# IN Carta

## Image Analysis Software

## SINAP User Guide

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# Help Topics

# Chapter 1: IN Carta Image Analysis Software

The IN Carta® Image Analysis Software combines powerful analytics with an intuitive interface to simplify workflows for advanced phenotypic classification and 3D image analysis. It provides robust, quantitative results from complex biological images and datasets utilizing 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

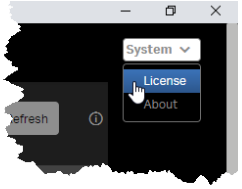
Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest level of technical service.

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 the license ID.

- To display the software version, in the top right corner of the IN Carta window, click **System > About**.
- To display the license ID, in the top right corner of the IN Carta window, click **System > License**.

### 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 discussed 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 IN Carta SINAP and describes how to use IN Carta SINAP to run your methods.

The information in this guide is valid for IN Carta software version 1.17 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

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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.

## Machine Learning

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 IN Carta 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.

## AI for Image Analysis

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.

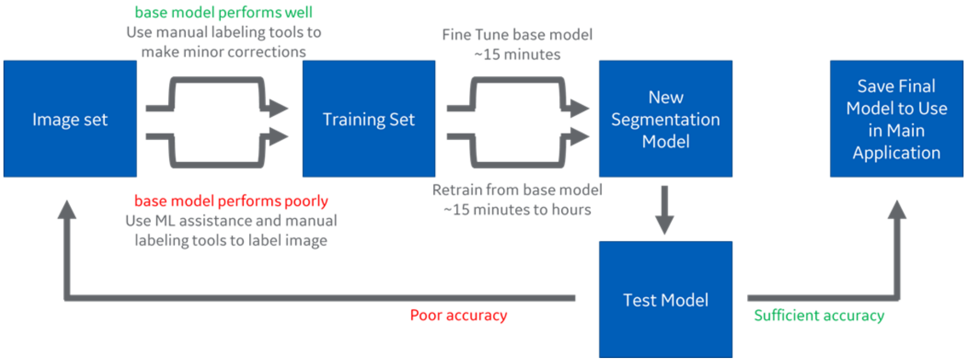
## Pre-Trained Models

The IN Carta 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 small number of 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 IN Carta 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 IN Carta SINAP

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

## Saving Data and Data Storage

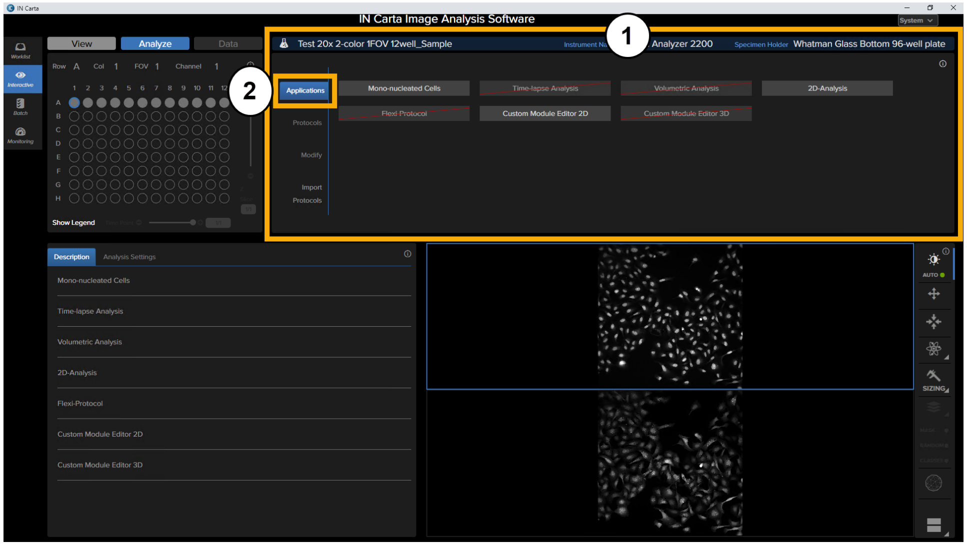
Consider the following when working with the IN Carta SINAP module:

