STAR Protocols

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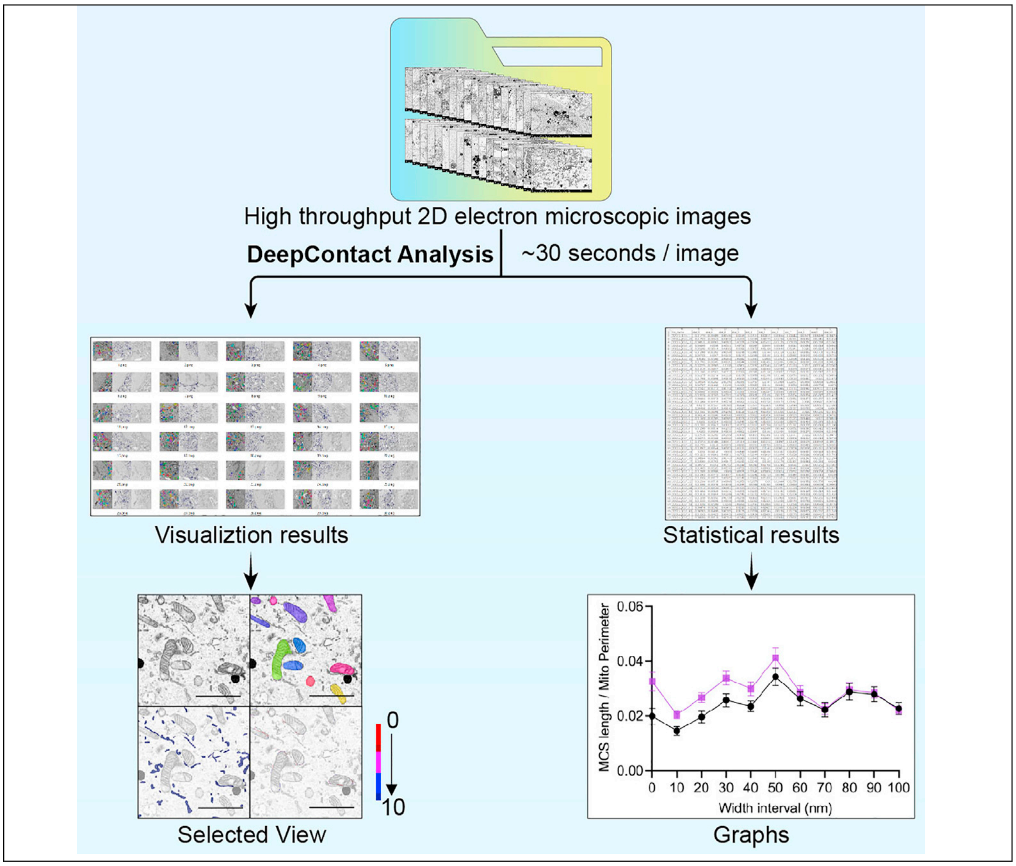
Protocol

Using DeepContact with Amira graphical user interface



DeepContact is a deep learning software for high-throughput quantification of membrane contact site (MCS) in 2D electron microscopy images. This protocol will guide users through incorporating available DeepContact models in Amira's artificial intelligence module, thereby allowing invoking of DeepContact functions in organelle segmentation and quantifying of MCS with a user-friendly graphical user interface of Amira software.

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Highlights

DeepContact is a software to quantify MCS

The protocol is for incorporating DeepContact models into Amira

Amira GUI can be used to perform DeepContact analysis of MCS after the installation

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Using DeepContact with Amira graphical user interface

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SUMMARY

DeepContact is a deep learning software for high-throughput quantification of membrane contact site (MCS) in 2D electron microscopy images. This protocol will guide users through incorporating available DeepContact models in Amira's artificial intelligence module, thereby allowing invoking of DeepContact functions in organelle segmentation and quantifying of MCS with a user-friendly graphical user interface of Amira software.

For complete details on the use and execution of this protocol, please refer to Liu et al. (2022).[1]

BEFORE YOU BEGIN

We have successfully incorporated DeepContact models into AI modules of Amira 2021.2 and Amira 2022.2.

Amira 2022.2 is commercially available and can be downloaded from its official website: https://www.fei-software-center.com/avizo/amira-avizo-packagedownload-97932f0f/.

We have successfully tested the procedure of installing DeepContact into Amira on Windows 10 and Windows 11.

"Deepcontact_Amira" installation package is publicly available at the figshare repository (https://doi.org/10.6084/m9.figshare.22655812).

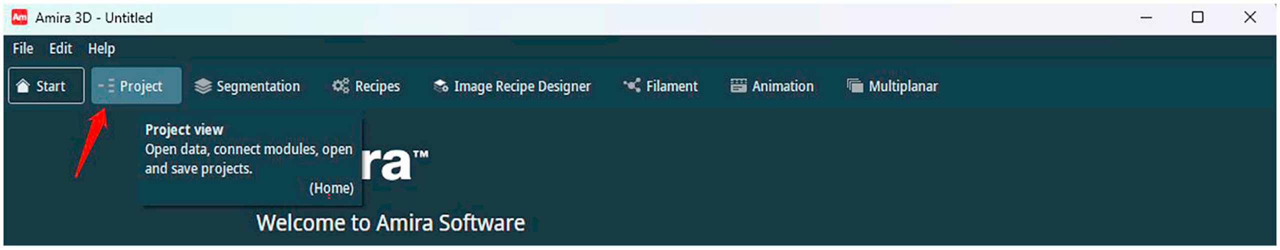
Example two-dimensional (2D) EM images for testing are publicly available at the figshare repository: https://doi.org/10.6084/m9.figshare.19898404.v3.

Preparation one: Operating environment preparation for DeepContact in Amira (2022.2)

Timing: ~30 min depending on the wire speed of the internet

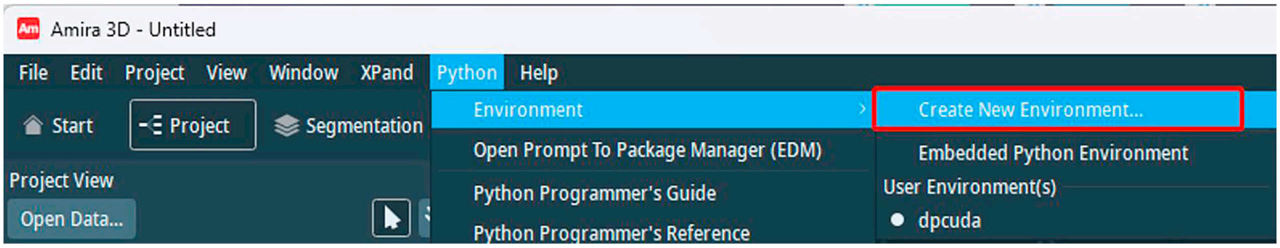
1. Download Amira 2022.2 from its official website: https://www.fei-software-center.com/avizo/amira-avizo-packagedownload-97932f0f/.

2. Install Amira according to the default settings.
3. Create a python3-based deep learning environment (Recommend to use Python 3.6).
   a. On the tool bar, click "Project".

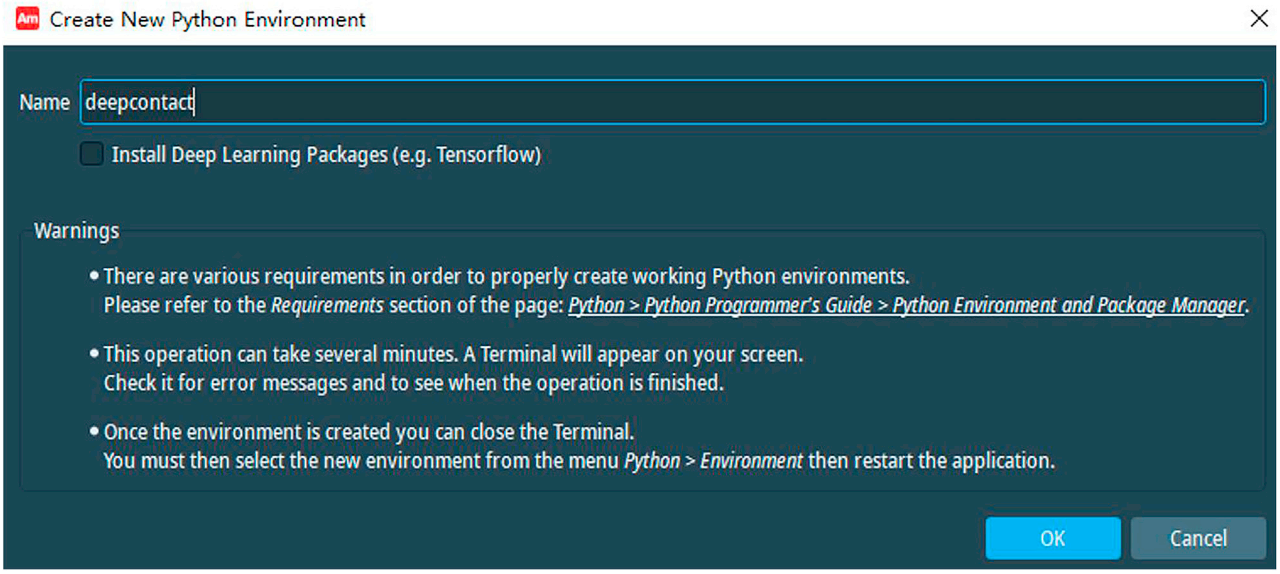


Project view
Open data, connect modules, open and save projects.
(Home)

   b. On the menu bar, click "Python" -> "Environment" -> "Create New Environment".



   c. Enter environment name, e.g., "deepcontact" as indicated below, then Click "OK".

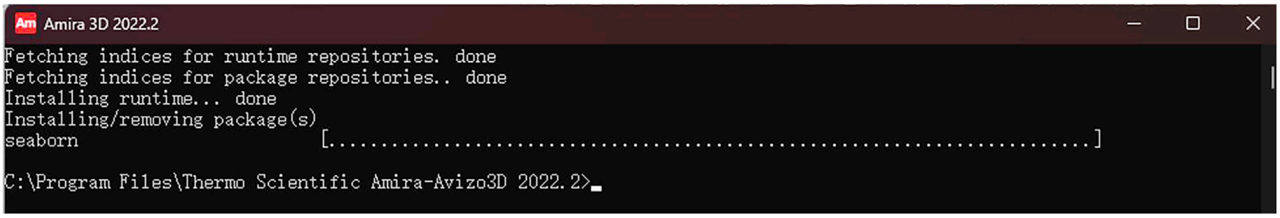


Name: deepcontact

☐ Install Deep Learning Packages (e.g. Tensorflow)

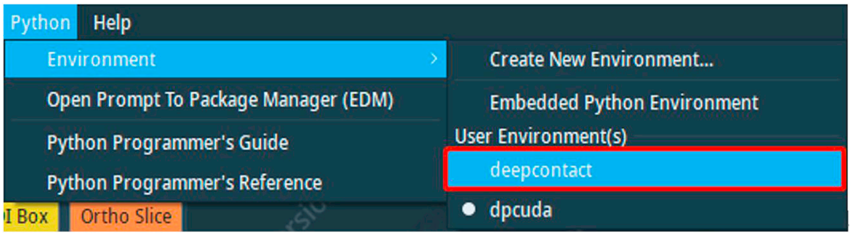
- There are various requirements in order to properly create working Python environments. Please refer to the *Requirements* section of the page: *Python > Python Programmer's Guide > Python Environment and Package Manager*.
- This operation can take several minutes. A Terminal will appear on your screen. Check it for error messages and to see when the operation is finished.
- Once the environment is created you can close the Terminal. You must then select the new environment from the menu *Python* > *Environment* then restart the application.

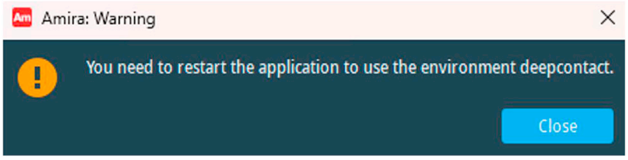
   d. The command-line interface popup and a new python environment which support running DeepContact will be installed automatically. Installation is accomplished if the interface below appears.



```
Fetching indices for runtime repositories. done
Fetching indices for package repositories.. done
Installing runtime... done
Installing/removing package(s)
seaborn                        [.........................................................................]

C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2>_
```

   e. After creating the deepcontact environment, select “Python”->“Environment” ->“deepcontact”, and then restart Amira.



