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Online Resource 2: Step-by-step AutoMorph protocol for automated retinal vascular morphology analysis.

Step-by-Step Guide to Running AutoMorph with Colab

The main scope of the following guide is to enhance AutoMorph usability by detailing critical steps.

Disclaimer: This guide is for educational and support purposes. We are not affiliated with, nor do we own any rights to AutoMorph, which was originally published by Zhou Y, Wagner SK, Chia MA, Zhao A, Woodward-Court P, Xu M, Struyven R, Alexander DC, Keane PA. AutoMorph: Automated Retinal Vascular Morphology Quantification Via a Deep Learning Pipeline. Transl Vis Sci Technol. 2022 Jul 8;11(7):12. doi: 10.1167/tvst.11.7.12.

1. Access the AutoMorph Repository:
    - Navigate to the AutoMorph GitHub page at: https://github.com/rmaphoh/AutoMorph.
2. Locate the "Running AutoMorph" Section:
    - Scroll to the bottom of the page to find the section titled "Running AutoMorph."
    - Here, you will see three available platform options.
3. Select Google Colab for Execution:
    - For this guide, we will use Google Colab. Click the corresponding link to launch the notebook.

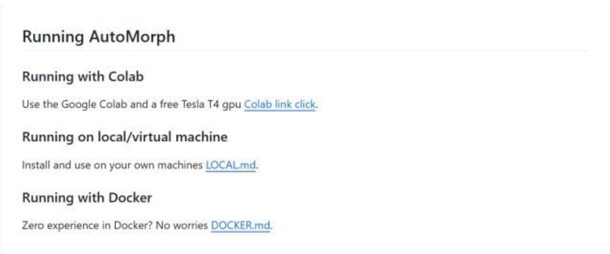

Figure 1. Running AutoMorph

4. AutoMorph Colab Notebook Initialization:
    - By clicking the link, the program's Google Colab notebook will open, enabling access to all necessary functions.

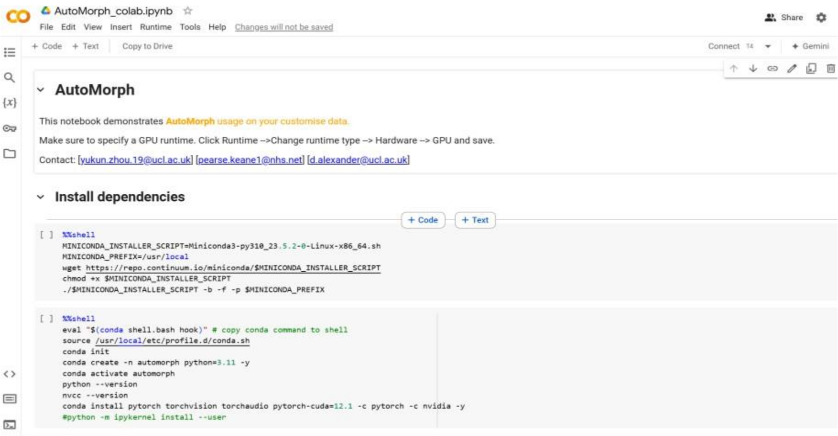

Figure 2. AutoMorph in Google Colab notebook

5. Enable GPU Acceleration
   - From the menu, choose Runtime → Change runtime type → GPU (Tesla T4).
   - Successful activation is denoted by a green verification tick in the Colab interface.

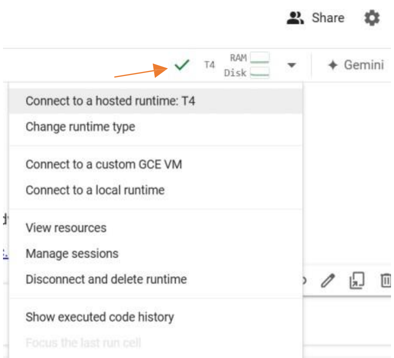

Figure 3. Selecting GPU runtime

6. Run the Complete Analysis:
   - Navigate to the Runtime dropdown menu in the toolbar.
   - Choose Run all to initiate all cells sequentially without manual intervention.

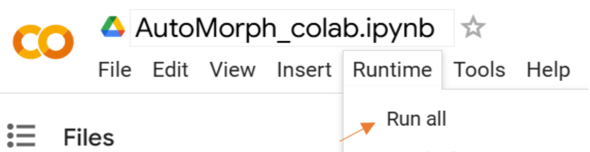

Figure 4. Running AutoMorph

7. Monitor automated processing
   - The system will now process all steps sequentially.
   - Scroll through the notebook to observe real-time progress.
   - Completed steps are marked with a green checkmark and their runtime.

