DATA NOTE

An image dataset related to automated macrophage detection in immunostained lymphoma tissue samples

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Abstract

Background: We present an image dataset related to automated segmentation and counting of macrophages in diffuse large B-cell lymphoma (DLBCL) tissue sections. For the classification of DLBCL subtypes, as well as for providing a prognosis of the clinical outcome, the analysis of the tumor microenvironment and, particularly, of the different types and functions of tumor-associated macrophages is indispensable. Until now, however, most information about macrophages has been obtained either in a completely indirect way by gene expression profiling or by manual counts in immunohistochemically (IHC) fluorescence-stained tissue samples while automated recognition of single IHC stained macrophages remains a difficult task. In an accompanying publication, a reliable approach to this problem has been established, and a large set of related images has been generated and analyzed. Results: Provided image data comprise (i) fluorescence microscopy images of 44 multiple immunohistostained DLBCL tumor subregions, captured at 4 channels corresponding to CD14, CD163, Pax5, and DAPI; (ii) "cartoon-like" total variation–filtered versions of these images, generated by Rudin-Osher-Fatemi denoising; (iii) an automatically generated mask of the evaluation subregion, based on information from the DAPI channel; and (iv) automatically generated segmentation masks for macrophages (using information from CD14 and CD163 channels), B-cells (using information from Pax5 channel), and all cell nuclei (using information from DAPI channel). Conclusions: A large set of IHC stained DLBCL specimens is provided together with segmentation masks for different cell populations generated by a reference method for automated image analysis, thus featuring considerable reuse potential.

Keywords: lymphoma; DLBCL; macrophage; multiple immunohistochemical staining; automated cell counting; ROF filtering; floating threshold; rule-based detection; image dataset

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Data Description

Background

We present an image dataset generated as a part of an accompanying publication, which is concerned with method development and comparison for automated segmentation and counting of macrophages in diffuse large B-cell lymphoma (DLBCL) tissue sections [1]. DLBCL is an aggressive cancer disease that is characterized by a large heterogeneity of pathological, clinical, and biological features [2]. Therefore, a crucial step for the classification of DLBCL subtypes, as well as for providing a prognosis of the clinical outcome, is the analysis of the tumor microenvironment in terms of counts, local distributions, and functions of the different cell populations and, particularly, of the tumor-associated macrophages occuring there [3].

Until now, most information about macrophages has been obtained either by gene expression profiling [4] or by manual counts in immunohistochemically (IHC) stained tissue microarrays or high-power fields, thus either gathering information in a completely indirect way or accepting extreme subsampling rates [5]. A reliable approach for fully automated segmentation, identification, and counting of IHC stained macrophages within whole tissue slides has been addressed by Wagner et al. [1].

Our dataset contains monochrome fluorescence microscopy images of 44 DLBCL tissue samples wherein different macrophage populations (using antibodies against CD14 and CD163) and B-cells (using antibody against Pax5) as well as all cell nuclei (using 4’,6-diamidino-2-phenylindole [DAPI]) have been stained and imaged at different wavelengths. Furthermore, we supply processed images, comprising "cartoon-like" total variation-filtered images (generated by Rudin-Osher-Fatemi [ROF] filtering), as well as results of the automated macrophage segmentation. For this publication, we completed these data by automated segmentation of B-cells and the cell nuclei, avoiding headline numbering at all.