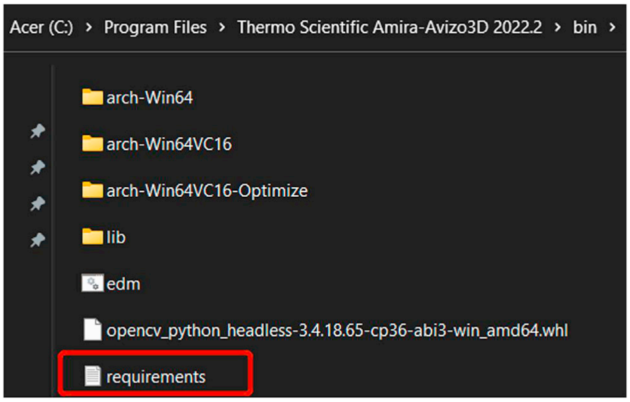


You need to restart the application to use the environment deepcontact.

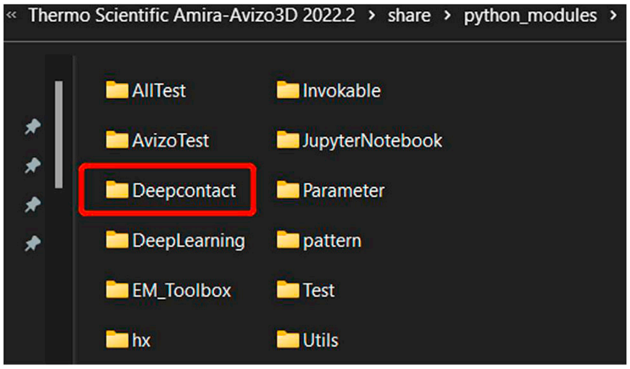
Preparation two: Installation of DeepContact in Amira

Timing: ~1–2 h depending on the wire speed of the internet

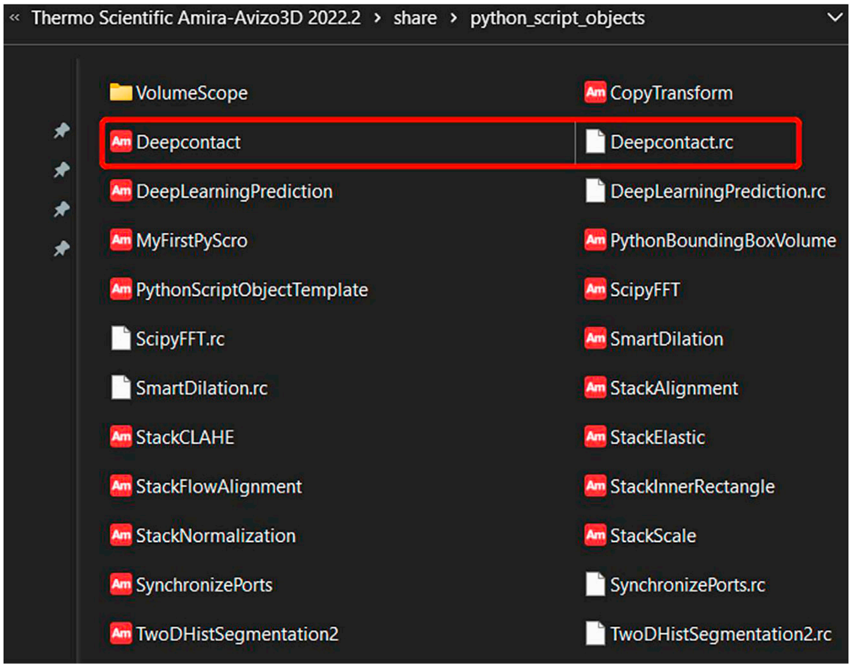
4. Download “Deepcontact_Amira” installation package (https://doi.org/10.6084/m9.figshare.22655812).
5. Decompress “Deepcontact_Amira” installation package and copy all components to the Amira installation directory. The package contains multiple directories or files: requirements.txt, python_modules, python_script_objects and xml.
   a. Copy "Deepcontact_Amira\requirements.txt" into "C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\bin".



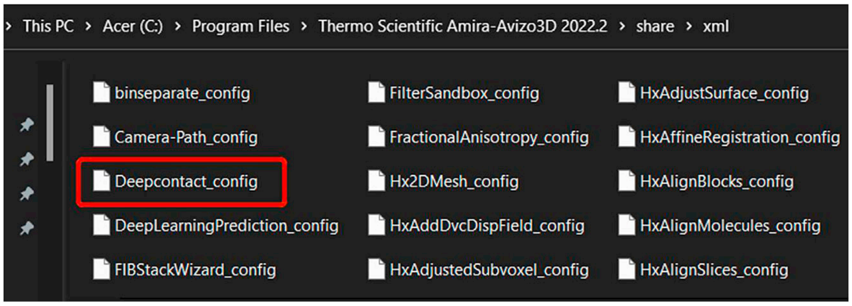
b. Copy all contents under "Deepcontact_Amira\python_modules" to "C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\share\python_modules".



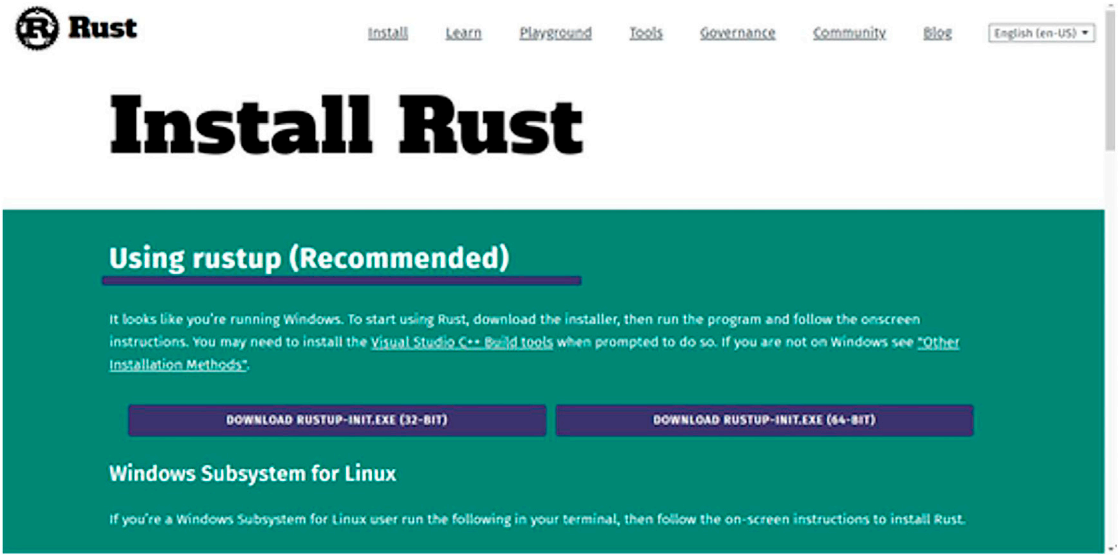
c. Copy all contents under "Deepcontact_Amira\python_script_objects" to "C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\share\python_script_objects".



d. Copy all contents under "Deepcontact_Amira\xml" to "C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\share\xml".



6. Download Rust at https://www.rust-lang.org/tools/install, and then run "rustupp-init.exe" to install it with the default configuration.



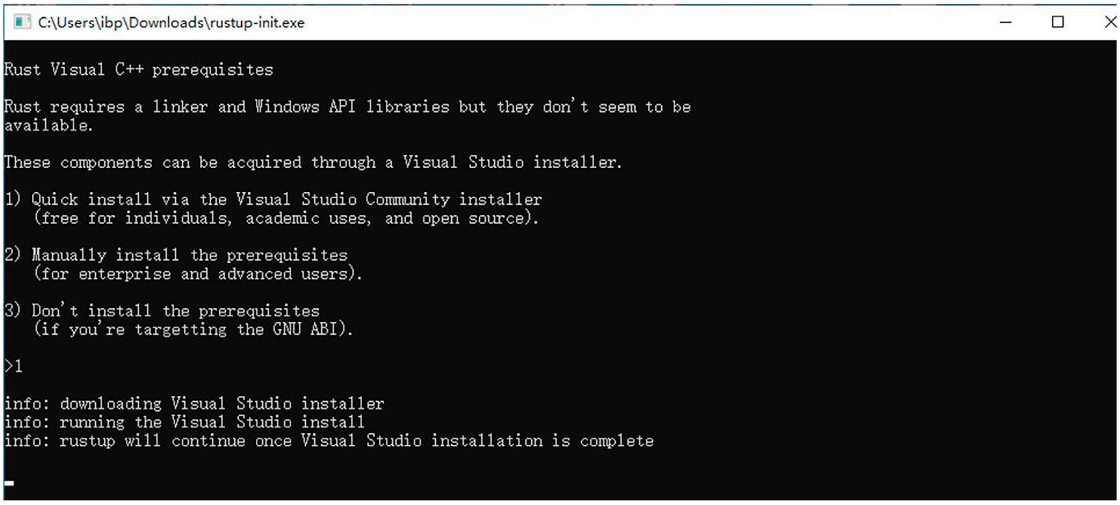
Install Rust

Using rustup (Recommended)

It looks like you're running Windows. To start using Rust, download the installer, then run the program and follow the onscreen instructions. You may need to install the Visual Studio C++ Build tools when prompted to do so. If you are not on Windows see "Other Installation Methods".

Windows Subsystem for Linux

If you're a Windows Subsystem for Linux user run the following in your terminal, then follow the on-screen instructions to install Rust.

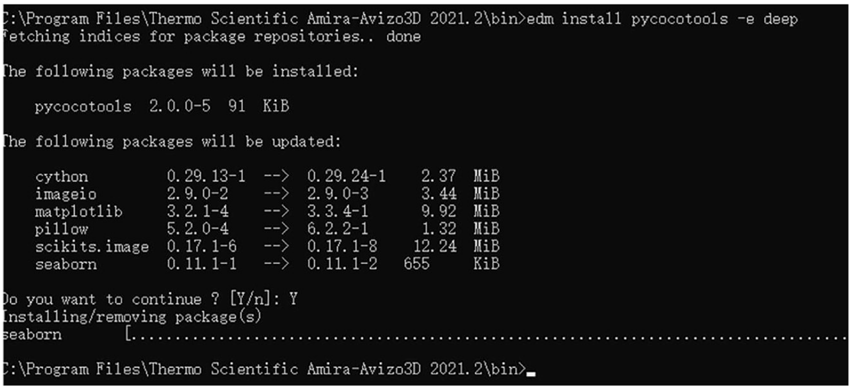


```
Rust Visual C++ prerequisites

Rust requires a linker and Windows API libraries but they don’t seem to be
available.

These components can be acquired through a Visual Studio installer.

1) Quick install via the Visual Studio Community installer
   (free for individuals, academic uses, and open source).

2) Manually install the prerequisites
   (for enterprise and advanced users).

3) Don’t install the prerequisites
   (if you’re targetting the GNU ABI).

>1

info: downloading Visual Studio installer
info: running the Visual Studio install
info: rustup will continue once Visual Studio installation is complete

_
```

7. Install the python dependencies required for Deepcontact
   a. Give Amira software administrator privileges before installation to avoid errors caused by insufficient permissions. If you are using the Windows 10 operating system, you can give software administrator privileges by following these steps:
      i. Select Amira software and right-click on it.
      ii. Select ‘Run as administrator’.
   b. Return to the Amira interface and click "project" -> "Python" -> "Open Prompt To Package Manager (EDM)" on the menu bar.