- 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 the SINAP Application

To launch the SINAP application:

From the **Applications Tab** in the **Protocol Editor Table**, click **2D-Analysis** to start the segmentation workflow.



| Part | Name |
|---|---|
| 1 | Protocol Editor Table |
| 2 | Applications Tab |

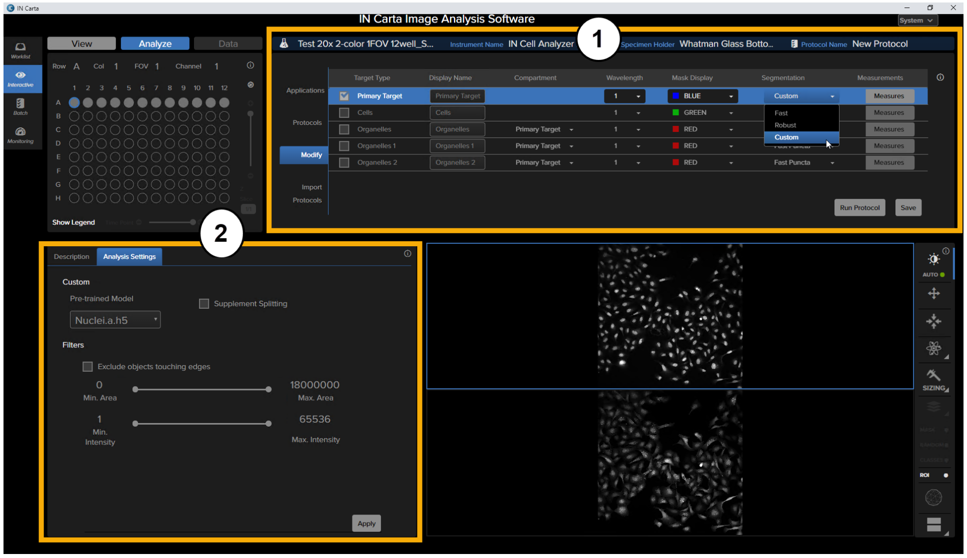
To use a pre-trained model with no further input or training, continue to Creating a New Protocol with Deep Learning Analysis on page 11.

To refine or create a new model, proceed to Refining a Pre-Trained Model on page 12.

## Creating a New Protocol with Deep Learning Analysis

To create a new protocol with deep learning analysis:

1. On the **Protocol Editor Table**, click **New** to create a new 2D-Analysis protocol.
2. In the row for the **Primary Target**, click the **Segmentation** drop-down list, and select **Custom**.
3. Click the **Wavelength** drop-down list, and select a channel of interest.
4. In the **Display Name** field, enter the display name for the protocol (for example, nuclei, puncta, mitochondria, other structure, and so on).
5. On the **Analysis Settings** tab, click the **Pre-trained Model** drop-down list, and select a model.



Test 20x 2-color 1FOV 12well_S... Instrument Name IN Cell Analyzer Specimen Holder Whatman Glass Botto... Protocol Name New Protocol

| Target Type | Display Name | Compartment | Wavelength | Mask Display | Segmentation | Measurements |
|---|---|---|---|---|---|---|
| ☑ Primary Target | Primary Target | | 1 | BLUE | Custom | Measures |
| ☐ Cells | Cells | | 1 | GREEN | | Measures |
| ☐ Organelles | Organelles | Primary Target | 1 | RED | | Measures |
| ☐ Organelles 1 | Organelles 1 | Primary Target | 1 | RED | | Measures |
| ☐ Organelles 2 | Organelles 2 | Primary Target | 1 | RED | Fast Puncta | Measures |

Custom

Pre-trained Model: Nuclei.a.h5

☐ Supplement Splitting

Filters

☐ Exclude objects touching edges

Min. Area: 0
Max. Area: 18000000
Min. Intensity: 1
Max. Intensity: 65536

| Part | Name |
|---|---|
| 1 | Protocol Editor Table |
| 2 | Analysis Settings |

**Tip:** More information on filters and *supplement splitting* is accessible through the information panel. Click the ⓘ icon in the **Analysis Settings** tab for details.

