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START

Original IHC image stained with DAB and Haematoxylin

1) Separate DAB from Haematoxylin

| DAB (Brown) | Haematoxylin (Blue) |

2) Segmentation of DAB positive nuclear patterns

4) Segmentation of DAB negative nuclear patterns

3) Learning of feature for DAB positive patterns

5) Discard non-tumour negative patterns

6) Assessment of final percentage of DAB positive tumour cells

END

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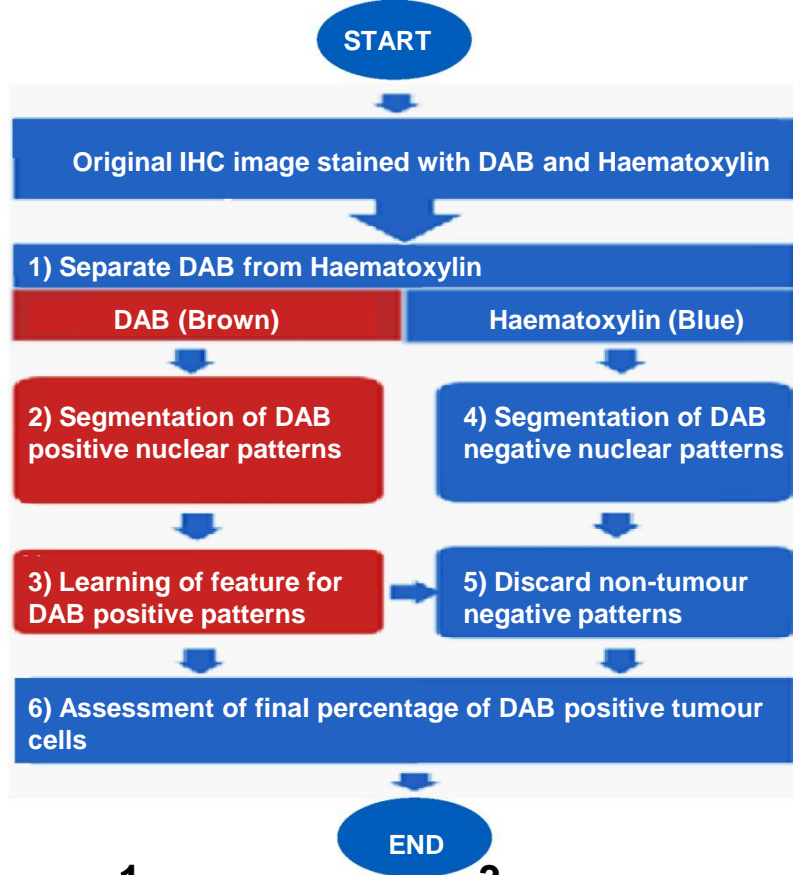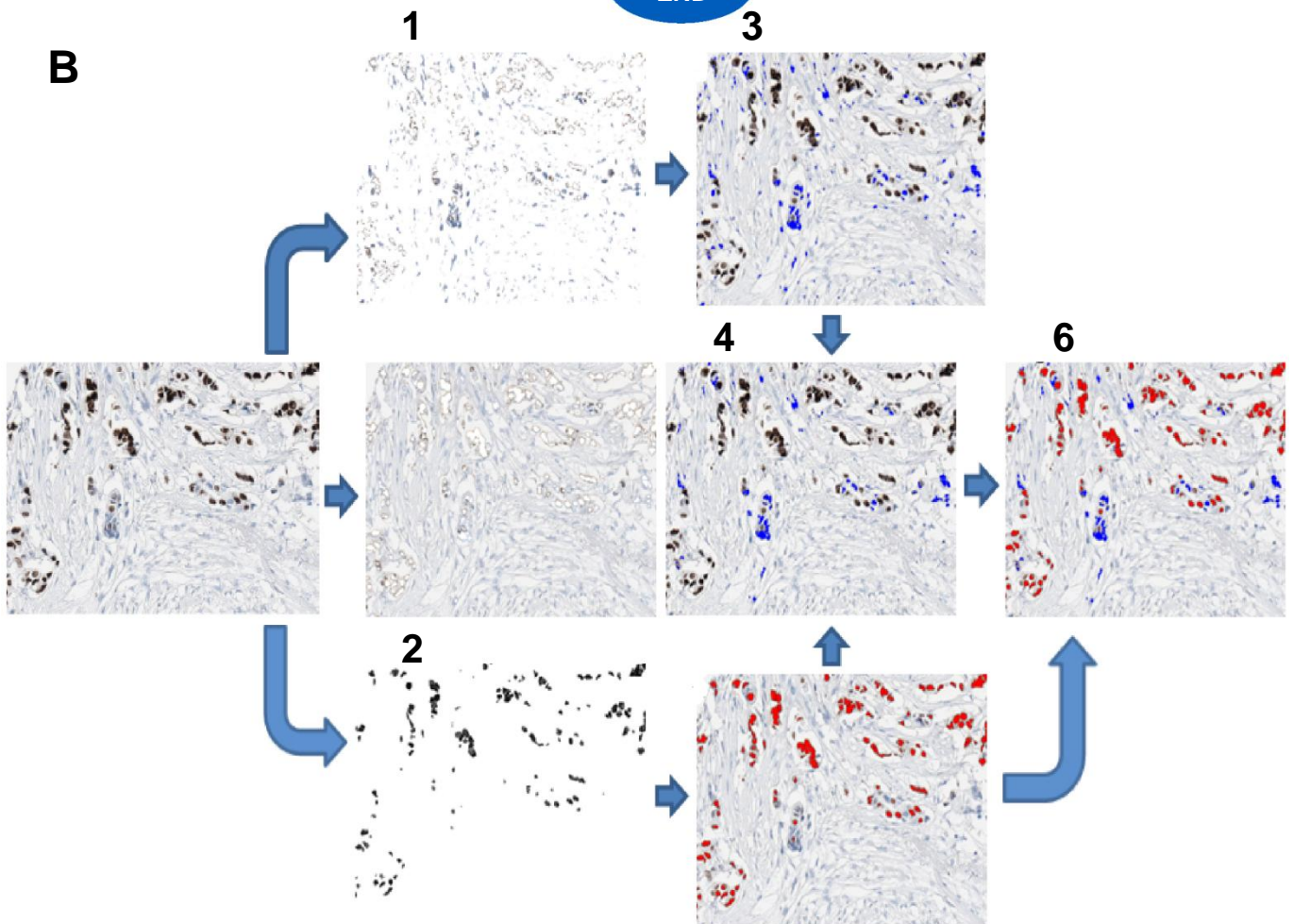2