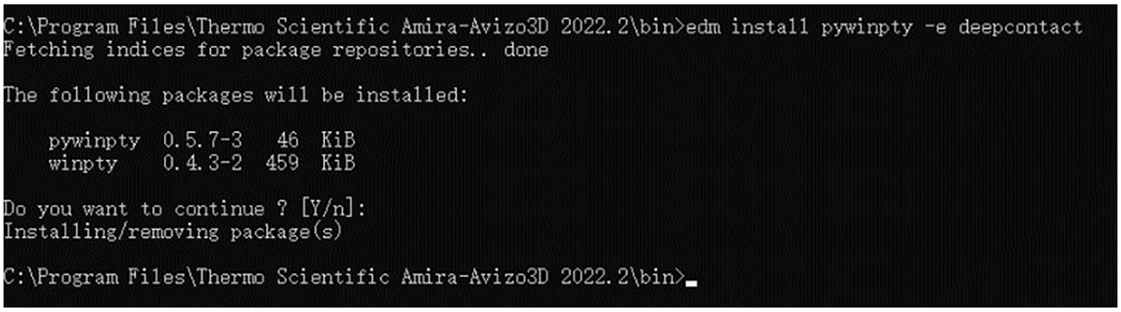
c. Make sure the current directory is in "C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\bin", if not, use "cd bin" to enter the directory.
d. Run the following command to install pycocotools and pywinpty,

```
>edm install pycocotools -e deepcontact ("STAR Protocols.Rdata")
```



```
:\Program Files\Thermo Scientific Amira-Avizo3D 2021.2\bin>edm install pycocotools -e deep
etching indices for package repositories.. done

he following packages will be installed:

   pycocotools  2.0.0-5  91  KiB

he following packages will be updated:

   cython         0.29.13-1  -->  0.29.24-1    2.37  MiB
   imageio        2.9.0-2    -->  2.9.0-3      3.44  MiB
   matplotlib     3.2.1-4    -->  3.3.4-1      9.92  MiB
   pillow         5.2.0-4    -->  6.2.2-1      1.32  MiB
   scikits.image  0.17.1-6   -->  0.17.1-8    12.24  MiB
   seaborn        0.11.1-1   -->  0.11.1-2   655     KiB

o you want to continue ? [Y/n]: Y
nstalling/removing package(s)
eaborn       [.............................................................................

:\Program Files\Thermo Scientific Amira-Avizo3D 2021.2\bin>
```

```
>edm install pywinpty -e deepcontact ("STAR Protocols.Rdata")
```

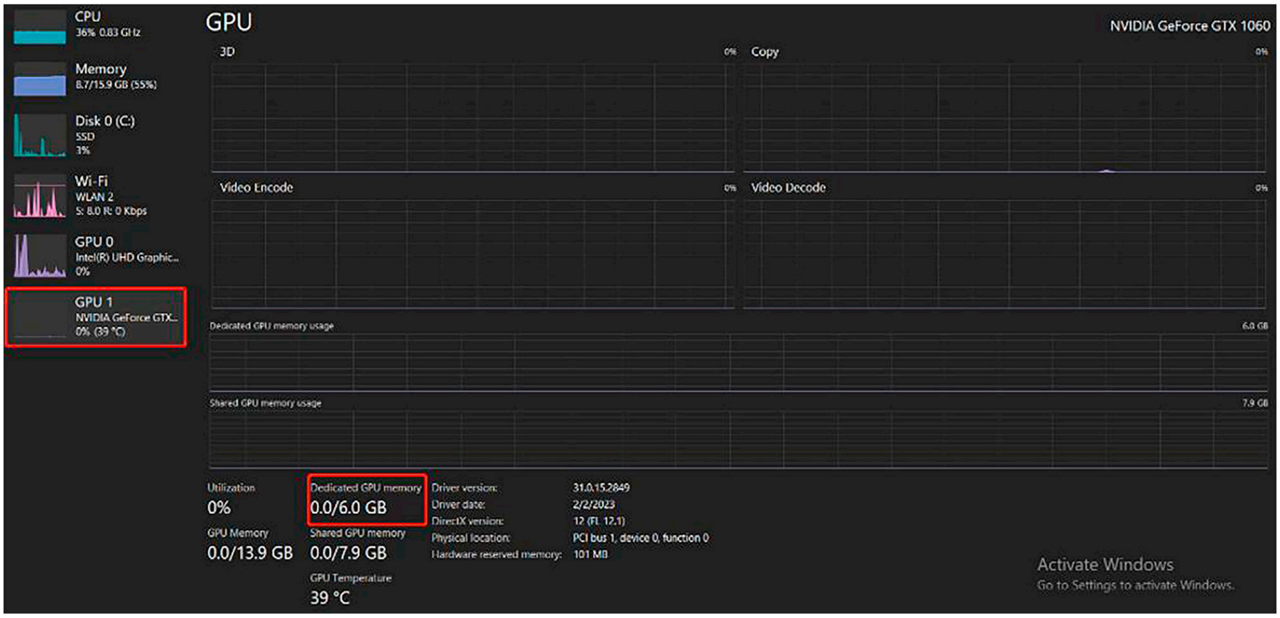


```
C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\bin>edm install pywinpty -e deepcontact
Fetching indices for package repositories.. done

The following packages will be installed:

    pywinpty  0.5.7-3   46  KiB
    winpty    0.4.3-2  459  KiB

Do you want to continue ? [Y/n]:
Installing/removing package(s)

C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\bin>
```

e. Enter "edm shell -e deepcontact" to switch to the "deepcontact" virtual environment,

```
>edm shell -e deepcontact ("STAR Protocols.Rdata")
```

f. Check if your GPU has at least 6 GB memory for Deepcontact inference. If not, skip the following step (step 4-h) to install CPU version instead. The pytorch-cpu can be installed directly via step 4-i. One way to use Task Manager to check the total memory for the Nvidia GPU is:
   i. Click the “More details” option at the bottom of the Task Manager window if you see the standard simple view,
   ii. In the full view of Task Manager, click the “Performance” tab,
   iii. Select your Nvidia GPU from the list on the left side,
   iv. You will see a graph and some numbers that show your GPU memory usage and total memory.



Utilization
0%
GPU Memory
0.0/13.9 GB
Dedicated GPU memory
0.0/6.0 GB
Shared GPU memory
0.0/7.9 GB
GPU Temperature
39 °C
Driver version: 31.0.15.2849
Driver date: 2/2/2023
DirectX version: 12 (FL 12.1)
Physical location: PCI bus 1, device 0, function 0
Hardware reserved memory: 101 MB

g. Run “pip install typing-extensions”,

```
>pip install typing-extensions ("STAR Protocols.Rdata")
```

h. To install a GPU version, run the following commands to install pytorch-gpu and torchvision,

```
>pip install torch==1.8.0+cu111 torchvision==0.9.0+cu111 -f https://download.pytorch.org/whl/
torch_stable.html ("STAR Protocols.Rdata")
```

```
(deepcontact)C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\bin>pip install torch==1.8.0+cu111 torchvision==0.9
.0+cu111 -f https://download.pytorch.org/whl/torch_stable.html
Looking in indexes: http://mirrors.aliyun.com/pypi/simple/
Looking in links: https://download.pytorch.org/whl/torch_stable.html
WARNING: Retrying (Retry(total=4, connect=None, read=None, redirect=None, status=None)) after connection broken by 'Prot
ocolError('Connection aborted.', ConnectionResetError(10054, '远程主机强迫关闭了一个现有的连接。', None, 10054, None))':
 /whl/torch_stable.html
Collecting torch==1.8.0+cu111
  Using cached https://download.pytorch.org/whl/cu111/torch-1.8.0%2Bcu111-cp36-cp36m-win_amd64.whl (3055.7 MB)
Collecting torchvision==0.9.0+cu111
  Using cached https://download.pytorch.org/whl/cu111/torchvision-0.9.0%2Bcu111-cp36-cp36m-win_amd64.whl (1.9 MB)
Requirement already satisfied: numpy in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from torch==1.8.0+cu111)
 (1.17.4)
Collecting dataclasses
  Downloading http://mirrors.aliyun.com/pypi/packages/fe/ca/75fac5856ab5cfa51bbbcefa250182e50441074fdc3f803f6e76451fab43
/dataclasses-0.8-py3-none-any.whl (19 kB)
Requirement already satisfied: typing-extensions in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from torch==
1.8.0+cu111) (3.10.0.2)
Requirement already satisfied: pillow>=4.1.1 in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from torchvision
==0.9.0+cu111) (7.2.0)
Installing collected packages: dataclasses, torch, torchvision
Successfully installed dataclasses-0.8 torch-1.8.0+cu111 torchvision-0.9.0+cu111
```

i. Run the following commands to install labelme and other required packages,

```
(deepcontact)C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\bin>pip install labelme
Looking in indexes: http://mirrors.aliyun.com/pypi/simple/
Collecting labelme
  Downloading http://mirrors.aliyun.com/pypi/packages/cf/2b/4a06be33ed86cc5227945b8dabb7d8f9d9c6e854f0de966a601738ceda69
/labelme-5.2.0.tar.gz (1.5 MB)
     |████████████████████████████████ 1.5 MB 384 kB/s
  Preparing metadata (setup.py) ... done
Collecting imgviz>=0.11
  Downloading http://mirrors.aliyun.com/pypi/packages/b1/b9/5c4f30e9685d24de44fcbc764d5e03d073710e29dbf25d6de25ba2839084
/imgviz-1.7.2-py3-none-any.whl (7.7 MB)
     |████████████████████████████████ 7.7 MB 384 kB/s
Requirement already satisfied: matplotlib in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from labelme) (3.3.
4)
Collecting natsort>=7.1.0
  Downloading http://mirrors.aliyun.com/pypi/packages/3e/58/61c4b4fd9e597affdcd3347d5991fa5be404af26f19932d3116b67e133da
/natsort-8.2.0-py3-none-any.whl (37 kB)
Requirement already satisfied: numpy in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from labelme) (1.17.4)
Requirement already satisfied: Pillow>=2.8 in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from labelme) (7.2
.0)
Requirement already satisfied: PyYAML in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from labelme) (5.1)
Collecting qtpy!=1.11.2
  Downloading http://mirrors.aliyun.com/pypi/packages/ae/b0/56e602873b05108f0ef9189a237fefcfbcd2fa3d84130b59e50c84fc90e8
/QtPy-2.0.1-py3-none-any.whl (65 kB)
     |████████████████████████████████ 65 kB 369 kB/s
Collecting termcolor
  Using cached termcolor-1.1.0-py3-none-any.whl
Requirement already satisfied: colorama in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from labelme) (0.4.4)
Requirement already satisfied: packaging in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from qtpy!=1.11.2->l
abelme) (20.9)
Requirement already satisfied: cycler>=0.10 in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from matplotlib->
labelme) (0.10.0)
Requirement already satisfied: kiwisolver>=1.0.1 in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from matplot
lib->labelme) (1.0.1)
Requirement already satisfied: pyparsing!=2.0.4,!=2.1.2,!=2.1.6,>=2.0.3 in c:\users\gwawh\.edm\envs\deepcontact\lib\site
-packages (from matplotlib->labelme) (2.2.0)
Requirement already satisfied: python-dateutil>=2.1 in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from matp
lotlib->labelme) (2.8.0)
Requirement already satisfied: six in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from cycler>=0.10->matplot
lib->labelme) (1.15.0)
Requirement already satisfied: setuptools in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from kiwisolver>=1.
0.1->matplotlib->labelme) (41.6.0)
Using legacy 'setup.py install' for labelme, since package 'wheel' is not installed.
Installing collected packages: termcolor, qtpy, natsort, imgviz, labelme
    Running setup.py install for labelme ... done
Successfully installed imgviz-1.7.2 labelme-5.2.0 natsort-8.2.0 qtpy-2.0.1 termcolor-1.1.0

(deepcontact)C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\bin>
```

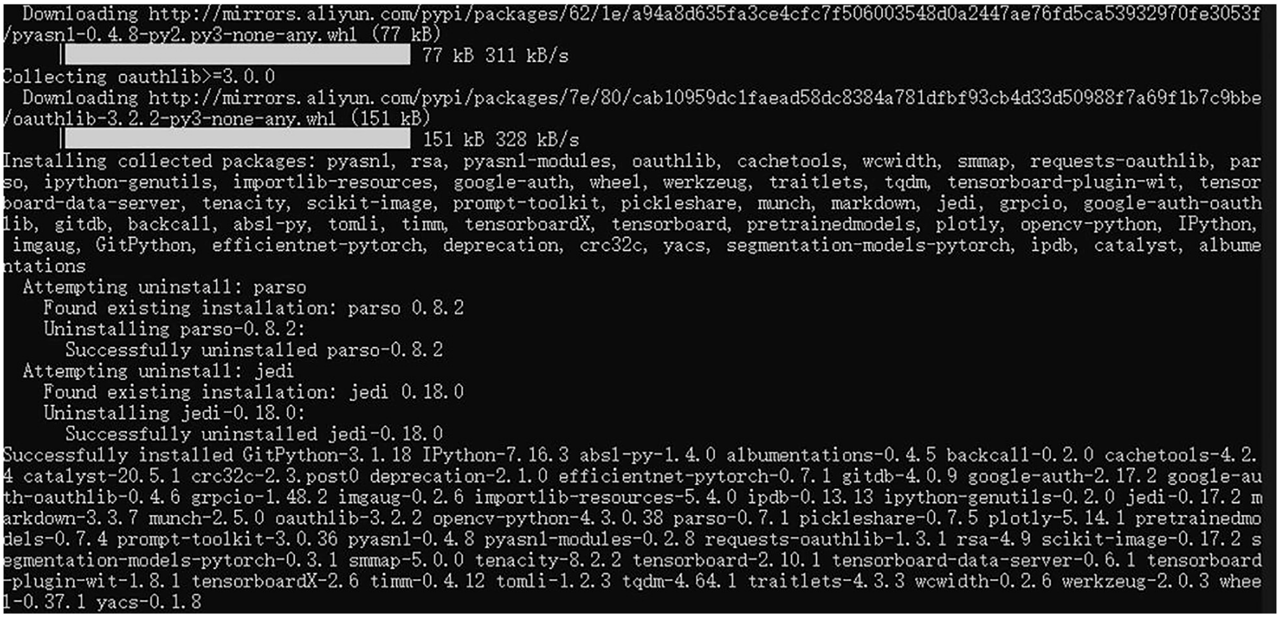
```
>pip install labelme ("STAR Protocols.Rdata")

>pip install -r requirements.txt ("STAR Protocols.Rdata")
```

Possible issues: "failure to install opencv-python-headless" or "fail to build wheel for opencv python" (refer to troubleshooting session, problem 1 also).

