays in blue when active.
   - Select the **Fill Holes** check box to fill holes in the annotated area.
   - Click within a biological object of interest. The calculated annotation mask displays within red box. At this point, the mask is not yet added as an annotation.
   - As needed, add more annotations to correct the segmentation. Hold **SHIFT** and click anywhere in the Image Viewer to add a mask as an annotation.
4. Click **Correct**.
5. Repeat steps 3 and 4, as needed.
6. When you are satisfied with the segmentation of the image, click **Add to Training Set** to add the FOV or ROI to the training set.

Using a Training Set to Train a Model

After adding images to the training set, you can use them to train a model.

To train a model using a training set:

1. In the **Neural Network Segmentation** pane, do one of the following:
   - Click **Default** to select a default model from the drop-down.
   - Click **Custom** to select a saved model from local or network storage. Saved models are stored in the `C:\ProgramData\TSModels` folder.
2. As needed, click **Advanced** to set parameters that further adjust segmentation options and optimize training:
   - Under **Image Segmentation**, select the **Supplement Splitting** check box to activate the algorithm used for additional splitting of objects. The same check box is available in the Analysis Settings for SINAP segmentation.
   - Under **Model Training**, select the **Fine Tune** option when the prediction model makes minor mistakes with a new dataset. Select the **Retrain** option when the biological structure to segment is significantly different from the structure used to generate the original model.
   - In the **Epochs** field, enter the number of iterations performed during the training process. Use a higher number of epochs to generate a more accurate model. Note that training time is dependent on the epoch number, so increasing the number of epochs increases training time. For fine-tuning, we recommend using between 30 and 200 epochs. For retraining, we recommend using between 200 and 1,000 epochs.
3. Click **Train** to train the model based on the images in the training set.
4. Test the segmentation on the new FOVs to check performance.

Note: If the segmentation results are not robust enough, add more images to the training set.

5. If you are satisfied with the segmentation results, click **Save**.
6. Enter the name of the new model, and click **OK**.

Note:
- Saved models are stored in the `c:\ProgramData\TSModels` folder.
- Click **Manage** to open the **Model Manager** dialog, which enables you to review a list of saved models and remove models that are no longer needed.
  Note that removed models are not deleted; they remain in the `c:\ProgramData\TSModels` folder.

You can now access the model in Analysis Settings when using the **Flexi-Protocol** application.

Chapter 4: Troubleshooting

This section contains troubleshooting information for the SINAP module.

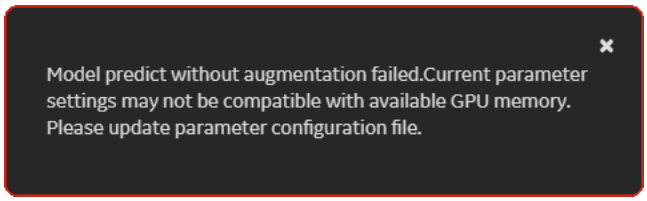
Updating Parameters for an Underpowered GPU

The SINAP module requires a supported Nvidia graphics processing unit (GPU) with the latest driver and a CUDA compute capability of at least 3.5.

Note:

- For more information on the compute capability of your Nvidia GPU, go to developer.nvidia.com/cuda-gpus.
- See the *IN Carta Installation Guide* for details on verifying the GPU driver for the IN Carta software.

If your Nvidia GPU has less than 24 GB memory, the IN Carta software attempts to automatically adjust the GPU-related settings. In some cases, however, you may need to update the neural network parameters in order for SINAP to run. If the neural network parameters have not been properly updated, the following message displays.



To update parameters for an underpowered GPU:

1. In Window File Explorer, browse to the **c:\Program Files\INCarta** folder.
2. Double-click **params_config.bat**.
3. Follow the on-screen instructions to update the neural network parameters. The following table lists the default and recommended values:

Type	Parameter	Default Value	Recommended Value
Train	crop_size	1024	512 (if fails, set to 256)
	batch_size	2	1
Prediction	num_blocks_x	1	2 (if fails, set to 4)
	num_blocks_y	1	2 (if fails, set to 4)

Diagnosing GPU Issues

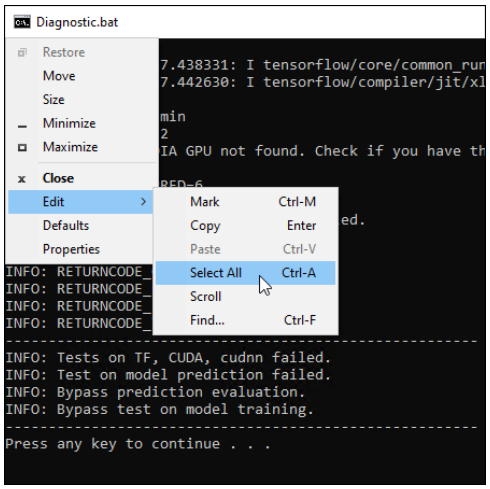
Use the Neural Network Diagnostic Tool to run a suite of experiments to test whether your graphics processing unit (GPU) hardware and software are appropriate for the SINAP module. If the initial tests do not succeed, the tool automatically runs the tests again with different settings. When it determines the best possible settings, the tool updates the IN Carta software accordingly.

If you are having issues running the SINAP module, run the Neural Net Diagnostic tool to test your GPU.

Note: The Neural Network Diagnostic Tool can take around 10 to 15 minutes to run.

To diagnose GPU issues with the Neural Network Diagnostic Tool:

1. In Window File Explorer, browse to the **c:\Program Files\INCarta** folder.
2. Right-click **diagnostic.bat** and select **Run as administrator**.
3. If the tests succeed, the GPU hardware and software are compatible with the SINAP module. Try running the SINAP module again.
4. If the tests fail, do the following:



   a. At the top left of the **Diagnostic.bat** window, click , and select **Edit > Select All**.

      Note: Do not press **CTRL** + **A**. Do this will clear the output and close the window.

   b. Click , and select **Edit > Copy**.
   c. Paste the output into any text editor (for example, Notepad).
   d. Save the text file. It will be helpful to diagnose the issue.
   e. Contact Molecular Devices Technical Support. See Obtaining Support on page 6 for details.

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