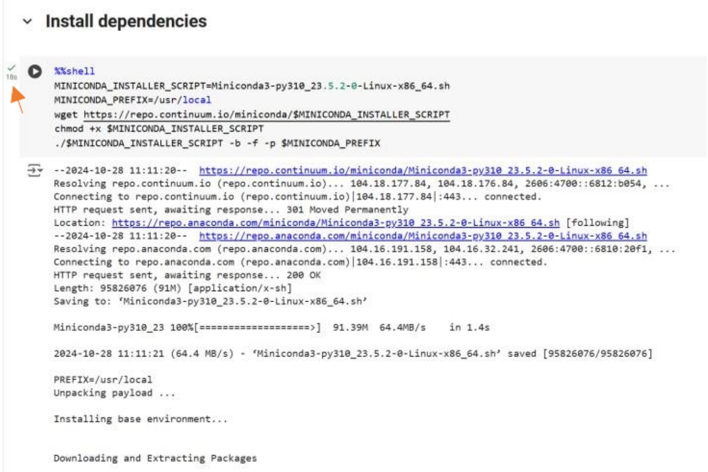

Figure 5. Monitoring automated execution – first step completed

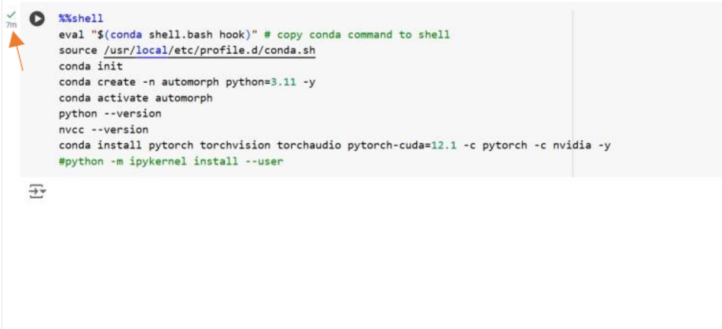

Figure 6. Monitoring automated execution – second step completed

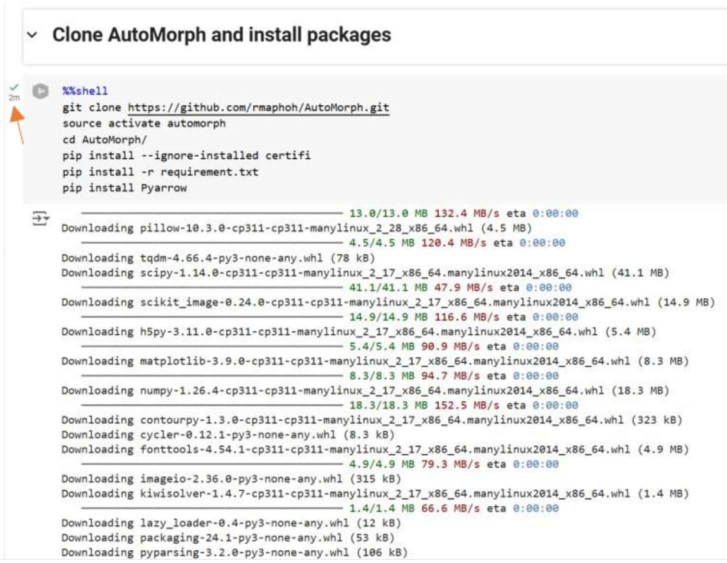

Figure 7. Monitoring automated execution – third step completed

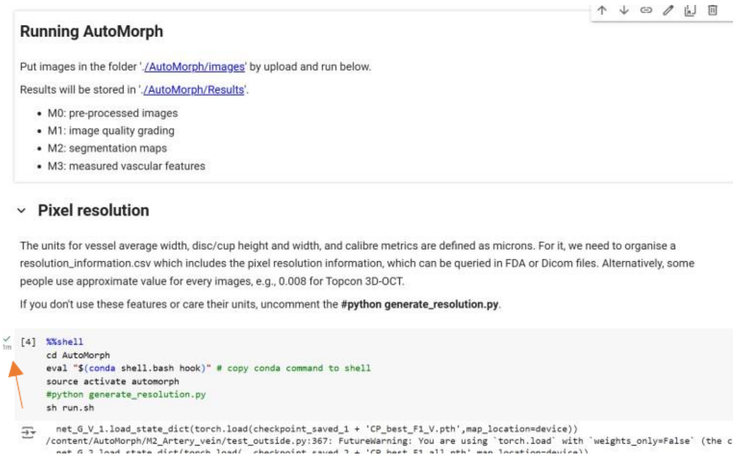

Figure 8. Monitoring automated execution – fourth step completed

8. Accessing Results folder
    - After completion, locate the folder icon on the left sidebar.
    - Select the AutoMorph folder containing all output files.