Choose one of the following solutions to resolve the issue:

(1) Update pip and cmake, and then reinstall opencv-python.
   - i. Update pip: enter "pip install –upgrade pip"
   - ii. Update cmake: enter "pip install cmake"
   - iii. Install opencv-python: enter "pip install opencv-python==4.3.0.38"

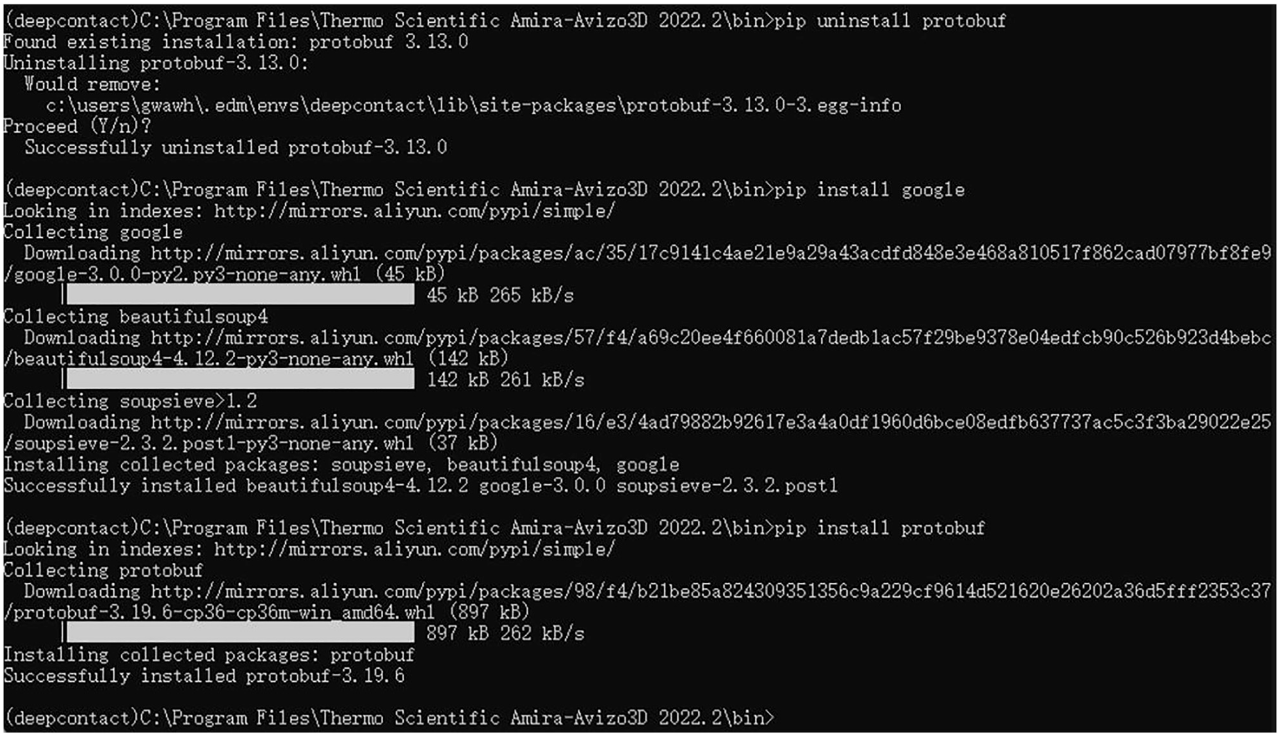
(2) Install opencv-python-headless manually
   - iv. Go to https://pypi.org/project/opencv-python-headless/3.4.18.65/#files ,
   - v. Download "opencv_python_headless-3.4.18.65-cp36-abi3-win_amd64.whl". If you are on a 32-bit machine, download "opencv_python_headless-3.4.18.65-cp36-abi3-win32.whl",
   - vi. Copy the downloaded Files to "C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\bin",
   - vii. Return to the Amira interface, On the menu bar, Click "project" -> "Python" -> "Open Prompt To Package Manager (EDM)",
   - viii. Enter "edm shell -e deepcontact",
   - ix. Enter "pip3 install opencv_python_headless-3.4.18.65-cp36-abi3-win_amd64.whl".



```
  Downloading http://mirrors.aliyun.com/pypi/packages/62/1e/a94a8d635fa3ce4cfc7f506003548d0a2447ae76fd5ca53932970fe3053f
/pyasn1-0.4.8-py2.py3-none-any.whl (77 kB)
     |████████████████████████████████| 77 kB 311 kB/s
Collecting oauthlib>=3.0.0
  Downloading http://mirrors.aliyun.com/pypi/packages/7e/80/cab10959dc1faead58dc8384a781dfbf93cb4d33d50988f7a69f1b7c9bbe
/oauthlib-3.2.2-py3-none-any.whl (151 kB)
     |████████████████████████████████| 151 kB 328 kB/s
Installing collected packages: pyasn1, rsa, pyasn1-modules, oauthlib, cachetools, wcwidth, smmap, requests-oauthlib, par
so, ipython-genutils, importlib-resources, google-auth, wheel, werkzeug, traitlets, tqdm, tensorboard-plugin-wit, tensor
board-data-server, tenacity, scikit-image, prompt-toolkit, pickleshare, munch, markdown, jedi, grpcio, google-auth-oauth
lib, gitdb, backcall, absl-py, tomli, timm, tensorboardX, tensorboard, pretrainedmodels, plotly, opencv-python, IPython,
 imgaug, GitPython, efficientnet-pytorch, deprecation, crc32c, yacs, segmentation-models-pytorch, ipdb, catalyst, albume
ntations
  Attempting uninstall: parso
    Found existing installation: parso 0.8.2
    Uninstalling parso-0.8.2:
      Successfully uninstalled parso-0.8.2
  Attempting uninstall: jedi
    Found existing installation: jedi 0.18.0
    Uninstalling jedi-0.18.0:
      Successfully uninstalled jedi-0.18.0
Successfully installed GitPython-3.1.18 IPython-7.16.3 absl-py-1.4.0 albumentations-0.4.5 backcall-0.2.0 cachetools-4.2.
4 catalyst-20.5.1 crc32c-2.3.post0 deprecation-2.1.0 efficientnet-pytorch-0.7.1 gitdb-4.0.9 google-auth-2.17.2 google-au
th-oauthlib-0.4.6 grpcio-1.48.2 imgaug-0.2.6 importlib-resources-5.4.0 ipdb-0.13.13 ipython-genutils-0.2.0 jedi-0.17.2 m
arkdown-3.3.7 munch-2.5.0 oauthlib-3.2.2 opencv-python-4.3.0.38 parso-0.7.1 pickleshare-0.7.5 plotly-5.14.1 pretrainedmo
dels-0.7.4 prompt-toolkit-3.0.36 pyasn1-0.4.8 pyasn1-modules-0.2.8 requests-oauthlib-1.3.1 rsa-4.9 scikit-image-0.17.2 s
egmentation-models-pytorch-0.3.1 smmap-5.0.0 tenacity-8.2.2 tensorboard-2.10.1 tensorboard-data-server-0.6.1 tensorboard
-plugin-wit-1.8.1 tensorboardX-2.6 timm-0.4.12 tomli-1.2.3 tqdm-4.64.1 traitlets-4.3.3 wcwidth-0.2.6 werkzeug-2.0.3 whee
l-0.37.1 yacs-0.1.8
```

j. Reinstalling the "protobuf" package. Run the following commands to update "protobuf" package and install the "google" package.

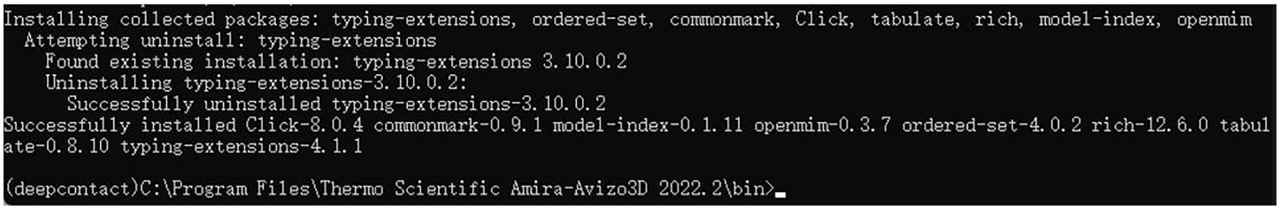
```
>pip uninstall protobuf

>pip install google

> pip install protobuf
```



```
(deepcontact)C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\bin>pip uninstall protobuf
Found existing installation: protobuf 3.13.0
Uninstalling protobuf-3.13.0:
  Would remove:
    c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages\protobuf-3.13.0-3.egg-info
Proceed (Y/n)?
  Successfully uninstalled protobuf-3.13.0

(deepcontact)C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\bin>pip install google
Looking in indexes: http://mirrors.aliyun.com/pypi/simple/
Collecting google
  Downloading http://mirrors.aliyun.com/pypi/packages/ac/35/17c9141c4ae21e9a29a43acdfd848e3e468a810517f862cad07977bf8fe9
/google-3.0.0-py2.py3-none-any.whl (45 kB)
     |████████████████████████████████| 45 kB 265 kB/s
Collecting beautifulsoup4
  Downloading http://mirrors.aliyun.com/pypi/packages/57/f4/a69c20ee4f660081a7dedb1ac57f29be9378e04edfcb90c526b923d4bebc
/beautifulsoup4-4.12.2-py3-none-any.whl (142 kB)
     |████████████████████████████████| 142 kB 261 kB/s
Collecting soupsieve>1.2
  Downloading http://mirrors.aliyun.com/pypi/packages/16/e3/4ad79882b92617e3a4a0df1960d6bce08edfb637737ac5c3f3ba29022e25
/soupsieve-2.3.2.post1-py3-none-any.whl (37 kB)
Installing collected packages: soupsieve, beautifulsoup4, google
Successfully installed beautifulsoup4-4.12.2 google-3.0.0 soupsieve-2.3.2.post1

(deepcontact)C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\bin>pip install protobuf
Looking in indexes: http://mirrors.aliyun.com/pypi/simple/
Collecting protobuf
  Downloading http://mirrors.aliyun.com/pypi/packages/98/f4/b21be85a824309351356c9a229cf9614d521620e26202a36d5fff2353c37
/protobuf-3.19.6-cp36-cp36m-win_amd64.whl (897 kB)
     |████████████████████████████████| 897 kB 262 kB/s
Installing collected packages: protobuf
Successfully installed protobuf-3.19.6

(deepcontact)C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\bin>
```

k. Deepcontact is implemented based on the mmdetection framework. Run the following commands to install "openmim" "mmengine" and "mmcv", which are the bases of the mmdetection framework.

```
>pip install -U openmim("STAR Protocols.Rdata")
```

