References

[1] Fons J. Verbeek. Seeing small things big. In *Leiden University*, 2018.

[2] Georgios C. Manikis, Kostas Marias, Eleftherios Alissandrakis, Louis Perrotto, Elisavet Savvidaki, and et al. Pollen grain classification using geometrical and textural features. In *2019 IEEE International Conference on Imaging Systems and Techniques (IST)*, pages 1–6, 2019.

[3] Frida Sommer, Vincenzo Torraca, and Annemarie H. Meijer. Chemokine receptors and phagocyte biology in zebrafish. *Frontiers Immunology*, 2020.

[4] Yufei Xie, Annemarie H. Meijer, and Marcel J. M. Schaaf. Modeling inflammation in zebrafish for the development of anti-inflammatory drugs. *Frontiers in Cell and Developmental Biology*, 8, 2021.

[5] Leiba Jade, Resul Özbilgiç, Liz Hernández, Maria Demou, Georges Lutfalla, Laure Yatime, and Mai Nguyen-Chi. Molecular actors of inflammation and their signaling pathways: Mechanistic insights from zebrafish. *Biology*, 12, 2023.

[6] Xiaoqin Tang. *Computational optimisation of optical projection tomography for 3D image analysis*. PhD thesis, Leiden University, 6 2020.

[7] Douglas E Chandler and Robert W Roberson. *Bioimaging: current concepts in light and electron microscopy*. Jones and Bartlett Publishers, 2009.

[8] Lu Cao. *Biological model representation and analysis*. PhD thesis, Leiden University, 11 2014.

[9] Fuyong Xing, Yuanpu Xie, Hai Su, Fujun Liu, and Lin Yang. Deep learning in microscopy image analysis: A survey. *IEEE Transactions on Neural Networks and Learning Systems*, 29(10):4550–4568, 2018.

[10] Gaudenz Danuser. Computer vision in cell biology. *Cell*, 147(5):973–978, 2011.

[11] Thomas M Ward, Pietro Mascagni, Yutong Ban, Guy Rosman, Nicolas Padoy, Ozanan Meireles, and et al. Computer vision in surgery. *Surgery*, 169(5):1253–1256, 2021.

[12] Jiechao Ma, Yang Song, Xi Tian, Yiting Hua, Rongguo Zhang, and Jianlin Wu. Survey on deep learning for pulmonary medical imaging. *Frontiers of medicine*, 14:450–469, 2020.

[13] Dinggang Shen, Guorong Wu, and Heung-II Suk. Deep learning in medical image analysis. *Annual Review of Biomedical Engineering*, 21(19):221–248, 2017.

[14] Mohamed A. Abdou. Literature review: efficient deep neural networks techniques for medical image analysis. *Neural Computing and Applications*, 34:5791–5812, 2022.

[15] Athanasios Voulodimos, Nikolaos Doulamis, Anastasios Doulamis, and Eftychios Protopapadakis. Deep learning for computer vision: A brief review. *Computational Intelligence and Neuroscience*, 2018.

[16] Niall O'Mahony, Sean Campbell, Anderson Carvalho, Suman Harapanahalli, Gustavo Velasco Hernandez, and et al. Deep learning vs. traditional computer vision. In *In Advances in Computer Vision: Proceedings of the 2019 Computer Vision Conference*, volume 943, pages 128–144, 2020.

[17] Seonwoo Min, Byunghan Lee, and Sungroh Yoon. Deep learning in bioinformatics. *Briefings in Bioinformatics*, 18(5):851–869, 2017.

[18] Bram van Ginneken. Fifty years of computer analysis in chest imaging: rule-based, machine learning, deep learning. *Radiological Physics and Technology*, 10:23–32, 2017.

[19] Zhichao Liu, Luhong Jin, Jincheng Chen, Qiuyu Fang, Sergey Ablameyko, Zhaozheng Yin, and Yingke Xu. A survey on applications of deep learning in microscopy image analysis. *Computers in Biology and Medicine*, 134, 2021.

[20] Vebjorn Ljosa, Katherine L Sokolnicki, and Anne E Carpenter. Annotated high-throughput microscopy image sets for validation. *Nature Methods*, 9(7):637–637, 2012.