Methods

Preparation and staining of DLBCL tissue

From the files of the Lymph Node Registry Kiel, 44 DLBCL biopsy specimens have been selected. For every specimen, from formalin-fixed paraffin-embedded tissue a slice of 2 μm thickness has been obtained. To detect specific macrophages and their relation to B-cells, a triple IHC staining was performed, using primary antibodies against CD14 (Cell Marque, Cat No. 114R-14, RRID:AB_2827391; 1:10), CD163 (Novus, Cat No. NB110-59935, RRID:AB_892323; 1:100), and Pax5 (Santa Cruz Biotechnology, Cat No. sc-1974, RRID:AB_2159678; 1:100) labeled with donkey anti-rabbit Alexa 488, donkey anti-mouse Alexa 555, and donkey anti-goat Alexa 647 (all from Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA; 1:100) as secondary antibodies. Subsequently, the slices were incubated with DAPI (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA; 1:5,000) and coverslipped with mounting medium. Use of tissue was in accordance with the guidelines of the internal review board of the Medical Faculty of the Christian-Albrechts-University Kiel, Germany (No. 447/10).

Selection of tumor subregions and image acquisition

Within every tissue sample, the tumor area was defined and marked by a pathologist on the basis of inspection of conventional hematoxylin-eosin (H&E) staining in a neighboring reference slice. Subsequently, within the IHC stained slice, a rectangular subregion of the tumor area was selected, taking care for acceptable tissue and staining quality. Maximum size of tumor subregions is 10 mm².

Images of tumor subregions within the IHC stained slides were captured by Hamamatsu Nanozoomer 2.0 RS slide scanner (Hamamatsu Photonics, Ammersee, Germany) with 20 × magnification at 4 wavelengths, resulting in single images for the CD14, CD163, Pax5, and DAPI channels, respectively, which were saved in .ndpi output format with default settings as used in clinical trial routine. Note that, at this point, moderate built-in compression by imaging device was accepted. Single-channel raw images were converted into .tif format without further compression and sliced into tiles of 1,000 × 1,000 pixel format (at right and lower border, the sizes may be smaller), using the software package ImageJ with the extension ndpi tools [6]. The resulting monochrome images have been further converted from RGB into greyscale mode using the modulus of the RGB vector and finally saved in losslessly compressed .png format. We refer to them as images of type "original." Note that image acquisition and tiling have been performed in such a way that no spatial misalignment between the scans at the different wavelengths occurred. Pixel size is 0.45 × 0.45 μm² in all images.

Image processing

For every tile, the segmentation method from Wagner et al. [1] has been applied to the CD14, CD163, Pax5, and DAPI channel images, resulting in ROF-filtered images (saved as type "cartoon"); a mask for the evaluation subregion within the tile, indicating the presence of tissue at all, as inferred from DAPI channel information (saved as type "evalmask"); and segmentations of macrophages within the CD14 and CD163 channels (saved as type "segment"). Owing to the large inhomogeneity of IHC staining, even across a single target macrophage, we provide 2 further masks containing the convex hulls of the segmented features instead of the features themselves (saved as type "convhull"). The segmentation masks for double-stained macrophages are saved as type "multiple." For a general description of the ROF filter-based segmentation method, see Wagner et al. [1]. Here, we describe in more detail the generation of segmentations for the Pax5 and DAPI channels, which are new in this article.

Let us recall the notation from [1] where the indices i and j count the current intensity threshold and the features to be inspected at this stage, s(Fi), c(Fi), and r(Fi), denote the size of a feature Fi itself, the size of its convex hull, and the ratio of the principal axes’ lengths of the smallest ellipse covering the feature, respectively. Meanwhile, smin, smax, cmin, and cmax denote the minimal and maximal feature size (in pixels), the maximal area excess of the convex hull (in percent), and the maximal ratio of axes, respectively.