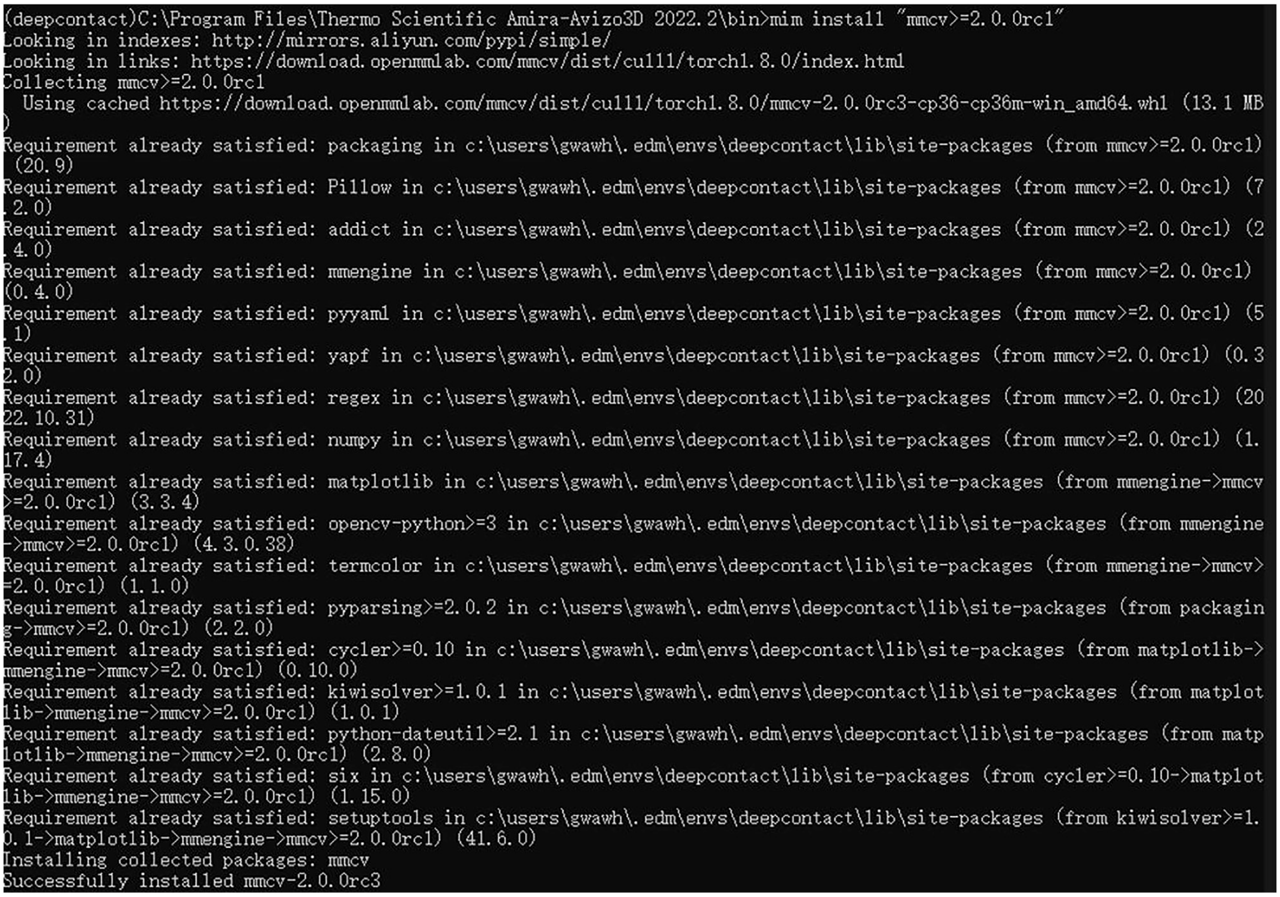


```
Installing collected packages: typing-extensions, ordered-set, commonmark, Click, tabulate, rich, model-index, openmim
  Attempting uninstall: typing-extensions
    Found existing installation: typing-extensions 3.10.0.2
    Uninstalling typing-extensions-3.10.0.2:
      Successfully uninstalled typing-extensions-3.10.0.2
Successfully installed Click-8.0.4 commonmark-0.9.1 model-index-0.1.11 openmim-0.3.7 ordered-set-4.0.2 rich-12.6.0 tabul
ate-0.8.10 typing-extensions-4.1.1

(deepcontact)C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\bin>_
```

```
>mim install mmengine("STAR Protocols.Rdata")
```

```
(deepcontact)C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\bin>mim install mmengine
Looking in indexes: http://mirrors.aliyun.com/pypi/simple/
Looking in links: https://download.openmmlab.com/mmcv/dist/cu111/torch1.8.0/index.html
Collecting mmengine
  Downloading http://mirrors.aliyun.com/pypi/packages/98/9b/1c0288d2e60af5f00bb9ed683b156f2b5b66e34e4937d8f3a46091ac1aab
/mmengine-0.4.0-py3-none-any.whl (317 kB)
     |████████████████████████████████ 317 kB 218 kB/s
Requirement already satisfied: termcolor in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from mmengine) (1.1.
0)
Collecting yapf
  Downloading http://mirrors.aliyun.com/pypi/packages/47/88/843c2e68f18a5879b4fbf37cb99fbabe1ffc4343b2e63191c8462235c008
/yapf-0.32.0-py2.py3-none-any.whl (190 kB)
     |████████████████████████████████ 190 kB 242 kB/s
Requirement already satisfied: pyyaml in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from mmengine) (5.1)
Requirement already satisfied: numpy in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from mmengine) (1.17.4)
Requirement already satisfied: opencv-python>=3 in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from mmengine
) (4.3.0.38)
Requirement already satisfied: matplotlib in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from mmengine) (3.3
.4)
Collecting regex
  Downloading http://mirrors.aliyun.com/pypi/packages/88/e0/d4251593cde041f3a9b249744da5b6e53d1ac4fa2542dfe251fe8070793b
/regex-2022.10.31-cp36-cp36m-win_amd64.whl (279 kB)
     |████████████████████████████████ 279 kB 242 kB/s
Collecting addict
  Downloading http://mirrors.aliyun.com/pypi/packages/6a/00/b08f23b7d7e1e14ce01419a467b583edbb93c6cdb8654e54a9cc579cd61f
/addict-2.4.0-py3-none-any.whl (3.8 kB)
Requirement already satisfied: cycler>=0.10 in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from matplotlib->
mmengine) (0.10.0)
Requirement already satisfied: kiwisolver>=1.0.1 in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from matplot
lib->mmengine) (1.0.1)
Requirement already satisfied: pillow>=6.2.0 in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from matplotlib-
>mmengine) (7.2.0)
Requirement already satisfied: pyparsing!=2.0.4,!=2.1.2,!=2.1.6,>=2.0.3 in c:\users\gwawh\.edm\envs\deepcontact\lib\site
-packages (from matplotlib->mmengine) (2.2.0)
Requirement already satisfied: python-dateutil>=2.1 in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from matp
lotlib->mmengine) (2.8.0)
Requirement already satisfied: six in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from cycler>=0.10->matplot
lib->mmengine) (1.15.0)
Requirement already satisfied: setuptools in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from kiwisolver>=1.
0.1->matplotlib->mmengine) (41.6.0)
Installing collected packages: yapf, regex, addict, mmengine
Successfully installed addict-2.4.0 mmengine-0.4.0 regex-2022.10.31 yapf-0.32.0
```

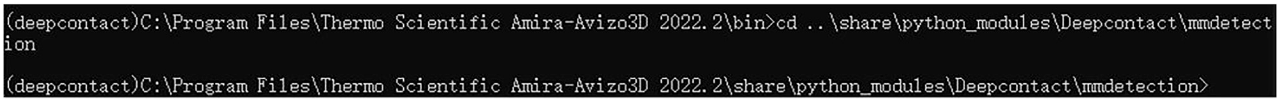
```
>mim install "mmcv>=2.0.0rc1"("STAR Protocols.Rdata")
```



```
(deepcontact)C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\bin>mim install "mmcv>=2.0.0rc1"
Looking in indexes: http://mirrors.aliyun.com/pypi/simple/
Looking in links: https://download.openmmlab.com/mmcv/dist/cu111/torch1.8.0/index.html
Collecting mmcv>=2.0.0rc1
  Using cached https://download.openmmlab.com/mmcv/dist/cu111/torch1.8.0/mmcv-2.0.0rc3-cp36-cp36m-win_amd64.whl (13.1 MB
)
Requirement already satisfied: packaging in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from mmcv>=2.0.0rc1)
 (20.9)
Requirement already satisfied: Pillow in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from mmcv>=2.0.0rc1) (7
.2.0)
Requirement already satisfied: addict in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from mmcv>=2.0.0rc1) (2
.4.0)
Requirement already satisfied: mmengine in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from mmcv>=2.0.0rc1)
(0.4.0)
Requirement already satisfied: pyyaml in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from mmcv>=2.0.0rc1) (5
.1)
Requirement already satisfied: yapf in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from mmcv>=2.0.0rc1) (0.3
2.0)
Requirement already satisfied: regex in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from mmcv>=2.0.0rc1) (20
22.10.31)
Requirement already satisfied: numpy in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from mmcv>=2.0.0rc1) (1.
17.4)
Requirement already satisfied: matplotlib in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from mmengine->mmcv
>=2.0.0rc1) (3.3.4)
Requirement already satisfied: opencv-python>=3 in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from mmengine
->mmcv>=2.0.0rc1) (4.3.0.38)
Requirement already satisfied: termcolor in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from mmengine->mmcv>
=2.0.0rc1) (1.1.0)
Requirement already satisfied: pyparsing>=2.0.2 in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from packagin
g->mmcv>=2.0.0rc1) (2.2.0)
Requirement already satisfied: cycler>=0.10 in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from matplotlib->
mmengine->mmcv>=2.0.0rc1) (0.10.0)
Requirement already satisfied: kiwisolver>=1.0.1 in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from matplot
lib->mmengine->mmcv>=2.0.0rc1) (1.0.1)
Requirement already satisfied: python-dateutil>=2.1 in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from matp
lotlib->mmengine->mmcv>=2.0.0rc1) (2.8.0)
Requirement already satisfied: six in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from cycler>=0.10->matplot
lib->mmengine->mmcv>=2.0.0rc1) (1.15.0)
Requirement already satisfied: setuptools in c:\users\gwawh\.edm\envs\deepcontact\lib\site-packages (from kiwisolver>=1.
0.1->matplotlib->mmengine->mmcv>=2.0.0rc1) (41.6.0)
Installing collected packages: mmcv
Successfully installed mmcv-2.0.0rc3
```

l. Enter "cd ..\share\python_modules\Deepcontact\mmdetection" to switch the current directory to "C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\share\python_modules\Deepcontact\mmdetection"

```
>cd ..\share\python_modules\Deepcontact\mmdetection ("STAR Protocols.Rdata")
```



```
(deepcontact)C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\bin>cd ..\share\python_modules\Deepcontact\mmdetect
ion

(deepcontact)C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\share\python_modules\Deepcontact\mmdetection>
```

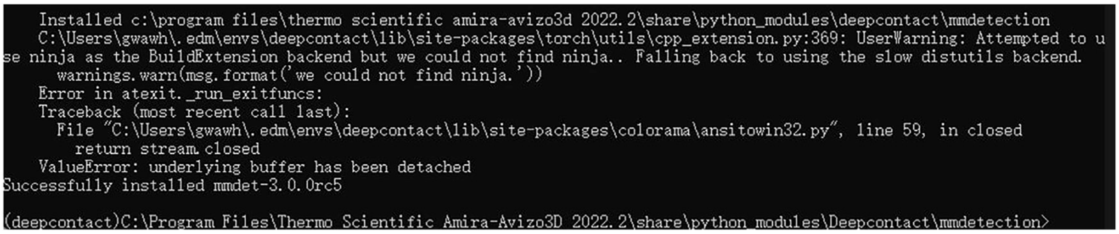
m. Run the following command.

```
>pip install packaging==20.9 ("STAR Protocols.Rdata")
```

```
(deepcontact)C:\Program Files\Thermo Scientific Amira-Avizo3D 2021.2\bin>pip install packaging==20.9
Collecting packaging==20.9
  Downloading packaging-20.9-py2.py3-none-any.whl (40 kB)
     |████████████████████████████████| 40 kB 1.3 MB/s
Requirement already satisfied: pyparsing>=2.0.2 in c:\users\ibp\.edm\envs\deepcontact\lib\site-packages (from packaging==20.9) (2.2.0)
Installing collected packages: packaging
  Attempting uninstall: packaging
    Found existing installation: packaging 16.8
    Uninstalling packaging-16.8:
      Successfully uninstalled packaging-16.8
Successfully installed packaging-20.9

(deepcontact)C:\Program Files\Thermo Scientific Amira-Avizo3D 2021.2\bin>_
```

n. Run the following commands to install mmdet.

```
>pip install -v -e .("STAR Protocols.Rdata")
```



```
    Installed c:\program files\thermo scientific amira-avizo3d 2022.2\share\python_modules\deepcontact\mmdetection
    C:\Users\gwawh\.edm\envs\deepcontact\lib\site-packages\torch\utils\cpp_extension.py:369: UserWarning: Attempted to u
se ninja as the BuildExtension backend but we could not find ninja.. Falling back to using the slow distutils backend.
      warnings.warn(msg.format('we could not find ninja.'))
    Error in atexit._run_exitfuncs:
    Traceback (most recent call last):
      File "C:\Users\gwawh\.edm\envs\deepcontact\lib\site-packages\colorama\ansitowin32.py", line 59, in closed
        return stream.closed
    ValueError: underlying buffer has been detached
Successfully installed mmdet-3.0.0rc5

(deepcontact)C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\share\python_modules\Deepcontact\mmdetection>
```

Possible issues: "PermissionError: [WinError 5] Access denied" (refer to troubleshooting session, problem 2).
Solution: Use administrator privileges to open Amira.
Select the Amira and right-click on it.
Select "Run as administrator".