6. Click **Apply**.
7. If you are satisfied with the segmentation, in the **Protocol Editor Table**, click **Run Protocol** to run the analysis on the dataset. Otherwise, proceed to Refining a Pre-Trained Model on page 12.

## Refining a Pre-Trained Model

Consider the following when generating a training set:

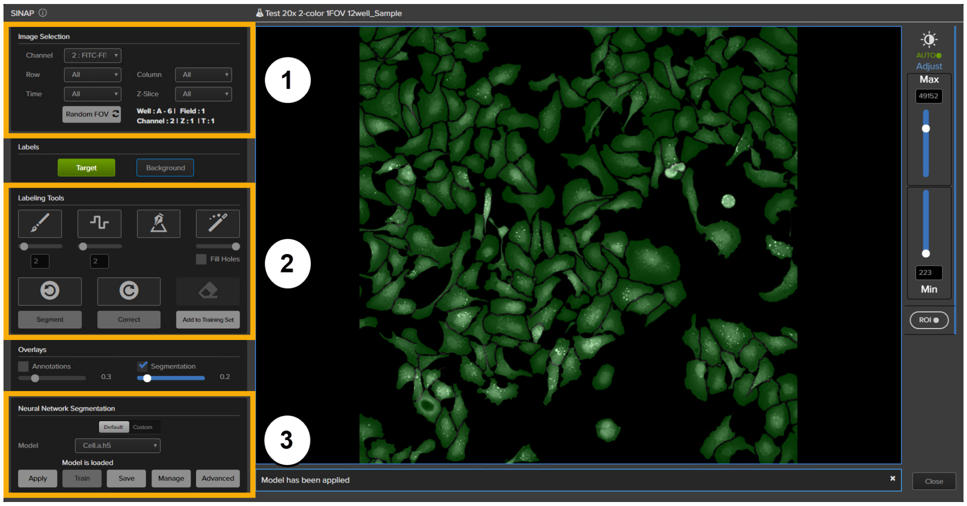
- Different methods of adding images to training set can be used to generate a training set.
- ROI can be used to specify a properly segmented region within an active image. When ROI is active and **Add to Training Set** is clicked, then only the ROI will be added to the training set. The ROI position can be changed by holding **CTRL** on the keyboard and dragging using the left mouse button.
- Multiple ROIs can be added to a training set for any given FOV.
- Consistent labeling of an image is the most important factor. 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. The most useful information is found at boundaries between target and background. Use the brush tool to precisely annotate these areas.

- Poor image quality, densely packed and overlapping objects, and rare object examples are tougher and typically require more annotated ground truth to be added to a training set.
- The same input images can be annotated differently to train a model to recognize a new target. In this way, models can be trained to recognize multiple targets from the same input images.

To refine a pre-trained model:

1. On the **Protocol Editor Table**, click **SINAP** to launch the IN Carta SINAP application window.
2. In the **Image Selection** pane, select **Channel**, **Row**, **Column**, **Time**, and **Z-slice** from the drop-down lists.



Channel 2 : FITC-FI
Row All
Column All
Time All
Z-Slice All
Random FOV
Well : A - 6 | Field : 1
Channel : 2 | Z : 1 | T : 1
2
2
Annotations 0.3
Segmentation 0.2
Model Cell.a.h5
Model is loaded
Max 49152
223 Min
Model has been applied

| Part | Name |
|---|---|
| 1 | Image Selection Pane |
| 2 | Labeling Tools Pane |
| 3 | Neural Network Segmentation Pane |

## AI-Assisted Segmentation

When a pre-trained model produces results that are close to expected and only minor corrections are required, you only need to fine-tune the model by correcting areas that have been mislabeled as target or background. This can be done using Workflow 1: Minor Corrections on this page.

