

Supplementary Note 1: MorphoCA Manual

1.1 Install MorphoCA

To install MorphoCA, first launch QuPath, then drag the plugin file **qupath-extension-MorphoCA.jar** into the QuPath interface or install it via *Extensions → Install extension*. The JAR file can be downloaded from [Github](#). After installation, QuPath needs to be restarted. Once restarted, the MorphoCA panel will be visible in the menu or sidebar (Figure 1).

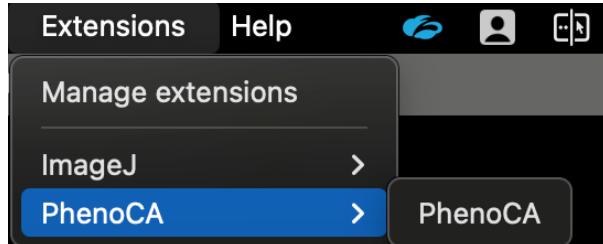


Figure 1 Installation of the MorphoCA extension in QuPath.

1.2 Load spatial coordinates

MorphoCA supports two modes of loading clustered patch coordinates: (i) single-slide import and (ii) project-level batch import, corresponding to the *Create File Selections* and *Create Project Selections* functions.

1.2.1 Single-slide import (Create File Selections)

In the single-slide mode, users need to open the target slide in QuPath and load a CSV file¹ that contains the spatial coordinates of clustered patches. This file records the locations of high-contribution patches within the slide. The CSV typically includes the following fields: *slide_name* (ending with .svs and matching the filename of the opened slide), *patch_id* (identifier of each patch, e.g., patch_2316), *hc_label* (cluster label, such as hc_1, hc_2, etc.), *x* and *y* (top-left coordinates of the patch), as well as width and height (the width and height of the patch). An example is shown in Figure 2.

A	B	C	D	E	F	G
ANONOLN0DJ16A_1.svs	patch_2316	hc_1	100679	14940	1367	1367
ANONOLN0DJ16A_1.svs	patch_2224	hc_1	97944	9470	1367	1367
ANONOLN0DJ16A_1.svs	patch_2235	hc_1	99311	16307	1367	1367
ANONOLN0DJ16A_1.svs	patch_2313	hc_1	100679	13572	1367	1367
ANONOLN0DJ16A_1.svs	patch_2234	hc_1	99311	14940	1367	1367
ANONOLN0DJ16A_1.svs	patch_2312	hc_1	100679	12205	1367	1367
ANONOLN0DJ16A_1.svs	patch_2401	hc_1	103414	16307	1367	1367
ANONOLN0DJ16A_1.svs	patch_2403	hc_1	104781	16307	1367	1367
ANONOLN0DJ16A_1.svs	patch_2227	hc_1	99311	10837	1367	1367
ANONOLN0DJ16A_1.svs	patch_2232	hc_1	97944	14940	1367	1367
ANONOLN0DJ16A_1.svs	patch_2193	hc_1	95209	49127	1367	1367
ANONOLN0DJ16A_1.svs	patch_1799	hc_1	85636	10837	1367	1367
ANONOLN0DJ16A_1.svs	patch_2442	hc_1	104781	42290	1367	1367
ANONOLN0DJ16A_1.svs	patch_2437	hc_1	103414	40922	1367	1367

Figure 2 Example of a CSV file containing spatial coordinates and cluster labels of patches.

After selecting the CSV file with *SelectFile* in the MorphoCA panel, the ‘In File:’ field displays the imported coordinate filename, whereas the ‘Out File:’ field specifies the filename for storing annotation results (see the following section “Saving Annotations”). By then clicking *Create File Selections*, the extension overlays the patches from the CSV onto the current slide as colored boxes or regions. Different clusters (*hc_label*) are displayed in distinct colors (Figure 3).

¹ For details on how to prepare the CSV file, please refer to the instructions provided in the [GitHub](#) README.