KEY RESOURCES TABLE

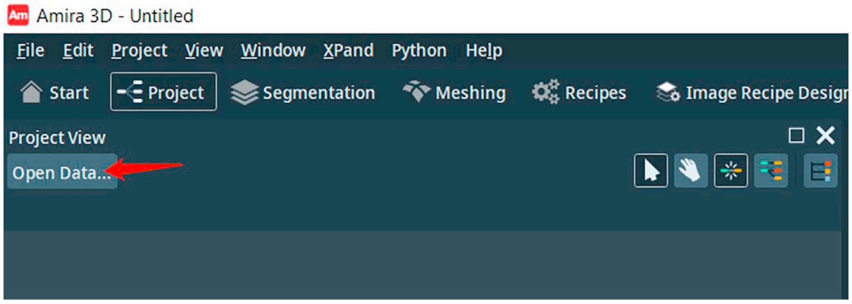
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
"Deepcontact_Amira" installation package, including model files of DeepContact	Figshare website	https://doi.org/10.6084/m9.figshare.22655812
Testing data	Figshare website	https://doi.org/10.6084/m9.figshare.19898404.v3

STEP-BY-STEP METHOD DETAILS

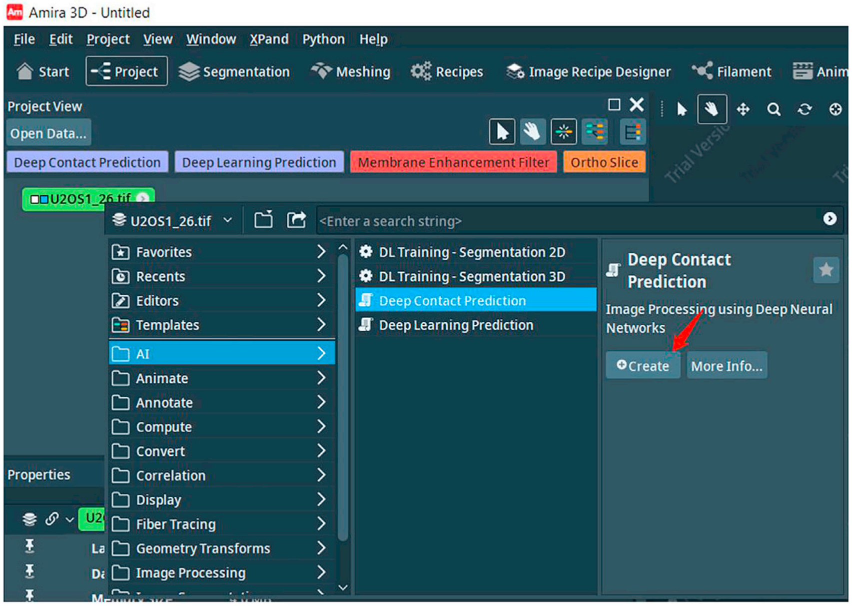
Timing: 15 min to analyze 30 electron microscopic images using a workstation with GPU NVIDIA Quadro RTX 8000

The following steps are for Usage of DeepContact in Amira GUI.

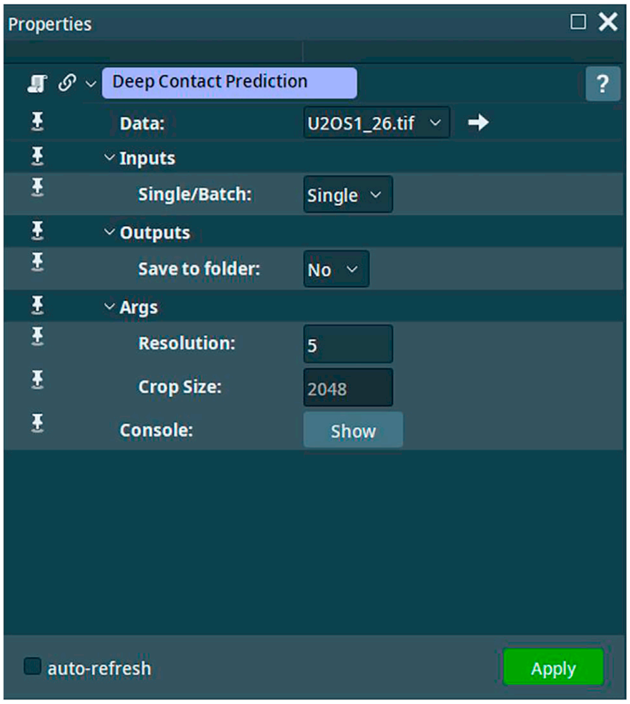
1. Select "Python" -> "Environment" -> "deepcontact" in the menu. Restart Amira when change python environment.
2. Select an image file: Click Project -> open data, and select an Image file in common EM image formats, e.g., Digital Micrograph, MRC and TIFF, for processing.



Create Deep Contact Prediction function: Right click on the image panel, select AI -> Deep Contact Prediction -> Create.



3. Define “Deep Contact Prediction” functional parameters in “Properties” interface.



Properties

Deep Contact Prediction

Data: U2OS1_26.tif

Inputs

Single/Batch: Single

Outputs

Save to folder: No

Args

Resolution: 5

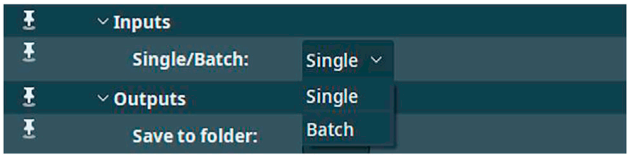
Crop Size: 2048

Console: Show

☐ auto-refresh

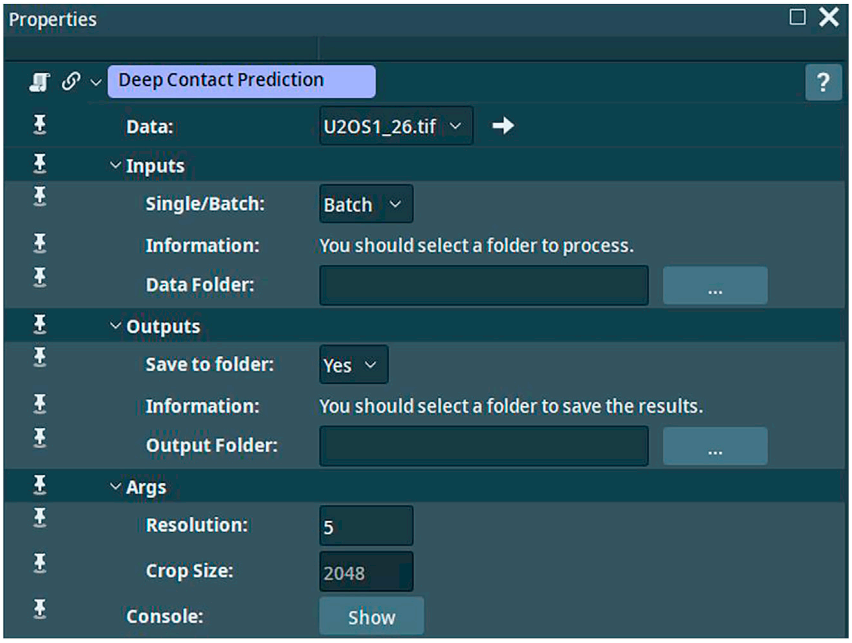
Apply

a. Inputs: “Single” or “Batch” can be selected here.



Inputs
Single/Batch: Single
Outputs
Save to folder:

Single: process initially selected data, and the visualization result will be shown directly in Amira; Batch: an input folder and an output folder must be selected respectively, and all the images in the input folder will be processed automatically.

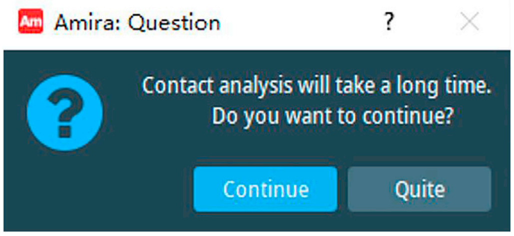


Properties
Deep Contact Prediction
Data: U2OS1_26.tif
Inputs
Single/Batch: Batch
Information: You should select a folder to process.
Data Folder:
Outputs
Save to folder: Yes
Information: You should select a folder to save the results.
Output Folder:
Args
Resolution: 5
Crop Size: 2048
Console:

b. Outputs: an output folder is selected to save the processed visual and statistical results.
c. Args:
   Resolution: the current system will transform the image into 10 nm/pixel resolution according to the resolution parameter.
   Crop Size: the size of the cropping file is calculated automatically according to the resolution of the inputting image, and which ensures the normalization of a patch size into 10 μm × 10 μm field of view. For example, if image data is in 5 nm/ pixel resolution the Crop Size will be automatically determined as 2048.
d. Console: click “Show” to display the status of the program operation in Console.

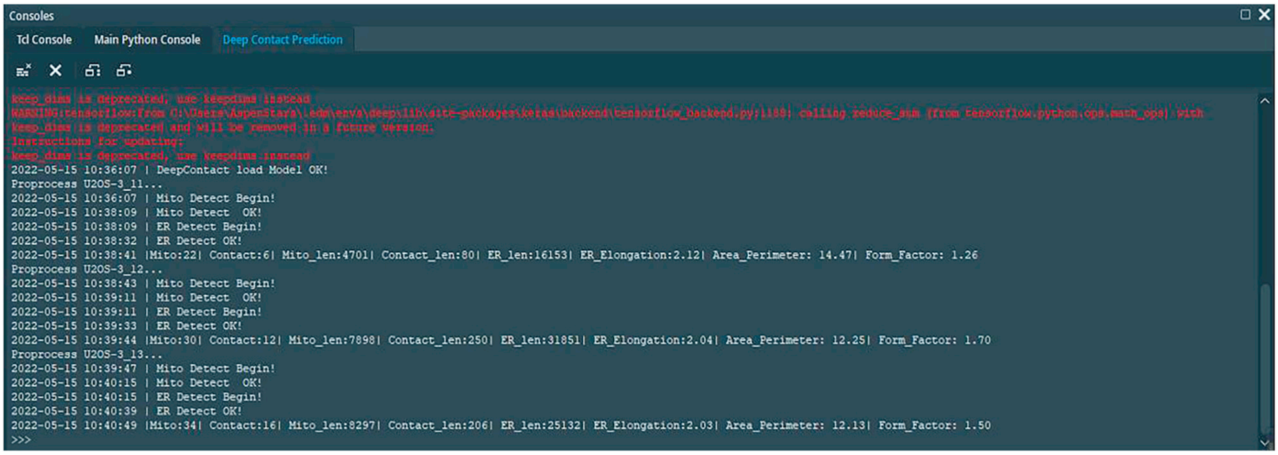
4. Click “apply” to execute the task

The loading of the program will take some time in the initial run, which requires user’s conformation. Click “Continue” to execute task immediately, Click “Quit” to quit the task.



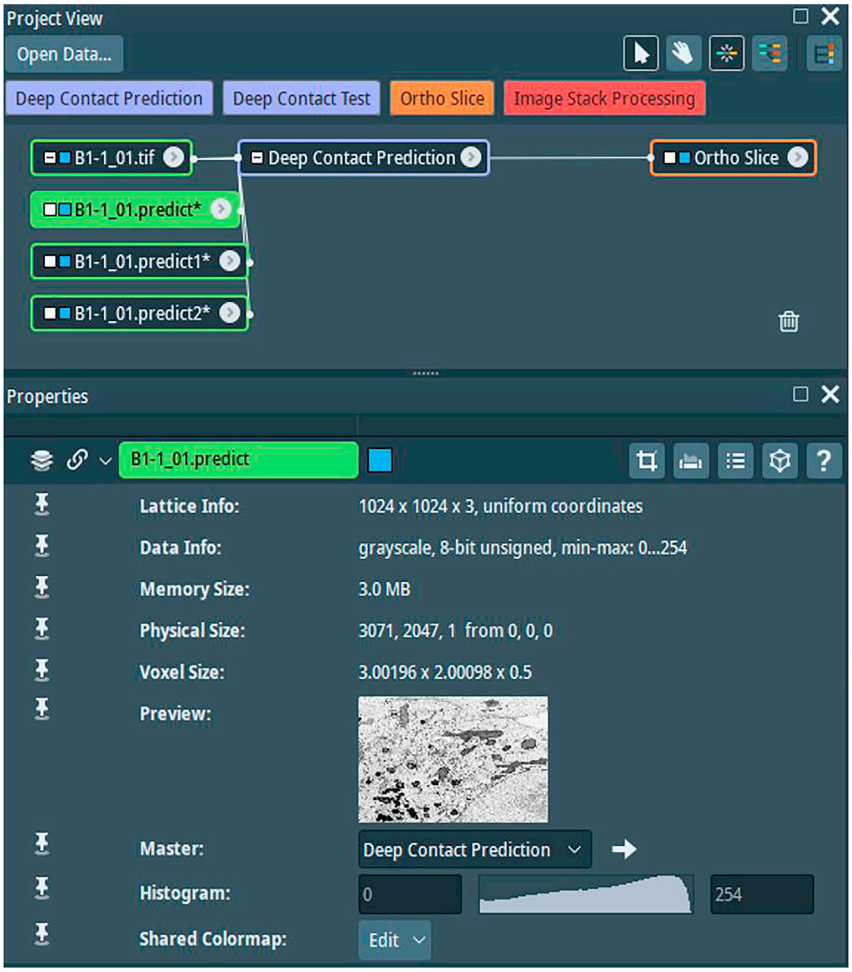
Amira: Question
Contact analysis will take a long time.
Do you want to continue?