[21] Vladimír Ulman and et al. An objective comparison of cell-tracking algorithms. *Nature Methods*, 14(12):1141–1152, 2017.

[22] Anubha Gupta and et al. Segpc-2021: A challenge dataset on segmentation of multiple myeloma plasma cells from microscopic images. *Medical Image Analysis*, 83, 2023.

[23] Cell tracking challenge. http://celltrackingchallenge.net/, 2012–2020.

[24] Fabrice Cordelieres. Manual tracking. https://imagej.nih.gov/ij/plugins/track/track.html, 2004–2005.

[25] Wei Zhou, Zhiwu Xia, Peng Dou, Tao Su, and Haifeng Hu. Aligning image semantics and label concepts for image multi-label classification. *ACM Transactions on Multimedia Computing, Communications, and Applications*, 19(2):1–23, 2023.

[26] Rui Liu, Wei Dai, Tianyi Wu, Min Wang, Song Wan, and Jun Liu. Aimic: Deep learning for microscopic image classification. *Computer Methods and Programs in Biomedicine*, 226, 2022.

[27] Hadi Rezaeilouyeh, Ali Mollahosseini, and Mohammad H. Mahoor. Microscopic medical image classification framework via deep learning and shearlet transform. *Journal of Medical Imaging*, 3(4), 2016.

[28] Long D. Nguyen, Dongyun Lin, Zhiping Lin, and Jiuwen Cao. Deep cnns for microscopic image classification by exploiting transfer learning and feature concatenation. In *2018 IEEE International Symposium on Circuits and Systems*, pages 1–5, 2018.

[29] William Meiniel, Jean-Christophe Olivo-Marin, and Elsa D. Angelini. Denoising of microscopy images: A review of the state-of-the-art, and a new sparsity-based method. *IEEE Transactions on Image Processing*, 27(8):3842–3856, 2018.

[30] Souad Larabi-Marie-Sainte, Reham Alskireen, and Sawsan Alhalawani. Emerging applications of bio-inspired algorithms in image segmentation. *Electronics*, 10(24):3226, 2021.

[31] Zhaozheng Yin, Kang Li, Takeo Kanade, and Mei Chen. Understanding the optics to aid microscopy image segmentation. In *Medical Image Computing and Computer-Assisted Intervention – MICCAI 2010*, volume 6361, 2010.

[32] Shervin Minaee, Yuri Boykov, Fatih Porikli, Antonio Plaza, Nasser Kehtarnavaz, and Demetri Terzopoulos. Image segmentation using deep learning: A survey. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, 44:3523–3542, 2022.

[33] Alper Yilmaz, Omar Javed, and Mubarak Shah. Object tracking: A survey. *ACM Computing Surveys*, 38(4):13–es, 2006.

[34] Martin Maška and et al. A benchmark for comparison of cell tracking algorithms. *Bioinformatics*, 30(11):1609–1617, 2014.

[35] Erik Meijering, Oleh Dzyubachyk, and Ihor Smal. Methods for cell and particle tracking. *Methods in Enzymology*, 504:183–200, 2012.

[36] Thomas Bieber and et al. Global allergy forum and 3rd davos declaration 2015: Atopic dermatitis/eczema: Challenges and opportunities toward precision medicine. *Allergy*, 71(5):588–592, 2016.

[37] Oliver Pfaar, Hongfei Lou, Yuan Zhang, Ludger Klimek, and Luo Zhang. Recent developments and highlights in rhinitis and allergen immunotherapy. *Allergy*, 73(12):2306–2313, 2018.

[38] Innes Asher and et al. World allergy organization guidelines for prevention of allergy and allergic asthma. *International Archives of Allergy and Immunology*, 135(1):83–92, 2004.

[39] Kostas D. Karatzas, Marina Riga, and Matt Smith. Presentation and dissemination of pollen information. In *Allergenic Pollen*, pages 217–247. Springer, 2012.

[40] Carmi Geller-Bernstein and Jay M Portnoy. The clinical utility of pollen counts. *Clinical Reviews in Allergy and Immunology*, 57(3):340–349, 2019.