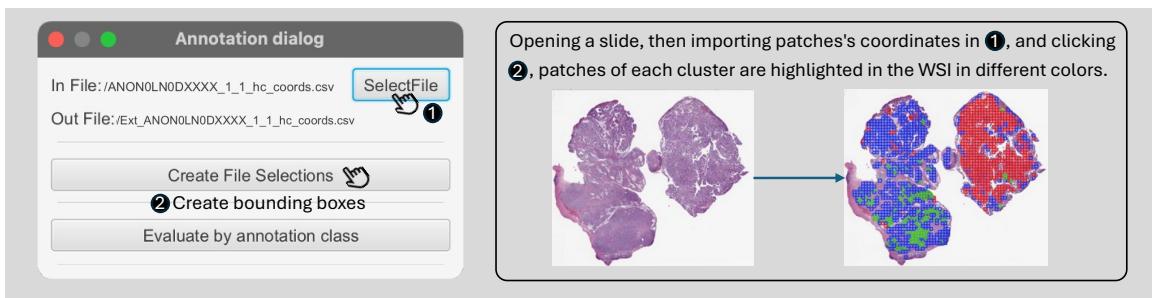


Figure 3 Example of loading a CSV file and visualizing clustered patches with distinct colors.

By default, QuPath also visualizes the patch_id on each patch after the coordinates are loaded (Figure 4), which may result in visual clutter. If the display of patch_id is not needed, users can disable it by clicking *View* → *Show annotation names*.

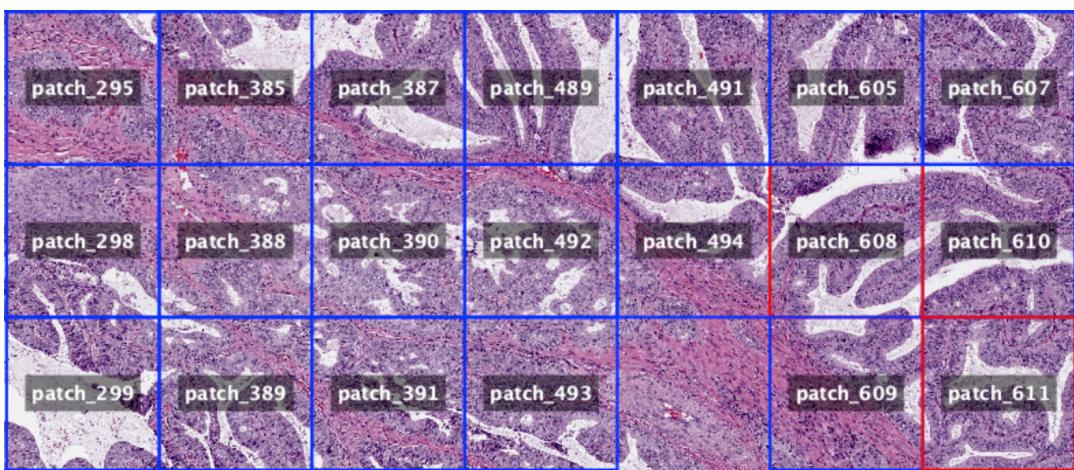


Figure 4 Visualization of clustered patches in QuPath, with patch IDs displayed by default.

1.2.2 Create Project Selections

In large-scale data scenarios, MorphoCA supports batch visualization by creating a QuPath project (Figure 5). The procedure involves merging the spatial coordinate CSV files of clustered patches from multiple slides into a single file. This file must contain a *slide_id* field that exactly matches the slide names in the QuPath project, while the other columns follow the same format as in the single-slide mode. Once a new project is created in QuPath and all relevant slides are imported, users can select the merged CSV file in the MorphoCA panel and click *Create Project Selections*. The extension will then automatically distribute the coordinates to each slide according to the *slide_id* and overlay the clustered results in distinct colors on the corresponding WSIs. This functionality is particularly useful for large cohorts, as it supports efficient batch visualization and streamlined annotation.