To obtain a segmentation of the DAPI channel, the ROF-filtered image has been further subjected to a local Narenra-Fitch contrast enhancement [7]

$$p(k, l)_{\text{enhanced}} = m(k, l) + \frac{c}{\sigma(k, l)} \cdot |p(k, l)_{\text{original}} - m(k, l)|,$$

(1)

where $c > 0$ is a weight parameter and $m(k, l)$, $\sigma(k, l)$ denote the mean and standard deviation of the intensities within a subregion centered at the pixel $p(k, l)_{\text{original}}$, respectively. We used $c = 0.75$ and a square subregion of 11 × 11 pixels size. Then, in a first
run, Steps 3–10 of the ROF filter–based segmentation have been applied, using the bounds $s_{\text{min}} = 60$ and $s_{\text{max}} = 119$ for the feature size but modifying geometrical Rule 3) for feature classification from [1] as follows: If $s_{\text{min}} \leq s(F) \leq s_{\text{max}}$, then test whether the feature satisfies both of the Criteria $3b)$ $r(F) \leq r_{\text{max}}$ (the feature is not too elongated) and $3d)$ $c(F)/s(F) \leq 1 + c_{\text{max}}/100$ (the deviation from circular shape is bounded from above). If yes, save the feature $F_j$ into the output mask, interpreting it as a cell nucleus, and mask it in $I^0(F_j)$. If not, then neglect the feature and mask it in $I^0(i)$ as well. Here, we used the parameter values $r_{\text{max}} = 2.5$ and $c_{\text{max}} = 150$. In a second run, Steps 3–10 of the ROF filter–based segmentation have been repeated with the parameter settings $s_{\text{min}} = 120$ and $s_{\text{max}} = 180$, using again the described modification of Rule 3) but saving only those features into the output mask that are completely disjoint to the output of the first run. Finally, the results of both runs have been combined into a single mask (saved as type “segment”). Within a further result mask of type convhull, the convex hulls of the detected features have been stored.

For the segmentation of the B-cells, the ROF–filtered image of the Pax5 channel has been subjected to a moderate Narendra–Fitch contrast enhancement as well, using the parameter $c = 0.1$ and a square subregion of $15 \times 15$ pixels size. To the result, Steps 3–10 of the ROF filter–based segmentation have been applied, using the bounds $s_{\text{min}} = 80$ and $s_{\text{max}} = 159$ as well as the described modification of Rule 3) with parameters $r_{\text{max}} = 2.5$ and $c_{\text{max}} = 150$ but saving into the output mask (of type segment) only features whose intersection with the convex hull of some cell nucleus, as obtained in the segmentation of the DAPI channel, is nonempty. Thus, numerous artifacts appearing in the Pax5 staining will be excluded. Again, the convex hulls of the detected features have been stored within a further mask of type convhull.

### BLC2 scoring

For all specimens, a BLC2 score is available (see Table 2). It is based on BCL2 staining for tissue slides obtained from the same biopsy specimens as before but not necessarily adjacent to the slides used for the generation of the image data presented here. Staining was microscopically examined and semi-quantitatively scored by an experienced pathologist. Each stained slide was evaluated for the percentage of stained tumor cells by visual estimation in a representative tumor area. The estimated value was graded into the following scores: 0, all cells negative; 1, $\leq 25\%$ positive cells; 2, 26–50% positive cells; 3, 51–75% positive cells; 4, $\geq 75\%$ positive cells.

### Dataset structure

Image data are organized by tissue specimens (top-level folders) and tiles (second-level folders), the latter ordered by position. Top-level folders are named specimen_01, ..., specimen_44; second-level folders are named, e.g., specimen_01_tile_01_01, ..., specimen_01_tile_09_08. Within each second-level folder, 19 image files in greyscale (gs) or black-and-white (bw) mode are stored in losslessly compressed .png format with 8-bit or 1-bit depth, respectively. Table 1 and Fig. 1 summarize the different images available at a given tile. The filenames are built as specimen_xx_tile_yy_zz_channel_[CD14, CD163, Pax5, DAPI]_type_[original, cartoon, segment, convhull, multiple, evalmask]_mode_[gs, bw].png. The size of losslessly compressed .png image files has been minimized by application of the OptiPNG routine [8]. Moreover, a logfile named