Figure 1

1) Separation of DAB from Haematoxylin

Representative DAB negative and positive tumour patterns are selected and converted into LUV colour space

⬇

Mean L levels for each pattern are computed based on the images selected above

⬇

Based on the thresholds defined above DAB is separated from Haematoxylin

⬇

Tissue is separated from slide background using a set of RGB thresholds optimised for IHC and the Aperio scanner

⬇

RGB values of Haematoxylin positive pixels are converted in the HSV colour space to separate connective tissue from nuclear negative staining

2) Segmentation of all nuclear patterns

Convert image from RGB to CIE LUV uniform colour space and then convert the L channel to gray level

⬇

Adjust contrast of the gray level representation above

⬇

Apply mean shift segmentation to separate nuclear staining from background cytoplasmic staining

⬇

Extract gray level values of pixels isolated above

⬇

Adjust contrast of the gray level representation above

⬇

Apply watershed segmentation to identify all nuclei

Figure 2

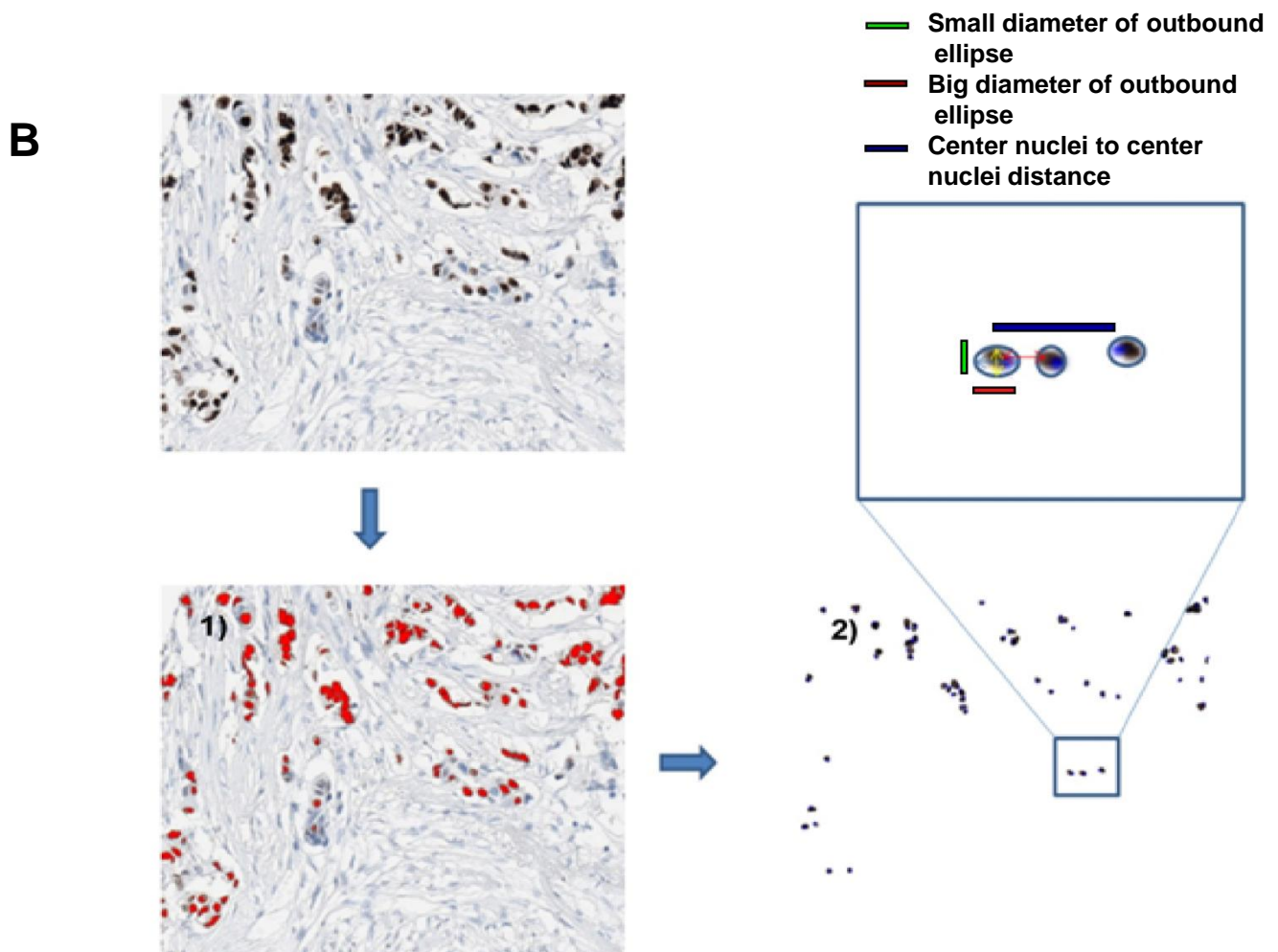# Extraction of DAB positive nuclei and their morphological and spatial properties

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1) Find for each DAB positive nuclei :
- X, Y position in the image
- Area
- The small and big axis of the outbound ellipse

⬇

Measure the distance between each nuclei and its neighbours

⬇

2) Nuclei-to-nuclei distances are clustered in two groups:
- Distances of nuclei belonging to nuclei part of the same tumour gland (cluster D1)
- Distances of isolated nuclei or nuclei part of different tumour glands (cluster D2)

⬇

Identify nuclei part of cluster D1 and discard nuclei in cluster D2

⬇

3) From the nuclei identified above, the following features are extracted:
Mean area (MAP), mean length of small and big axis, mean (MDN) and max nuclei-to-nuclei distance, total number of nuclei and mean intensity of DAB positive nuclei