5. Result.
   a. The statistical result will be displayed in Console, and the information marked in red can be ignored, which will not affect the execution of the program.



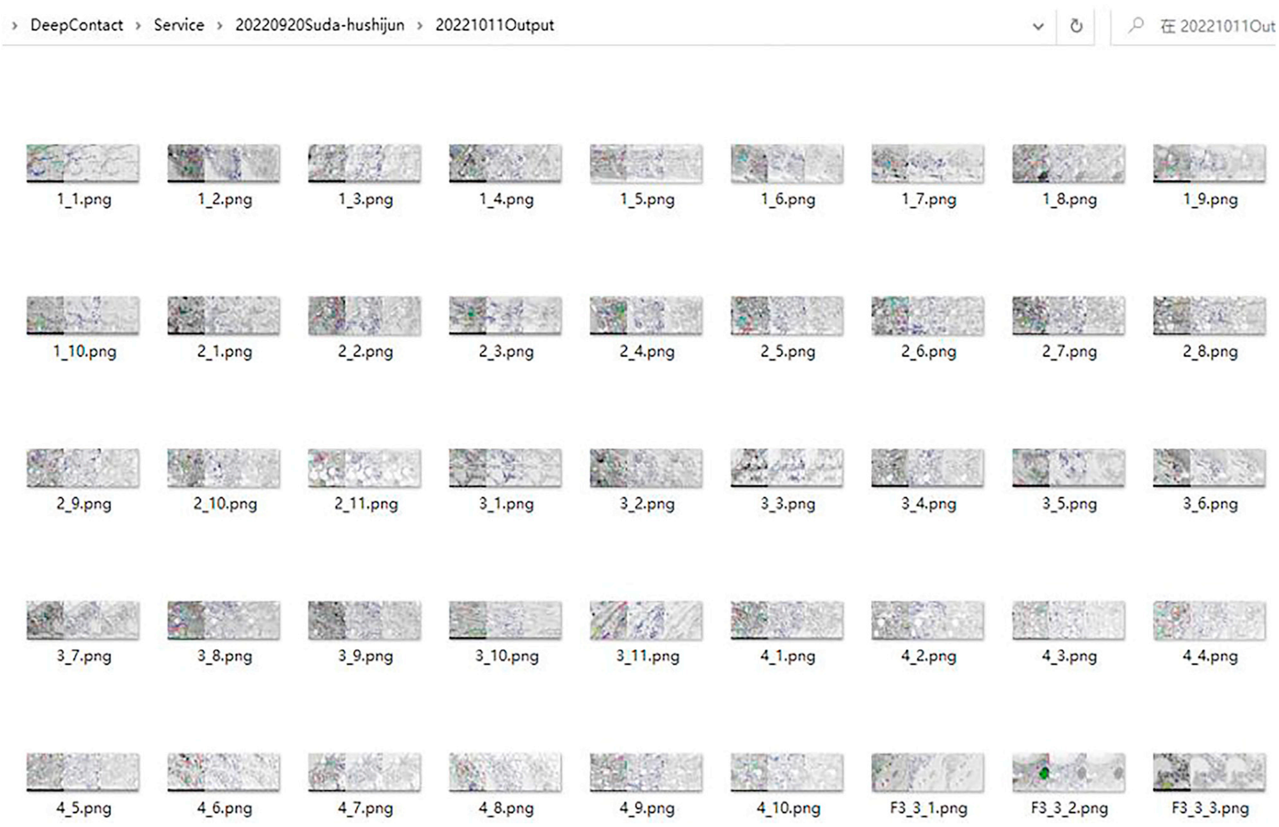
```
keep_dims is deprecated, use keepdims instead
WARNING:tensorflow:From C:\Users\AsperStars\.edm\envs\deep\lib\site-packages\keras\backend\tensorflow_backend.py:1188: calling reduce_sum (from tensorflow.python.ops.math_ops) with
keep_dims is deprecated and will be removed in a future version.
Instructions for updating:
keep_dims is deprecated, use keepdims instead
2022-05-15 10:36:07 | DeepContact load Model OK!
Proprocess U2OS-3_11...
2022-05-15 10:36:07 | Mito Detect Begin!
2022-05-15 10:38:09 | Mito Detect  OK!
2022-05-15 10:38:09 | ER Detect Begin!
2022-05-15 10:38:32 | ER Detect OK!
2022-05-15 10:38:41 |Mito:22| Contact:6| Mito_len:4701| Contact_len:80| ER_len:16153| ER_Elongation:2.12| Area_Perimeter: 14.47| Form_Factor: 1.26
Proprocess U2OS-3_12...
2022-05-15 10:38:43 | Mito Detect Begin!
2022-05-15 10:39:11 | Mito Detect  OK!
2022-05-15 10:39:11 | ER Detect Begin!
2022-05-15 10:39:33 | ER Detect OK!
2022-05-15 10:39:44 |Mito:30| Contact:12| Mito_len:7898| Contact_len:250| ER_len:31851| ER_Elongation:2.04| Area_Perimeter: 12.25| Form_Factor: 1.70
Proprocess U2OS-3_13...
2022-05-15 10:39:47 | Mito Detect Begin!
2022-05-15 10:40:15 | Mito Detect  OK!
2022-05-15 10:40:15 | ER Detect Begin!
2022-05-15 10:40:39 | ER Detect OK!
2022-05-15 10:40:49 |Mito:34| Contact:16| Mito_len:8297| Contact_len:206| ER_len:25132| ER_Elongation:2.03| Area_Perimeter: 12.13| Form_Factor: 1.50
>>>
```

   b. When you select "Single" in the "Input" panel, the visualization result will be displayed in Amira with "Ortho Slice".

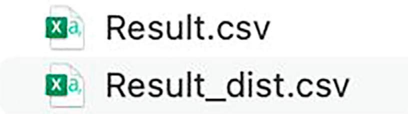


Lattice Info: 1024 x 1024 x 3, uniform coordinates
Data Info: grayscale, 8-bit unsigned, min-max: 0...254
Memory Size: 3.0 MB
Physical Size: 3071, 2047, 1 from 0, 0, 0
Voxel Size: 3.00196 x 2.00098 x 0.5
Preview:
Master: Deep Contact Prediction
Histogram: 0 254
Shared Colormap: Edit

c. When select "Batch" in the Input panel, both the visualization result and two *.csv files with statistical results will be given in the folder named in "Outputs" panel.
d. Visualization result will be given in *.png format in 10 μm × 10 μm field of view in the "output folder".



e. Statistical result will be given as Result.csv and Result_dist.csv in the "output folder" folder.
f. Parameters in Result.csv are listed as below:
   i. Mito_length: perimeter of all segmented mitochondrial in an EM image.
   ii. Mito_length_mean: mean perimeter of the mitochondria.
   iii. Contact_length: length of all segmented contact in an EM image.
   iv. Ratio_number: the number of segmented contact in an EM image / the total number of segmented mitochondrial in an EM image.
   v. Ratio_length: length of all segmented contact in an EM image / perimeter of all segmented mitochondrial in an EM image.
   vi. ER_Length: the perimeter of all segmented ER in an EM image.
   vii. Mito_Elongation: mitochondria_perimeter^2 / (4π×area).
   viii. ER_Elongation: ER_perimeter^2 / (4π×area).
g. Parameters in Result_dist.csv are listed as below:



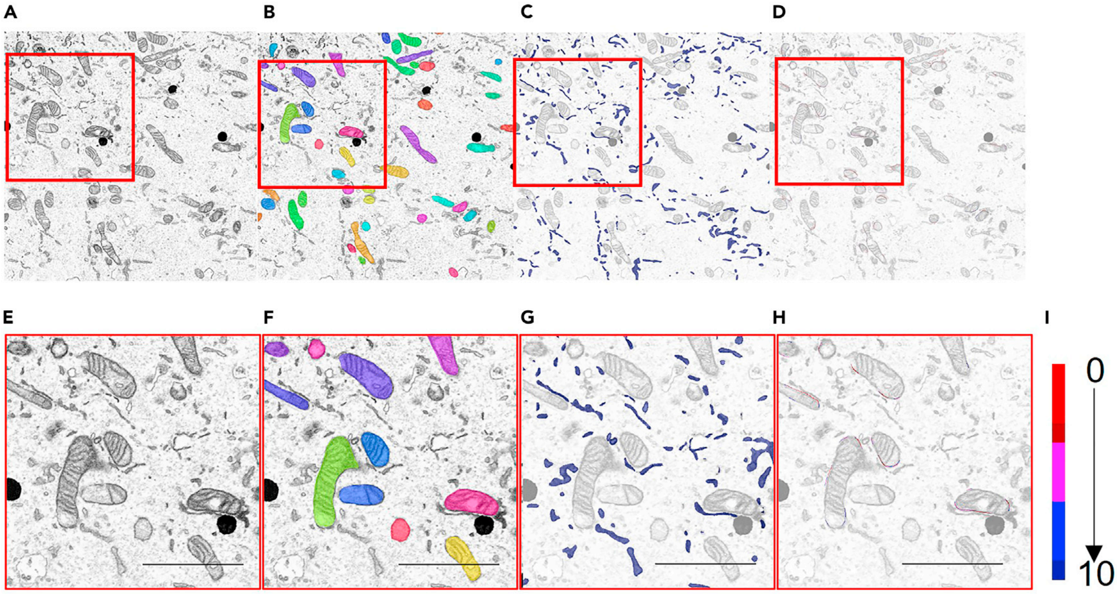


Figure 1. Visualization result

   i. dist_N: N is pixel-based contact width intervals. One pixel corresponds to a length of 10 nm. dist_N give the value of contact length ratio in of N pixel (N×10 nm) width interval.

EXPECTED OUTCOMES

Visualization result (Figure 1).

a, Original patch image in 1024 X 1024 pixels with 10 nm/pixel sampling size. Segmentation of mitochondria (b), ER (c) and Membrane Contact Sites (MCSs, d) on a. e-h, enlarged area framed in a-d. Scale bar = 2 μm. i, the color gradients of color bar indicate 0 pixel to 10 pixel width intervals, corresponding to a width range from 0 nm to 100 nm of the ER-Mito MCS in d and h.

Statistical result (Figure 2) refers to Figure 2 of Liu et al., 2022 (https://doi.org/10.1083/jcb.202106190),[1] as represented below.

a, Total ER perimeter in control and HBSS-treated U2OS cells. b, Total ER area in control and HBSS-treated U2OS cells. c, ER elongation condition in control and HBSS-treated U2OS cells. Elongation factor, perimeter2 / ($4\pi \times$area). d, Total number of Mito in control and HBSS-treated U2OS cells. e, Total Mitoperimeter in control and HBSS-treated U2OS cells. f, Mean Mito perimeter in control and HBSS-treated U2OS cells. g, Total Mito area in control and HBSS-treated U2OS cells. h, Mito elongation condition in control and HBSS-treated U2OS cells. Elongation factor, perimeter2 / ($4\pi \times$ area). i, Ratio of MCS length / Mito perimeter is plotted with width of contact sites ranges from 0 to 100-nm in 10 nm width intervals with HBSS treatment. The sample size of each experimental setting is 30, and individual dots in the plot represent the mean value of a 15 μm × 10 μm cellular image. Bars indicate 95% CIs in a-j.