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**Table 1. Image files available within a given second-level folder**

| Channel | Description | Type | Mode |
|---------|-------------|------|------|
| CD14    | CD14 staining, original single-channel image | original | gs   |
|         | ROF–filtered image derived from original | cartoon  | gs   |
|         | Mask highlighting the segmented macrophages | segment  | bw   |
|         | Mask highlighting the convex hulls of the segmented macrophages | convhull | bw   |
|         | Mask highlighting the segmented macrophages bearing CD163 staining as well | multiple | bw   |
| CD163   | CD163 staining, original single-channel image | original | gs   |
|         | ROF–filtered image derived from original | cartoon  | gs   |
|         | Mask highlighting the segmented macrophages | segment  | bw   |
|         | Mask highlighting the convex hulls of the segmented macrophages | convhull | bw   |
|         | Mask highlighting the segmented macrophages bearing CD14 staining as well | multiple | bw   |
| Pax5    | Pax5 staining, original single-channel image | original | gs   |
|         | ROF–filtered image derived from original | cartoon  | gs   |
|         | Mask highlighting the segmented B-cells | segment  | bw   |
|         | Mask highlighting the convex hulls of the segmented B-cells | convhull | bw   |
| DAPI    | DAPI staining, original single-channel image | original | gs   |
|         | ROF–filtered image derived from original | cartoon  | gs   |
|         | Mask representing the evaluation subregion | evalmask |   |
|         | Mask highlighting the segmented cell nuclei | segment  | bw   |
|         | Mask highlighting the convex hulls of the segmented cell nuclei | convhull | bw   |

**Table 2. BLC2 scores**

| Score | Specimen No. |
|-------|--------------|
| 0     | 03, 05, 18, 32, 37, 43, 44 |
| 1     | – |
| 2     | 36 |
| 3     | 28 |
| 4     | 01, 02, 04, 06 – 17, 19 – 27, 29 – 31, 33 – 35, 38 – 42 |
Figure 1 Summary of images available at a given tile (cutouts of 500 × 500 pixels size). Contrast enhanced in A by a factor of 3.5, in E by a factor of 7, and in F–H by a factor of 2. Originals (A–D): A: species:02_tile:01_06_channel:CD14_type:original_mode:gs.png, B: species:02_tile:01_06_channel:CD163_type:original_mode:gs.png, C: species:02_tile:01_06_channel:Pax5_type:original_mode:gs.png, D: species:02_tile:01_06_channel:DAPI_type:original_mode:gs.png. Cartoons (E–H): E: species:02_tile:01_06_channel:CD14_type:cartoon_mode:gs.png, F: species:02_tile:01_06_channel:CD163_type:cartoon_mode:gs.png, G: species:02_tile:01_06_channel:Pax5_type:cartoon_mode:gs.png, H: species:02_tile:01_06_channel:DAPI_type:cartoon_mode:gs.png. Segmentations (I–L): I: species:02_tile:01_06_channel:CD14_type:segment_mode:bw.png, J: species:02_tile:01_06_channel:CD163_type:segment_mode:bw.png, K: species:02_tile:01_06_channel:Pax5_type:segment_mode:bw.png, L: species:02_tile:01_06_channel:DAPI_type:segment_mode:bw.png. Convex hulls (M–P): M: species:02_tile:01_06_channel:CD14_type:convhull_mode:bw.png, N: species:02_tile:01_06_channel:CD163_type:convhull_mode:bw.png, O: species:02_tile:01_06_channel:Pax5_type:convhull_mode:bw.png, P: species:02_tile:01_06_channel:DAPI_type:convhull_mode:bw.png. Various (Q–S): Q: species:02_tile:01_06_channel:CD14_type:multiple_mode:bw.png, R: species:02_tile:01_06_channel:CD163_type:multiple_mode:bw.png, S: species:02_tile:01_06_channel:DAPI_type:evalmask_mode:bw.png.
Figure 2 Examples of combined information from several images, based on specimen_xx_tile_yy_zz_logfile.txt. Image size is 1,000 × 1,000 pixels (450 × 450 μm²). A: Original image at CD14 channel (greyscale, contrast enhanced by a factor of 3.5, inverted) (channel_CD14_type_original_mode_gs.png) with superimposition of the mask of the evaluation subregion, as obtained from the DAPI channel (light blue) (channel_DAPI_type_evalmask_mode_bw.png) and the segmentation of the CD14-stained macrophages (olive green) (channel_CD14_type_segment_mode_bw.png). B: The same tile as imaged at the Pax5 channel (greyscale, inverted) (channel_Pax5_type_original_mode_gs.png) with superimposition of the cell nuclei segmentation from the DAPI channel (light blue, convex hulls) (channel_DAPI_type_convhull_mode_bw.png) and the segmentation of the CD163-stained macrophages (dark yellow) (channel_CD163_type_segment_mode_bw.png). Examples of B-cells positioned inside macrophages indicated by arrows. C: Combination of both macrophage segmentations (olive green or dark yellow, convex hulls) for the same tile in order to reveal double-stained parts (light yellow) (channel_CD14_type_convhull_mode_bw.png / channel_CD163_type_convhull_mode_bw.png). D: Segmentation of B-cells from the Pax5 channel (magenta and grey, convex hulls) (channel_Pax5_type_convhull_mode_bw.png) superimposed to Fig. 2C. Examples of B-cells positioned inside macrophages indicated by arrows (the same cells as in Fig. 2B).