[41] JM Hirst. An automatic volumetric spore trap. *Annals of Applied Biology*, 39:257–265, 1952.

[42] Beug Hans-Jürgen. Leitfaden der pollenbestimmung für mitteleuropa und angrenzende gebiete. In *Verlag Dr. Friedrich Pfeil*. 2004.

[43] Chiara Ziello, Tim H. Sparks, Nicole Estrella, Jordina Belmonte, Karl C. Bergmann, Edith Bucher, and et al. Changes to airborne pollen counts across europe. *Plos One*, 7(4):e34076, 2012.

[44] Erdtman G. The acetolysis method-a revised description. *Svensk Botanisk Tidskrift*, 54(4):561–564, 1960.

[45] Gretchen D. Jones. Pollen analyses for pollination research, acetolysis. *Journal of Pollination Ecology*, 13:203–217, 2014.

[46] Angelica Tiotiu, Andrea Brazdova, Cyril Longé, Patrice Gallet, Martine Morisset, Virginie Leduc, and et al. Urtica dioica pollen allergy: Clinical, biological, and allergomics analysis. *Annals of Allergy, Asthma Immunology*, 117(5):527–534, 2016.

[47] D'Amato G. and Liccardi G. Pollen-related allergy in the european mediterranean area. *Clinical and Experimental Allergy*, 24(3):210–219, 1994.

[48] Giorgio Ciprandi, Paola Puccinelli, Cristoforo Incorvaia, and Simonetta Masieri. Parietaria allergy: An intriguing challenge for the allergist. *Medicina*, 54(6):106, 2018.

[49] Bass D.A. and Bass D.J. Parietaria judaica l. a cause of allergic disease in sydney. a study of habit and spread of the weed. *Review of Palaeobotany and Palynology*, 64(1):97–101, 1990.

[50] Christina Fotiou, Athanasios Damialis, Nikolaos Krigas, John M Halley, and Despoina Vokou. Parietaria judaica flowering phenology, pollen production, viability and atmospheric circulation, and expansive ability in the urban environment: impacts of environmental factors. *Internation Journal of Biometeorology*, 55(1):35–50, 2011.

[51] D'Amato G, Ruffilli A, Sacerdoti G, and Bonini S. Parietaria pollinosis: a review. *Allergy*, 47(5):443–449, 1992.

[52] Roser GuardiaCentre and Jordina BelmonteBotanical. Phenology and pollen production of parietaria judaica l. in catalonia (ne spain). *Grana*, 43(1):57–64, 2010.

[53] Corbi A.L., Pelaez A., Errigo E., and Carreira J. Cross-reactivity between parietaria judaica and parietaria officinalis. *Annals of Allergy*, 54(2):142–147, 1985.

[54] Bousquet J., Hewitt B., Guérin B., Dhivert H., and Michel F.B. Allergy in the mediterranean area. ii: Cross-allergenicity among urticaceae pollens (parietaria and urtica). *Clinical Allergy*, 16(1):57–64, 1986.

[55] D'Amato G., Cecchi L., Bonini S., Nunes C., Annesi-Maesano I., Behrendt H., and et al. Allergenic pollen and pollen allergy in europe. *Allergy*, 62(9):976–990, 2007.

[56] Rodríguez A.M., Palacios I.S., Molina R.T, and Corchero A.M. Urtica membranacea and the importance of its separation from the rest of the urticaceae in aeropalynological studies carried out in the mediterranean region. *Plant Biosystems*, 140(3):321–332, 2006.

[57] Punt W. and Malotaux Marieke. Cannabaceae, moraceae and urticaceae. *Review of Palaeobotany and Palynology*, 42(1-4):23–44, 1984.

[58] Holt K.A. and Bennett K.D. Principles and methods for automated palynology. *New Phytologist*, 203(3):735–742, 2014.

[59] De Sá otero Ma Pilar, González Amelia, Rodríguez-Damián M., and Cernadas E. Computer-aided identification of allergenic species of urticaceae pollen. *Grana*, 43(4):224–230, 2004.