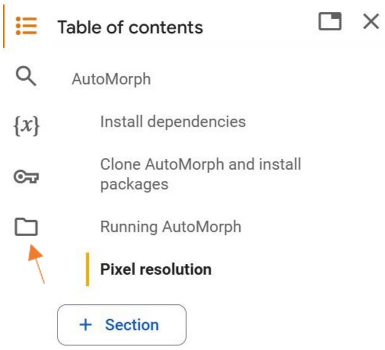

Figure 9. Accessing the AutoMorph folder

9. Essential output subfolders within the AutoMorph directory
    - /images: Stores sample fundus images (1.png, 100.png, 11.png) for analysis (Figure 10, black arrow).
    - /resolution_information.csv: Contains resolution data (Figure 10, green arrow)
    - /results: Holds processed data from all AutoMorph analysis modules (Figure 10, blue arrow).

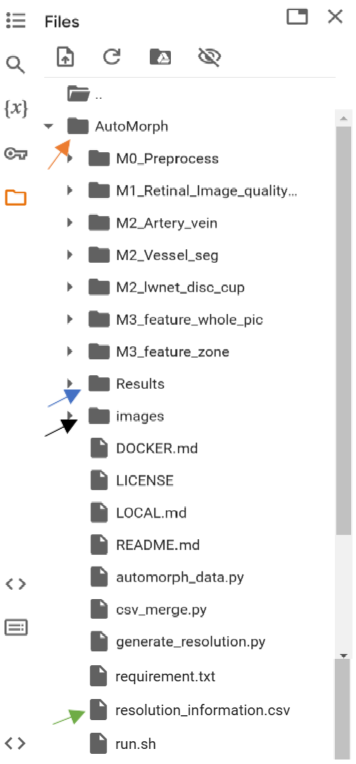

Figure 10. Essential output folders

10. Custom Image Analysis – 2 options

10.1 Using Sample Images

- Run Pixel Resolution step by clicking the play icon next to the pixel resolution module.
- Wait for step to complete (green checkmark ✓ appears).
- Access the saved results by selecting the /results folder.

10.2 Using Your Own Fundus Images

- Delete Sample Images (1.png, 100.png, 11.png) by clicking the three dots next to the filename and selecting “Delete”.
- Ensure the /images folder is empty afterward.
- By selecting the three dots, and clicking “Upload”, choose and upload your own fundus images into the /images folder.

11. Generate Resolution Information Chart

- Navigate to the resolution_information.csv file in the AutoMorph folder (Figure 10, Green arrow).
- Recreate the chart (see tables 1 and 2) in a spreadsheet program.
- Preserve the header format of the first row: Keep "fundus" (column A)

and "res" (column B).

- In the second row of column A, enter the exact filename of your uploaded image, including the extension.
- In the second row of column B, fill in the resolution value of the fundus images. If the resolution value is not known, obtain the pixel size in microns from the manual of the device used to create the fundus photographs. Microns will then be the unit in the results section. In case the resolution remains unknown, enter a default value of "1". In this case, the resulting measurement unit will be pixels.
- Save the file and ensure the file remains in CSV format and retains its original name (resolution_information.csv).
- Note: This file must match the images in your /images folder for proper processing.

fundus	res
Image name.extension	1
Image name.extension	1

Table 1. Creating resolution_information.csv

fundus	res
OD.tif	1
OS.tif	1

Table 2: Example image names in resolution_information.csv

12. Replace default Resolution Data File
    - In the AutoMorph folder, locate the default resolution_information.csv.
    - Click the three dots (⋮) next to the file and select "Delete"
    - Upload Custom CSV File by selecting the three dots (⋮) next to the AutoMorph folder. Choose "Upload" and select your prepared resolution_information.csv.
    - Critical Requirement: names and extensions in the CSV must exactly match those in the /images folder. For error prevention, avoid spaces, typos, or extra characters.

13. Execute Pixel Resolution Analysis
    - Now run the pixel resolution program
    - in some cases, the hash symbol (#) before the command python generate_resolution.py has to be deleted prior to running pixel resolution.
    - Wait for completion while the system processes all fundus photographs until successful execution is confirmed by a green checkmark (✓).

14. Results
    - To access results, navigate to /AutoMorph/Results/. Then visualize the four modules
    - M0: contains the pre-processed images
    - M1, Quality Grading of the analysed images: /Good_quality/, Images passing

quality control proceed to further analysis. /Bad_quality/: Rejected images

- M2: displays different vessel segmentation forms
- M3: shows quantitative vascular features. Disc-Centered Images receive metrics in Disc_features, Macular-Centered Images receive metrics in Macular_features.