Reuse potential

Although there is a vast number of publications concerned with the composition of the tumor microenvironment in various types of lymphoma disease, image datasets of IHC stained cancer tissue are rarely publicly accessible if at all (cf. the discussion in Kostopoulos et al. [9]). Most data generated for the purpose of such analyses are not findable or not even accessible. For example, the Genomic Data Commons Data Portal of the National Cancer Institute [10, 11] currently lists only 48 cases of mature B-cell lymphoma with an image of an H&E-stained slide available, while IHC stainings are completely missing. In this situation, the
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We outline the most important options for further use of the dataset. First, it allows for a detailed morphometrical investigation of the imaged macrophages and B-cells with respect to the distribution of geometrical parameters such as size, diameter, and perimeter, as well as to overall shape patterns. Second, the data may be used for validation, calibration, and comparison of cell segmentation methods (manual, automated) and related software packages, making available a large reference dataset together with the output of a reference method as described in [1]. Note that, for these purposes, it is particularly adequate to use data featuring a routine quality level. Third, the original images as well as the segmentations presented here could be used for the generation of a sufficiently large training set for automated macrophage detection by machine learning methods. Fourth, the data may be used for study of co-localization and clustering of macrophages and B-cells within lymphoma tissue and cancer microenvironment, using appropriate methods of point-pattern statistics [12, 13]. Finally, the dataset enables a closer study of the double-stained macrophage subpopulation. To facilitate a possible further processing of the obtained features (e.g., extraction of barycenters, replacement of the features by equally sized circles or squares), not only the masks for the segmented features themselves but as well those for their convex hulls are provided.

To illustrate the described reuse potential, we include a set of composite figures, each combining information from several separate images. Fig. 2A shows an original image at CD14 channel (grayscale, original contrast-enhanced by a factor of 3.5 and inverted) with superimposition of the mask of the evaluation subregion, as obtained from the DAPI channel (light blue), and the segmentation of the CD14-stained macrophages (olive green). Fig. 2B shows the same tile as imaged at the Pax5 channel (grayscale, original inverted) with superimposition of the cell nuclei segmentation from the DAPI channel (light blue, convex hulls) and the segmentation of the CD163-stained macrophages (dark yellow). In Fig. 2C, for the same tile, both macrophage segmentations (olive green or dark yellow, convex hulls) are combined to reveal double-stained parts (light yellow). In Fig. 2D, we superimposed to Fig. 2C the segmentation of B-cells from the Pax5 channel (magenta and grey, convex hulls) on the double-stained macrophage subpopulation. To facilitate a possible further processing of the obtained features (e.g., extraction of barycenters, replacement of the features by equally sized circles or squares), not only the masks for the segmented features themselves but as well those for their convex hulls are provided.