[60] Ariadne Barbosa Gonçalves, Junior Silva Souza, Gercina Gonçalves da Silva, Marney Pascoli Cereda, Arnildo Pott, and Marco Hiroshi Naka. Feature extraction and machine learning for the classification of brazilian savannah pollen grains. *Plos One*, 11(6):e0157044, 2016.

[61] Hanane Menad, Farah Ben-Naoum, and Abdelmalek Amine. Deep convolutional neural network for pollen grains classification. In *National Study Day on Research on Computer Sciences*, 2019.

[62] Sevillano Víctor and Aznarte José L. Improving classification of pollen grain images of the polen23e dataset through three different applications of deep learning convolutional neural networks. *Plos One*, 13(9):e0201807, 2018.

[63] Sevillano Víctor, Holt Katherine, and Aznarte José L. Precise automatic classification of 46 different pollen types with convolutional neural networks. *Plos One*, 15(6):e0229751, 2020.

[64] Eraldo Ribeiro Amar Daood and Mark Bush. Pollen grain recognition using deep learning. *Advances in Visual Computing*, 10072(6):321–330, 2016.

[65] Surangi W. Punyasena, David K. Tcheng, Cassandra Wesseln, and Pietra G. Mueller. Classifying black and white spruce pollen using layered machine learning. *New Phytologist*, 196(3):937–944, 2012.

[66] Yılmaz Kaya, S. Mesut Pınar, M. Emre Erez, Mehmet Fidan, and James B. Riding. Identification of onopordum pollen using the extreme learning machine, a type of artificial neural network. *Palynology*, 38(1):129–137, 2014.

[67] Amirreza Mahbod, Gerald Schaefer, Rupert Ecker, and Isabella Ellinger. Pollen grain microscopic image classification using an ensemble of fine-tuned deep convolutional neural networks. *arXiv preprint arXiv:2011.07428*, 2020.

[68] Duistermaat Leni. Heukels' flora van nederland 24th edition. Groningen/Utrecht, Noordhoff Uitgevers, 2020.

[69] Rasband W.S. Imagej, US National Institutes of Health, Bethesda, MD, U.S.A. http://rsb.info.nih.gov/ij/, 1997–2006.

[70] Wheeler R. Extended depth of field. http://www.richardwheeler.net.

[71] Simonyan Karen and Zisserman Andrew. Very deep convolutional networks for large-scale image recognition. *arXiv preprint arXiv:1409.1556*, 2014.

[72] Howard G. Andrew, Zhu Menglong, Chen Bo, Kalenichenko Dmitry, Wang Weijun, and Weyand Tobias. Mobilenets: Efficient convolutional neural networks for mobile vision applications. *arXiv preprint arXiv:1704.04861*, 2017.

[73] Sandler Mark, Howard Andrew, Zhu Menglong, Zhmoginov Andrey, and Chen Liang-Chieh. Mobilenetv2: Inverted residuals and linear bottlenecks. In *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition*, pages 4510–4520, 2018.

[74] Chollet F.K. https://github.com/fchollet/keras, 2015.

[75] Geus André R. de, Barcelos Celia A.Z., Batista Marcos A., and Silva Sérgio F. da. Large-scale pollen recognition with deep learning. In *2019 27th European Signal Processing Conference (EUSIPCO)*, pages 1–5, 2019.

[76] Amar I. Daood, Eraldo Ribeiro, and Mark B. Bush. Sequential recognition of pollen grain z-stacks by combining cnn and rnn. In *The Florida AI Research Society*, 2018.

[77] Ingrid C. Romero, Shu Kong, Charless C. Fowlkes, and Surangi W. Punyasena. Improving the taxonomy of fossil pollen using convolutional neural networks and superresolution microscopy. In *Proceedings of the National Academy of Sciences of the United States of America*, volume 117, pages 28496–28505, 2020.

[78] Rodriguez-Damian M., Cernadas E., Formella A., Fernandez-Delgado M., and Pilar De Sa-Otero. Automatic detection and classification of grains of pollen based on shape and texture. *IEEE Transactions on Systems, Man, and Cybernetics, Part C (Applications and Reviews)*, 36(4):531–542, 2006.