QUANTIFICATION AND STATISTICAL ANALYSIS

Significance was calculated by two-tailed unpaired t-test with Welsh's correction (nonparametric, do not assume equal SDs). Data distribution was assumed to be normal but this was not formally tested. Plots and statistics were generated using GraphPad Prism 8.3.0. Individual values in each plot indicate a summed or averaged value of the corresponding parameters from one micrograph,

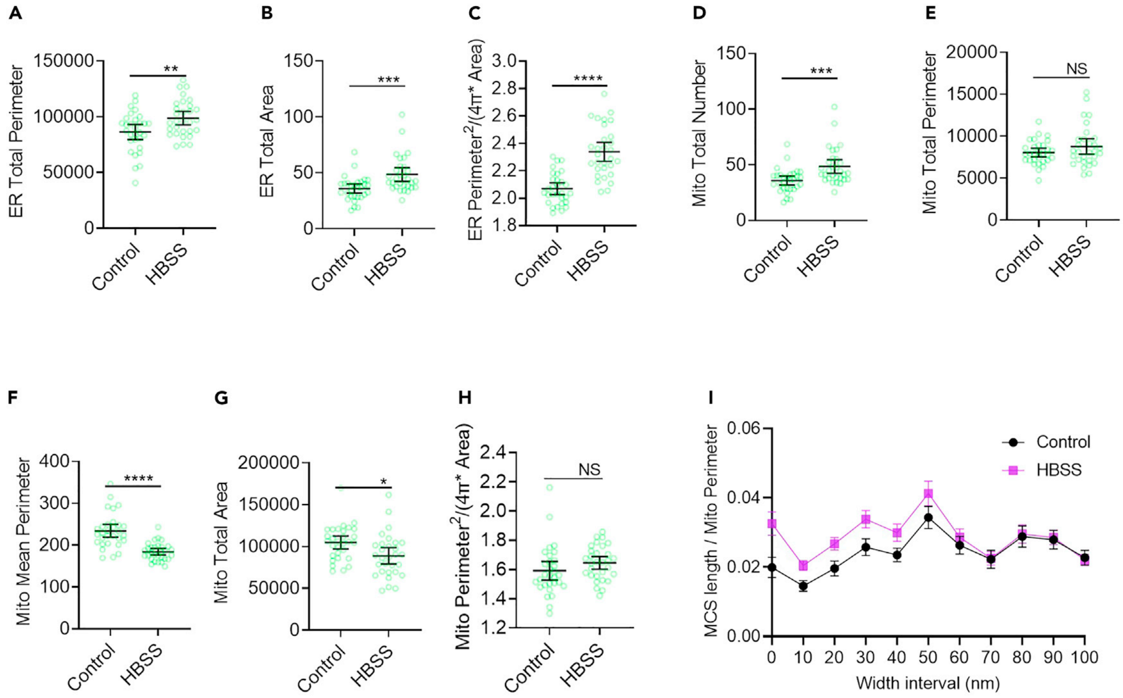


Figure 2. Statistical result

representing the organelle information from the main body part of a cell. The sample size was 30 unless otherwise indicated. 30 Measurements for each experimental setting were from independent EM images from different batches of cells.

LIMITATIONS

- Training functions of DeepContact are not incorporated into Amira yet. Prediction function of DeepContact could be accomplished in Amira by pre-trained models.
- Current model of DeepContact for each organelle is not generalized, and contains morphologies of Mito, ER of several cell lines (e.g., U2OS, Cos7, TM4) and tissues (Liver cell). Most of the samples for cultured cell were prepared by ROTO[2] method, which ensure a more accurate segmentation of the organelles boundary with DeepContact models by enhancing lipid membrane staining of the organelles. Currently we offer services for model refinement upon request for new morphologies or new types of organelles, which we plan to release in the next version of DeepContact.
- Image data were normalized by Resize/Crop/affine transformation to 10 nm resolution in the pre-processing steps before training/prediction process, which is relatively low resolution to represent the nanoscale size of the contact width. Resolution of the models will be optimized in the higher version of DeepContact.
- Unified python environment of different organelle models has been achieved, which enhances the flexibility of DeepContact installation. The training function of DeepContact will be further incorporated on this basis, which will allow the users to build their own models or refine the current models.

TROUBLESHOOTING

Problem 1

The installation of opencv-python failed. The error "failure to install opencv-python-headless" or "fail to build wheel for opencv python" occurred in the command line window of the EDM.

Potential solution

Choose one of the following solutions to resolve the issue.

- Update pip and cmake, and then reinstall opencv-python.
  - Update pip: enter "pip install –upgrade pip"
  - Update cmake: enter "pip install cmake"
  - Install opencv-python: enter "pip install opencv-python==4.3.0.38"
- Install opencv-python-headless manually
  - Go to https://pypi.org/project/opencv-python-headless/3.4.18.65/#files ,
  - Download "opencv_python_headless-3.4.18.65-cp36-abi3-win_amd64.whl". If you are on a 32-bit machine, download "opencv_python_headless-3.4.18.65-cp36-abi3-win32.whl",
  - Copy the downloaded Files to "C:\Program Files\Thermo Scientific Amira-Avizo3D 2022.2\bin",
  - Return to the Amira interface, On the menu bar, Click "project" -> "Python" -> "Open Prompt To Package Manager (EDM)",
  - Enter "edm shell -e deepcontact",
  - Enter "pip3 install opencv_python_headless-3.4.18.65-cp36-abi3-win_amd64.whl".

Problem 2

The installation of mmdet failed. The error "PermissionError: [WinError 5] Access denied" occurred in the command line window of the EDM.

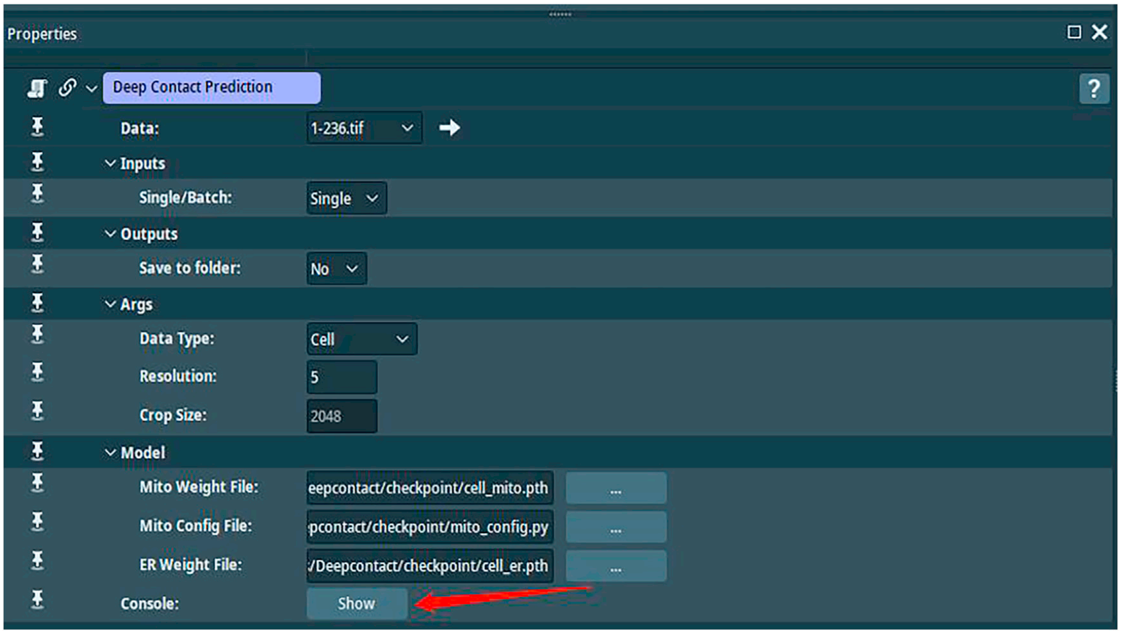
Potential solution

Use administrator privileges to open Amira.

- Select the Amira and right-click on it.
- Select "Run as administrator".

Problem 3

Missing of "AI" module when right click on the inputting image data if the data is in RGB format, e.g., *.jpg, due to the limitation of "AI" module of Amira software. Consequently, DeepContact prediction function within "AI" module cannot be invoked.



Data: 1-236.tif

Inputs

Single/Batch: Single

Outputs

Save to folder: No

Args

Data Type: Cell

Resolution: 5

Crop Size: 2048

Model

Mito Weight File: eepcontact/checkpoint/cell_mito.pth

Mito Config File: pcontact/checkpoint/mito_config.py

ER Weight File: /Deepcontact/checkpoint/cell_er.pth

Console: Show

Potential solution

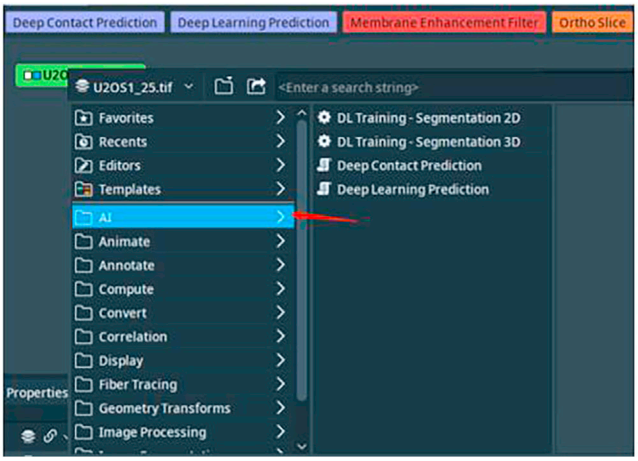
Open an 8bit image in "tif" format, and invoke DeepContact Prediction function by Right click on the image panel and select "AI -> Deep Contact Prediction -> Create". Apply "Deep Contact Prediction" in "Single" inputs mode to load the DeepContact model, then Select RGB image data folder as an "batch" inputs option.

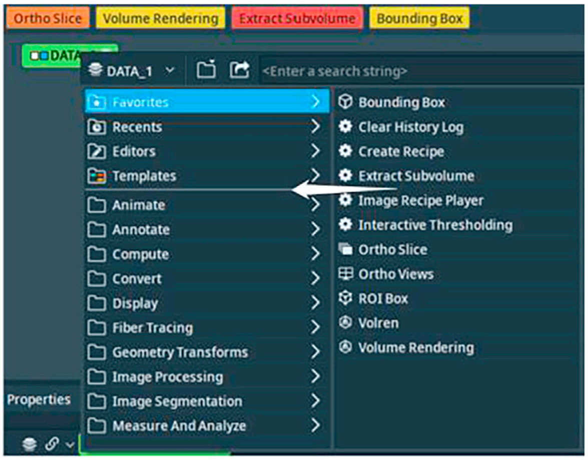
Problem 4

Fail to process the input data with the DeepContact model. An window pops up with an error "An error has occurred during parsing of the script-object class or during the __init__(). Please see error in python console."

Potential solution

Click the "show" button at the bottom of "Properties" window to show the python console, and then check the specific problem. Possible problems are listed below with corresponding solutions.





- "AttributeError: 'Version' object has no attribute 'release'".
  - Enter "edm shell -e deepcontact" in EDM to switch to "deepcontact" virtual environment, and then enter "pip install packaging==20.9" to install packaging.
- "ModuleNotFoundError: No module named 'google.protobuf'".
- Enter "edm shell -e deepcontact" in EDM to switch to "deepcontact" virtual environment, and then enter the following commands to reinstall protobuf.
  - pip uninstall protobuf.
  - pip uninstall google.
  - pip install google.
  - pip install protobuf.

Problem 5

DeepContact option is missing or could not be invoked from the sublist of AI module. (step 1, a).

Possible solution

- Reselect Python environment: Click menu bar "Python" -> "Environment" -> deepContact", and restart Amira software. Python environment should be switched between deepcontact environment and default deeplearning environment when invoking different submodules.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Liqing Liu (liuliqing@ibp.ac.cn).

Materials availability

"Deepcontact_Amira" installation package is publicly available at the figshare repository (https://doi.org/10.6084/m9.figshare.22655812).

Example images for testing (https://doi.org/10.6084/m9.figshare.19898404.v3) are publicly available at the figshare repository.

The DeepContact source code is available on GitHub: https://github.com/LX-doctorAI1/DeepContact.

Data and code availability

Example images for testing (https://doi.org/10.6084/m9.figshare.19898404.v3) are publicly available at the figshare repository.

The DeepContact source code is available on GitHub: https://github.com/LX-doctorAI1/DeepContact.

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AUTHOR CONTRIBUTIONS

Scientific evaluation, T.X. and J.H.; conceptualization, J.H., T.X., L.X., and L.L.; protocol elaboration, H.W., K.Y., S.Y., L.L., L.X., and J.H.; software development, L.X., S.Y., and H.W.; writing – review and editing, L.L., S.Y., H.W., K.Y., and L.X.; funding acquisition, T.X., J.H., L.X., and L.L.

DECLARATION OF INTERESTS

A patent (ZL 2022 1 0141093.4) related to this work has been authorized.

REFERENCES

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2. Tapia, J.C., Kasthuri, N., Hayworth, K.J., Schalek, R., Lichtman, J.W., Smith, S.J., and Buchanan, J. (2012). High-contrast en bloc staining of neuronal tissue for field emission scanning electron microscopy. Nat. Protoc. *7*, 193–206.