To improve reusability, BLC2 scores for the biopsy specimens are provided.

Availability of Supporting Data and Materials

All image data are made publicly accessible under CC0 1.0 license at the Leipzig Health Atlas (LHA) repository [14] and can be reached from the website [15]. Each top-level folder can be downloaded as .zip file and bears a separate identifier, e.g., https://health-atlas.de/lha/7YXMMFNPDG-0 within the repository (see Table 3). Two folders with total size >1 GB (Nos. 04 and 44) have been split into a pair of files. Snapshots of the datasets are available in the GigaScience GigaDB repository as well [16].

### Table 3. Datasets available at the Leipzig Health Atlas

| Name       | Size (MB) | Identifier         |
|------------|-----------|--------------------|
| specimen_01.zip | 161       | 7YXMMFNPDG-0       |
| specimen_02.zip | 142       | 7YYXUTYPYN-9       |
| specimen_03.zip | 121       | 7YYX2MUWDK-3       |
| specimen_04a.zip | 630      | 7YYXECKXQM-0       |
| specimen_04b.zip | 709       | 7YYX6V6Y7-9        |
| specimen_05.zip | 653       | 7YYX0G00AD-4       |
| specimen_06.zip | 168       | 7YY0NHX8F-2        |
| specimen_07.zip | 409       | 7YY0X073KU7-4      |
| specimen_08.zip | 396       | 7YY146X8HE-4       |
| specimen_09.zip | 283       | 7YY19ARW7C-8       |
| specimen_10.zip | 368       | 8004HF6Q6R-5       |
| specimen_11.zip | 708       | 8004RHW6X-6        |
| specimen_12.zip | 360       | 800516XMC9         |
| specimen_13.zip | 150       | 8005NPDNX-6        |
| specimen_14.zip | 283       | 8005QXY7QG-0       |
| specimen_15.zip | 124       | 8005TY4388-4       |
| specimen_16.zip | 146       | 8005X2U355-5       |
| specimen_17.zip | 364       | 80062H7C7J-4       |
| specimen_18.zip | 164       | 800HNWTVTX-5       |
| specimen_19.zip | 105       | 800HR8G5PE-5       |
| specimen_20.zip | 418       | 800JSA4V17-7       |
| specimen_21.zip | 431       | 800JDA4XHV-6       |
| specimen_22.zip | 465       | 802X2RAVTV-8       |
| specimen_23.zip | 333       | 802XYVYQ27-0       |
| specimen_24.zip | 462       | 802XR67D0U-2       |
| specimen_25.zip | 749       | 802Y1JKFPO-6       |
| specimen_26.zip | 635       | 803AHCEAH-5        |
| specimen_27.zip | 137       | 803AHWGT39-4       |
| specimen_28.zip | 225       | 803AUJ2NYK-8       |
| specimen_29.zip | 549       | 803C11MPMP-3       |
| specimen_30.zip | 334       | 803C4Q4N4P-5       |
| specimen_31.zip | 217       | 803CB09ERJ-6       |
| specimen_32.zip | 293       | 803CF4U96-9        |
| specimen_33.zip | 330       | 803CR62YA-8        |
| specimen_34.zip | 474       | 803NYKMO9P-9       |
| specimen_35.zip | 286       | 803PJH0HTQ-2       |
| specimen_36.zip | 225       | 803PKLTZG-7        |
| specimen_37.zip | 563       | 803PKW9X5G9-9      |
| specimen_38.zip | 524       | 803PVR444-8        |
| specimen_39.zip | 879       | 804FGWCGP2-1       |
| specimen_40.zip | 382       | 804FGUSJG-0        |
| specimen_41.zip | 421       | 804GIXAZAE-8       |
| specimen_42.zip | 114       | 804G4F4H4Q-4       |
| specimen_43.zip | 596       | 804G42PMN-9        |
| specimen_44a.zip | 506      | 804HE6EM8W-2       |
| specimen_44b.zip | 452       | 804H7C4T1P-0       |