[79] Amar Daood, Eraldo Ribeiro, and Mark Bush. Classifying pollen using robust sequence alignment of sparse z-stack volumes. In *International Symposium on Visual Computing*, pages 331–340, 2016.

[80] Chloe He, Gerard Glowacki, and Alexis Gkantiragas. Unsupervised representations of pollen in bright-field microscopy. *arXiv preprint arXiv:1908.01866*, 2019.

[81] Kadaikar Aysha, Pan Yan, Zhang Qiaoxi, Conde-Céspedes Patricia, Trocan Maria, Amiel Frédéric, and et al. Variable complexity neural networks comparison for pollen classification. *International Journal of Biology and Biomedical Engineering*, 2019.

[82] Shaoning Zeng, Bob Zhang, Jianping Gou, and Yong Xu. Regularization on augmented data to diversify sparse representation for robust image classification. *IEEE Transactions on Cybernetics*, 52(6):4935–4948, 2022.

[83] Ramón Gallardo-Caballero, Carlos J García-Orellana, Antonio García-Manso, Horacio M González-Velasco, Rafael Tormo-Molina, and Miguel Macías-Macías. Precise pollen grain detection in bright field microscopy using deep learning techniques. *Sensors*, 19(16):3583, 2019.

[84] Katherine Angharad Holt, G. P. Allen, R.M. Hodgson, and et al. Progress towards an automated trainable pollen location and classifier system for use in the palynology laboratory. *Review of Palaeobotany and Palynology*, 167(3-4):175–183, 2011.

[85] Jose Oteros, Gudrun Pusch, Ingrid Weichenmeier, Ulrich Heimann, Rouven Möller, Stefani Röseler, and et al. Automatic and online pollen monitoring. *International Archives of Allergy and Immunology*, 167(3):158–166, 2015.

[86] Eric Sauvageat, Yanick Zeder, Kevin Auderset, Bertrand Calpini, Bernard Clot, Benoît Crouzy, and et al. Real-time pollen monitoring using digital holography. *Atmospheric Measurement Techniques*, 13(3):1539–1550, 2020.

[87] Gennaro D'Amato, Herberto Jose Chong-Neto, Olga Patricia Monge Ortega, and et al. The effects of climate change on respiratory allergy and asthma induced by pollen and mold allergens. *In Allergy: European Journal of Allergy and Clinical Immunology*, 75(9):2219–2228, 2020.

[88] Biedermann T., Winther L., Till S.J., Panzner P., Knulst A., and Valovirta E. Birch pollen allergy in europe. In *Allergy: European Journal of Allergy and Clinical Immunology*, volume 74, pages 1237–1248, 2019.

[89] Ariadne Barbosa Gonçalves, Junior Silva Souza, Gercina Gonçalves Da Silva, Marney Pascoli Cereda, Arnildo Pott, Marco Hiroshi Naka, and Hemerson Pistori. Feature extraction and machine learning for the classification of brazilian savannah pollen grains. *Plos One*, 11(6), 2016.

[90] Battiato Sebastiano, Ortis Alessandro, Trenta Francesca, Ascari Lorenzo, Politi Mara, and Siniscalco Consolata. Detection and classification of pollen grain microscope images. In *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition Workshops*, pages 4220–4227, 2020.

[91] Dunker Susanne, Motivans Elena, Rakosy Demetra, Boho David, Mäder Patrick, Hornick Thomas, and Knight Tiffany M. Pollen analysis using multispectral imaging flow cytometry and deep learning. *New Phytologist*, 229(1):593–606, 2021.

[92] Pospiech Matej, Javůrková Zdeňka, Hrabec Pavel, Štarha Pavel, Ljasovská Simona, Bednář Josef, and Tremlová Bohuslava. Identification of pollen taxa by different microscopy techniques. *Plos One*, 16(9):e0256808, 2021.

[93] Manikis G.C., Marias K., Alissandrakis E., Perrotto L., Savvidaki E., and Vidakis N. Pollen grain classification using geometrical and textural features. In *IST 2019 - IEEE International Conference on Imaging Systems and Techniques, Proceedings*, pages 1–6, 2019.