In cases where a model produces suboptimal segmentation and requires training, refer to Workflow 2: Major Corrections on page 14.

### Workflow 1: Minor Corrections

Use this workflow if a model produces optimal segmentation and requires only 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, use the **Adjust** sliders to modify the brightness and contrast.
3. (Optional) Define a region of interest by clicking **ROI** to speed up image segmentation.
4. In the **Neural Network Segmentation** pane, click the **Model** drop-down list, and select the model.
5. Click **Apply**.
6. In the **Overlays** pane, use the **Annotation** and **Segmentation** sliders to adjust the opacity of the masks.
7. In the **Labels** pane, select one of the following:
    - Select the **Background** label to train the model to remove false-positive regions.
    - Select the **Target** label to train the model to remove false-negative regions.
8. In the **Labeling Tools** pane, select the labeling tool that best fits the objects/areas to be labeled. The following table describes the tool options:

| Labeling Tool | Description |
|---|---|
| Brush | Allows free-hand annotation – the circle size of the cursor corresponds to the size of the brush stroke. |
| Line | Allows annotating a series of lines using mouse clicks. |
| Polygon | Allows for annotation of larger regions. |
| Connected Component | Labels image regions with intensities like selected pixels. It can be used for quick annotation of structures of interest.<br>a. Adjust the slider bar to change the sensitivity.<br>b. Use the **Fill Holes** check box to include gaps in the annotated area.<br>c. Add more annotations to correct the segmentation of desired structures. |

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** in the **Labeling Tools** pane 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, use the **Adjust** sliders to modify the brightness and contrast.
3. Using the **Labeling Tools** described in step 9 of Workflow 1: Minor Corrections on page 13, annotate example regions for Target and Background. ROI may be used to speed up segmentation. ROI must include annotations for both Target and Background.
4. Click **Segment** in the **Labeling Tools** panel to segment the current FOV.

   **Note:** A machine learning model is used to predict all unlabeled pixels in an image, assigning them as either Target or Background. This model can be re-used for any new FOV that is loaded or when the ROI is moved to a new position.

   a. If the resulting segmentation mask is not satisfactory, more annotations can be added, followed by clicking **Segment**.
   b. (Optional) Corrections can be added to a segmentation mask as described in steps 6 to 10 of Workflow 1: Minor Corrections on page 13.
5. When the segmentation of the image is satisfactory, the FOV or ROI can be added to the training set by clicking **Add to Training Set** in the **Labeling Tools** panel.

## Manual Segmentation

When applying a segmentation model or using the **Segment** button in the **Labeling Tools** panel 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, use the **Adjust** sliders to modify the brightness and contrast.
3. Using the **Labeling Tools**, annotate only the structures of interest as **Target**. All unlabeled pixels will be assigned as **Background**.
4. Click **Correct**.
5. Repeat steps 3 and 4, if needed.
6. When the segmentation of the image is satisfactory, the FOV or ROI can be added to the training set by clicking **Add to Training Set** in the **Labeling Tools** panel.

## Using a Training Set to Train a Model

After adding images to the training set, they can be used to train a model.

To use a training set to train a module:

1. Select either a **Default** model from the drop-down list or a **Custom** model using file browse dialog in the **Neural Network Segmentation** panel.

   **Tip:** Consider adjusting **Advanced** parameters using the options seen in the table below. (More information on advanced parameters is accessible through the information panel – to access click the ⓘ icon.)

   | Advanced Parameters | Functionality |
   |---|---|
   | Fine tune | Use when the base model is closely related to images in the training set. |
   | Retrain | Use when creating a model for a distinct biological structure. |
   | Number of epochs | Increase to allocate more time to obtain a potentially more robust model. |

2. Click **Train** to train the model based on the images in the training set.
3. Test the segmentation on the new FOVs to check performance.