**Abbreviations**

DAPI: 4’,6-diamidino-2-phenylindole; DLBCL: diffuse large B-cell lymphoma; H&E: hematoxylin-eosin; IHC: immunohistochemistry(ly); LHA: Leipzig Health Atlas; ROF: Rudin-Osher-Fatemi.

**Ethical approval**

Tissue usage is covered by statement No. 447/10 of the internal review board of the Medical Faculty of the Christian-Albrechts-University Kiel, Germany.
Competing interests
The authors declare that they have no competing interests.

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Authors’ contributions
M.W. performed the image processing and wrote the manuscript. S.R. performed the IHC fluorescence staining and image generation. R.H. curated the large-size image datasets and managed the storage within the Leipzig Health Atlas repository. W.K. identified the cohort. U.D.B. contributed to the Background, Methods, and Reuse Potential sections. All authors read and approved the final manuscript.

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References
1. Wagner M, Hänsel R, Reinke S, et al. Automated macrophage counting in DLBCL tissue samples: a ROF filter based approach. Biol Proced 2019;21:13.
2. Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of tumours of haematopoietic and lymphoid tissues. In: WHO Classification of Tumours, Vol. 2. Lyon: International Agency for Research on Cancer; 2017. 4th, rev. ed.
3. Scott DW, Gascoyne RD. The tumour microenvironment in B cell lymphomas. Nat Rev Cancer 2014;14:517–34.
4. Scott DW, Wright GW, Williams PM, et al. Determining cell-of-origin subtypes of diffuse large B-cell lymphoma using gene expression in formalin-fixed paraffin embedded tissue. Blood 2014;123:1214–7.
5. Lozanski G, Pennell M, Shana’ah A, et al. Inter-reader variability in follicular lymphoma grading: conventional and digital reading. J Pathol Inform 2013;4:30.
6. Deroulers C, Ameisen D, Badoual M, et al. Analyzing huge pathology images with open source software. Diagn Pathol 2013;8:92.
7. Narendra PM, Fitch RC. Real-time adaptive contrast enhancement. IEEE Trans Pattern Anal Mach Intell 1981;3:655–61.
8. OptiPNG: Advanced PNG Optimizer. http://optipng.sourceforge.net. Accessed 6 February 2020.
9. Lozanski G, Pennell M, Shana’ah A, et al. Inter-reader variability in follicular lymphoma grading: conventional and digital reading. J Pathol Inform 2013;4:30.
10. https://portal.gdc.cancer.gov/repository. Accessed 6 February 2020.
11. Cooper LAD, Demicco EG, Saltz JH, et al. PanCancer insights from The Cancer Genome Atlas: the pathologist's perspective. J Pathol 2018;244:512–24.
12. Ripley BD. Spatial Statistics. New York: Wiley; 1981.
13. Møller J, Waagepetersen RP. Modern statistics for spatial point processes. Scand J Stat 2007;34:643–84.
14. Meineke FA, Löbe M, Stäubert S. Introducing technical aspects of research data management in the Leipzig Health Atlas. Stud Health Technol Inform 2018;247:425–30.
15. An image dataset related to automated macrophage detection in immunostained lymphoma tissue samples. https://health-atlas.de/lha/7XWCURQFP8K-8. Accessed 6 February 2020.
16. Wagner M, Reinke S, Hänsel R, et al. Supporting data for "An image dataset related to automated macrophage detection in immunostained lymphoma tissue samples." GigaScience Database 2020. http://dx.doi.org/10.5524/100703.