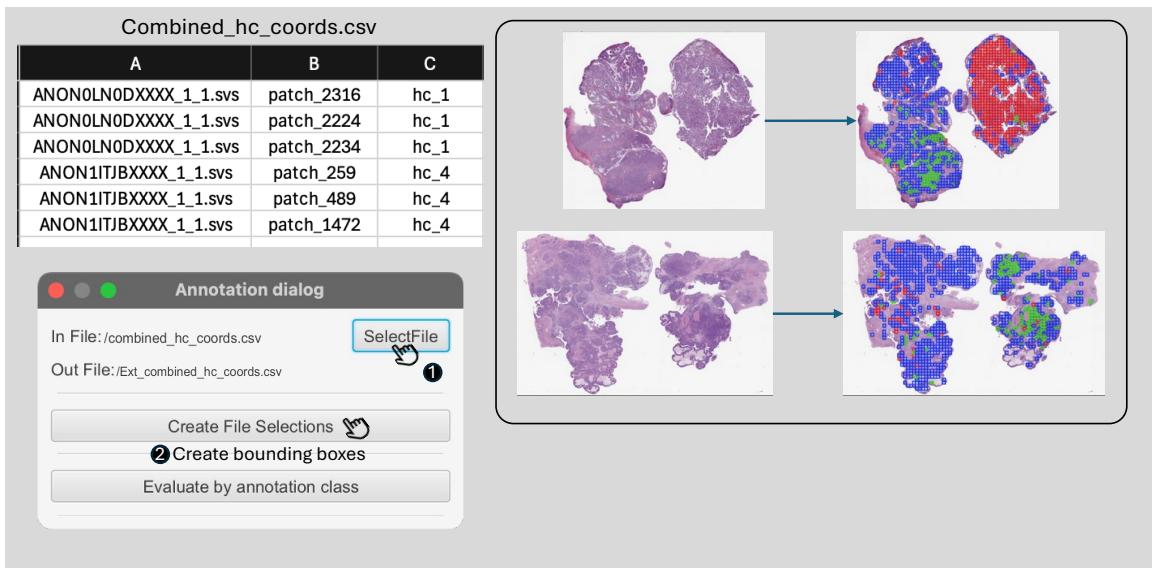


Figure 5 Example of batch visualization of clustered patches in MorphoCA.

1.3 Interactive Annotation

1.3.1 Entering Annotation Mode

After the spatial coordinates have been loaded in MorphoCA, users can click *Evaluate by annotation class* to enter the annotation mode (Figure 6). By selecting a cluster from the class list (for example, hc_1) and then clicking *Process selected class*, the chosen cluster will remain highlighted while other clusters are automatically hidden. This allows users to focus on the distribution and morphological features of the selected cluster patches. At this stage, users enter a whole-slide contextual environment for visualization and annotation, where they can freely zoom or pan to examine features at both the cellular and tissue levels.