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— Small diameter of outbound ellipse
— Big diameter of outbound ellipse
— Center nuclei to center nuclei distance

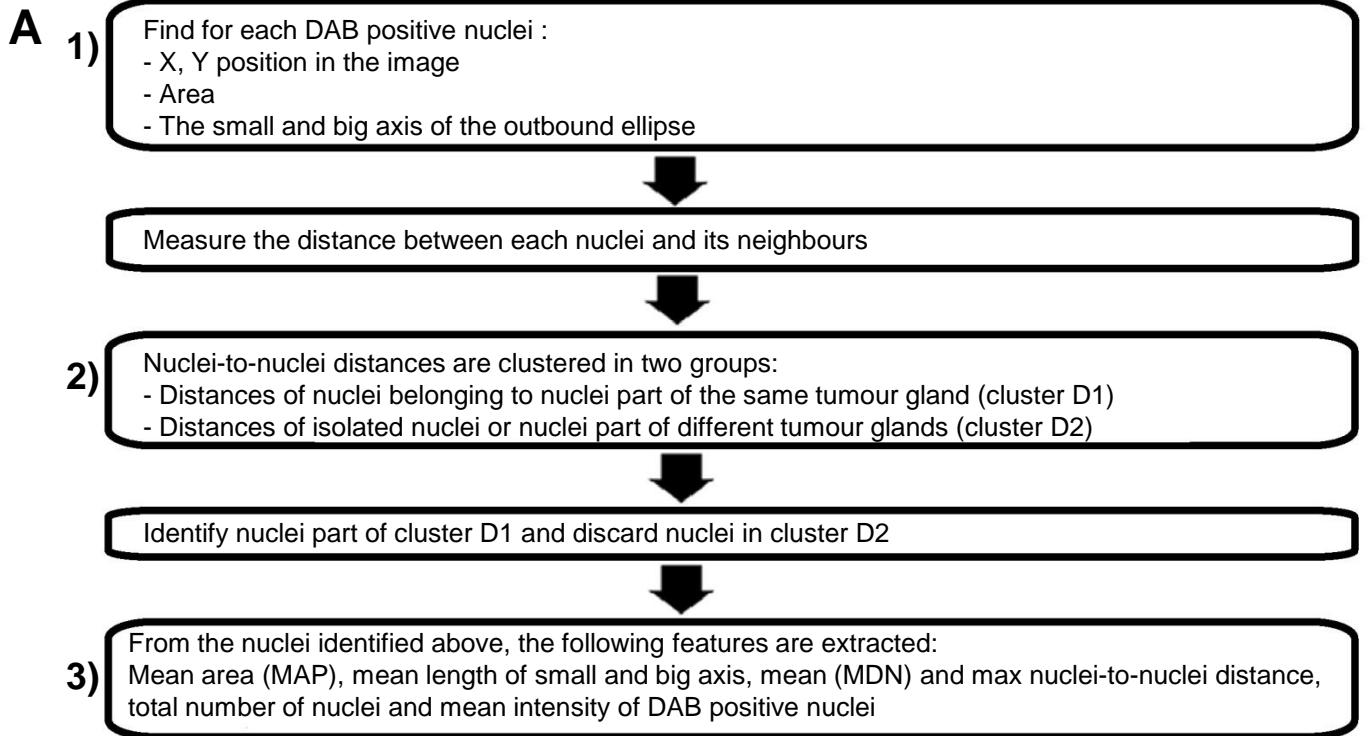Figure 3

Extraction of DAB tumor negative nuclei

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1) For each DAB negative nuclei find:
- X, Y position in the image
- Area
- The small and big axis of the outbound ellipse

⬇

Find the negative DAB nuclei area standard deviation (STD)

⬇

2) Discard all DAB negative nuclei with an area smaller then 2*STD as being either lymphocytes or connective tissue

⬇

Discard all DAB negative nuclei with area bigger then MAP+2*STD as being image artifacts