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

4. If you are satisfied with the segmentation results, in the **Neural Network Segmentation** panel, click **Save**.
5. 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.

| Model Name | Date Created |
|---|---|
| zebrafish_TL-2020322130327.a.h5 | 22-Mar-20 13:03 |
| zebrafish_GFP-2020322130408.a.h5 | 22-Mar-20 19:03 |
| iPSC_green-2020722100439.a.h5 | 22-Jul-20 10:04 |
| iPSC_DsRed-2020722100509.a.h5 | 4-Aug-20 09:04 |
| FYVE_GFP-202084090554.a.h5 | 4-Feb-21 11:05 |
| FYVE_RFP-202124150604.a.h5 | 4-Feb-21 15:06 |
| H2B_GFP-202124150647.a.h5 | 12-Apr-21 13:21 |
| LaminA_Alx488-2021416152159.a.h5 | 16-Apr-21 15:21 |
| tubulin_GFP-2021823153435.a.h5 | 23-Aug-21 15:34 |
| woundHealing_BD-2021823153511.a.h5 | 23-Nov-21 13:26 |
| Bovine_cells-2022310145526.a.h5 | 10-Mar-22 14:55 |

Note that removed models are not deleted; they remain in the `c:\ProgramData\TSModels` folder.

You can now access the model from **2D Analysis** in the **Analyze** dashboard.

# Chapter 4: Troubleshooting

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This section contains troubleshooting information for the IN Carta SINAP module.

- Updating Parameters for an Underpowered GPU, see below
- Diagnosing GPU Issues, see page 18

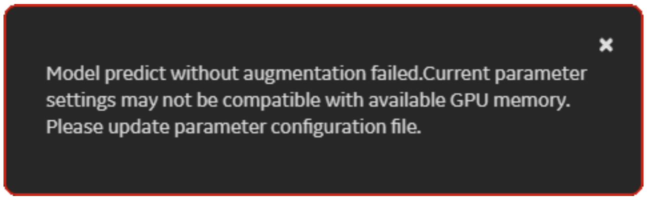
## Updating Parameters for an Underpowered GPU

The IN Carta 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-gpu.
- 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.



Model predict without augmentation failed.Current parameter settings may not be compatible with available GPU memory. Please update parameter configuration file.

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

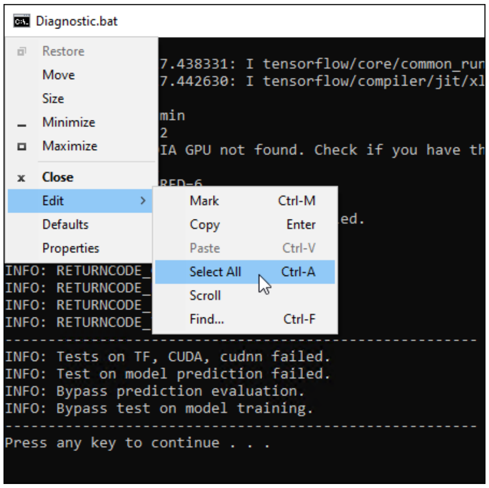
The Neural Network Diagnostic tool runs a suite of experiments to test whether your graphics processing unit (GPU) hardware and software are appropriate for the SINAP module. If the initial tests do not succeed, the tool automatically runs the tests again with different settings. When it determines the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



```
7.438331: I tensorflow/core/common_run
7.442630: I tensorflow/compiler/jit/xl

min
2
IA GPU not found. Check if you have th

ed.
INFO: RETURNCODE_
INFO: RETURNCODE_
INFO: RETURNCODE_
INFO: RETURNCODE_
-------------------------------------------------------
INFO: Tests on TF, CUDA, cudnn failed.
INFO: Test on model prediction failed.
INFO: Bypass prediction evaluation.
INFO: Bypass test on model training.
-------------------------------------------------------
Press any key to continue . . .
```

   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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