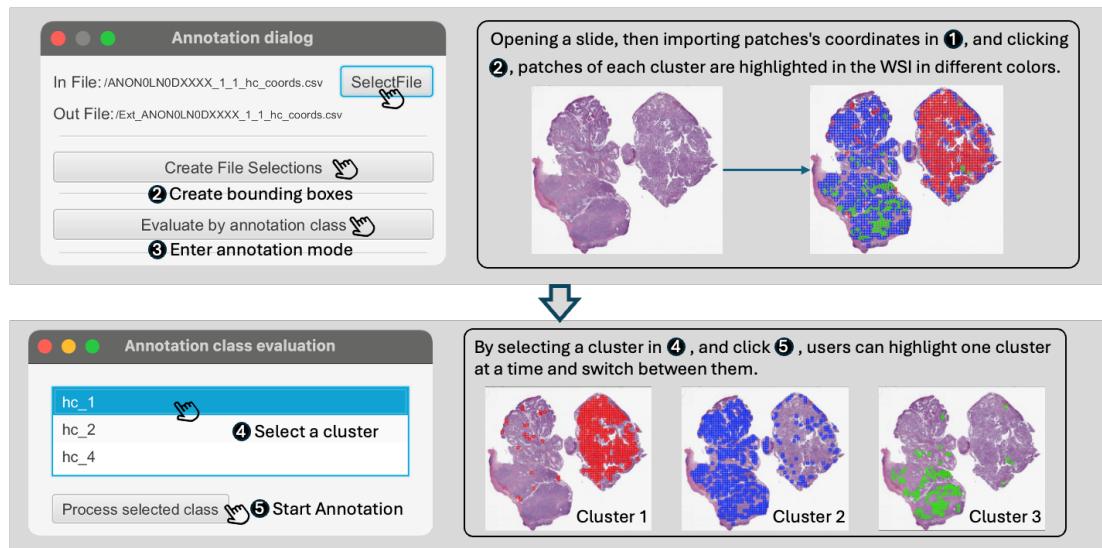


Figure 6 Entering annotation mode in MorphoCA

1.3.2 Multiscale Review

At high magnification, users can examine the cytological features of individual patches. At low magnification, they can evaluate the spatial distribution of the clustered patches across the whole slide and their relationship with histological structures. This combination of high-

and low-power views provides a comprehensive morphological context and forms the basis for subsequent annotation questions (Figure 7 right).

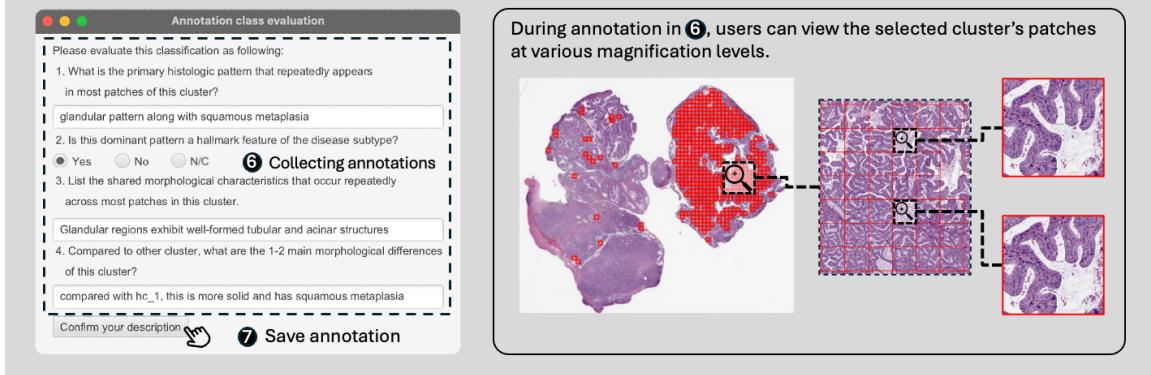


Figure 7 Workflow of annotation in MorphoCA

1.3.3 Four Annotation Questions

In the pop-up panel, users are required to fill in four types of information for the current cluster (Figure 7 left). The first is **Represented Phenotype**, which describes the main phenotype of the cluster, recorded using standard terminology (e.g., glandular architecture, papillary architecture, solid pattern, squamous metaplasia). If uncertain, users should input N/C (Not Certain). The second is **Subtype-Defining Phenotype**, which determines whether the phenotype is a core diagnostic feature for the subtype. The options are Yes, No, or N/C, and the judgment should follow diagnostic criteria or guidelines. The third is **Detailed Morphological Descriptions (Intra-cluster Features)**, which require listing the consistent detailed features that appear across most patches in the cluster, such as prominent nucleoli, pleomorphism, or stromal fibrosis. The last is **Inter-cluster Morphological Differences**, which compare the current cluster with other clusters of the same phenotype, requiring 2–3 distinguishing traits. For example, “compared with other glandular clusters, this cluster shows richer stroma or denser solid regions.” If uncertain, users should enter N/C. It is important to note that responses should reflect features consistently observed across the majority of patches, rather than being biased by a few outliers..

1.3.4 Saving Annotations

Once the four questions have been completed, users can click *Confirm your description* to save the structured annotation results locally. A new CSV file will be created in the same directory as the imported CSV, with filenames beginning with ‘Ext_’. Users should repeat the same process for all clusters and slides. In batch annotation scenarios, it is recommended to save after completing several clusters at a time to reduce the risk of data loss caused by accidental operations or software crashes.