⬇

Measure nuclei-to-nuclei distance of remaining nuclei
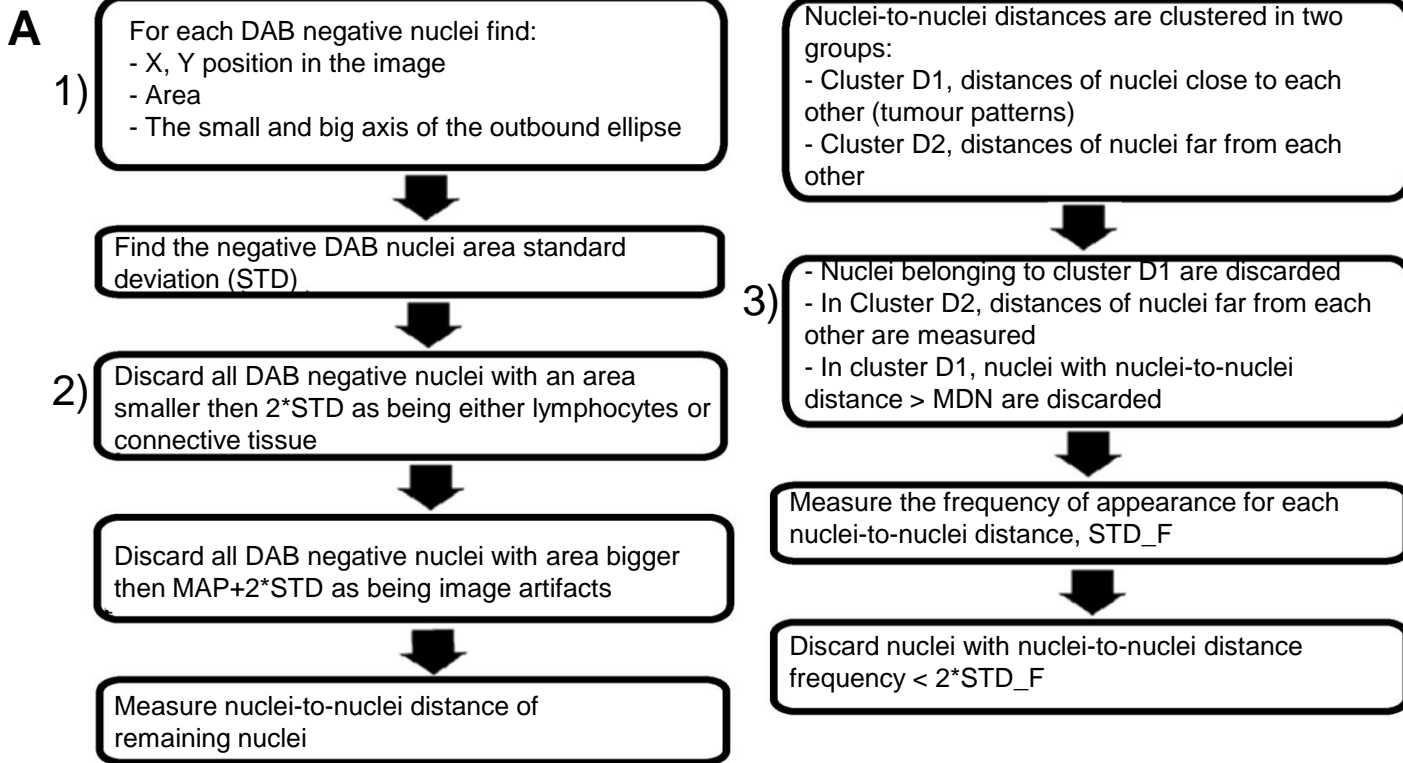
Nuclei-to-nuclei distances are clustered in two groups:
- Cluster D1, distances of nuclei close to each other (tumour patterns)
- Cluster D2, distances of nuclei far from each other

⬇

3) - Nuclei belonging to cluster D1 are discarded
- In Cluster D2, distances of nuclei far from each other are measured
- In cluster D1, nuclei with nuclei-to-nuclei distance > MDN are discarded

⬇

Measure the frequency of appearance for each nuclei-to-nuclei distance, STD_F

⬇

Discard nuclei with nuclei-to-nuclei distance frequency < 2*STD_F

B

Figure 4