[94] Daood Amar, Ribeiro Eraldo, and Bush Mark. Pollen grain recognition using deep learning. In *Lecture Notes in Computer Science (including subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics)*, volume 10072, pages 321–330, 2016.

[95] Schiele Julian, Rabe Fabian, Schmitt Maximilian, Glaser Manuel, Haring Franziska, Brunner Jens O., Bauer Bernhard, Schuller Bjorn, Traidl-Hoffmann Claudia, and Damialis Athanasios. Automated classification of airborne pollen using neural networks. In *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS*, pages 4474–4478, 2019.

[96] Marcel Polling, Chen Li, Lu Cao, Fons J. Verbeek, Letty A. de Weger, Jordina Belmonte, Concepción De Linares, Joost Willemse, Hugo de Boer, and Barbara Gravendeel. Neural networks for increased accuracy of allergenic pollen monitoring. *Scientific Reports*, 11, 2021.

[97] del Pozo-Baños Marcos, Ticay-Rivas Jaime R., Alonso Jesús B., and Travieso Carlos M. Features extraction techniques for pollen grain classification. *Neurocomputing*, 150:377–391, 2015.

[98] Marcos J. Víctor, Nava Rodrigo, Cristóbal Gabriel, Redondo Rafael, Escalante-Ramírez Boris, Bueno Gloria, Déniz Óscar, González-Porto Amelia, Pardo Cristina, Chung François, and Rodríguez Tomás. Automated pollen identification using microscopic imaging and texture analysis. *Micron*, 68:36–46, 2015.

[99] Astolfi Gilberto, Gonçalves Ariadne Barbosa, Menezes Geazy Vilharva, Borges Felipe Silveira Brito, Astolfi Angelica Christina Melo Nunes, Matsubara Edson Takashi, Alvarez Marco, and Pistori Hemerson. Pollen73s: An image dataset for pollen grains classification. *Ecological Informatics*, 60, 2020.

[100] Rodrìuez-Damián Marìa, Cernadas Eva, Formella Arno, Fernández-Delgado Manuel, and De Sá-Otero Pilar. Automatic detection and classification of grains of pollen based on shape and texture. *IEEE Transactions on Systems, Man and Cybernetics Part C: Applications and Reviews*, 36(4):531–542, 2006.

[101] Aguet François, Van De Ville Dimitri, and Unser Michael. Model-based 2.5-d deconvolution for extended depth of field in brightfield microscopy. *IEEE Transactions on Image Processing*, 17(7):1144–1153, 2008.

[102] Bountris P, Farantatos E, and Apostolou N. Advanced image analysis tools development for the early stage bronchial cancer detection. *World Academy of Science, Engineering and Technology*, 1(9), 2007.

[103] Lu Cao, Marjo de Graauw, Kuan Yan, Leah Winkel, and Fons J. Verbeek. Hierarchical classification strategy for phenotype extraction from epidermal growth factor receptor endocytosis screening. *BMC Bioinformatics*, 17(196), 2016.

[104] Dunn G.A. and Brown A.F. Alignment of fibroblasts on grooved surfaces described by a simple geometric transformation. *Journal of Cell Science*, 83:313–340, 1986.

[105] Chudyk Celeste, Castaneda Hugo, Leger Romain, Yahiaoui Islem, and Boochs Frank. Development of an automatic pollen classification system using shape, texture and aperture features. In *LWA 2015 Workshops: KDML, FGWM, IR, and FGDB*, pages 65–74, 2015.

[106] Rodríguez-Damián M., Cernadas E., Formella A., and Sá-Otero P. Pollen classification using brightness-based and shape-based descriptors. In *Proceedings - International Conference on Pattern Recognition*, volume 2, pages 212–215, 2004.

[107] Dalal Navneet and Triggs Bill. Histograms of oriented gradients for human detection. In *Proceedings - 2005 IEEE Computer Society Conference on Computer Vision and Pattern Recognition, CVPR 2005*, 2005.

[108] Haralick Robert M., Dinstein Its'hak, and Shanmugam K. Textural features for image classification. *IEEE Transactions on Systems, Man and Cybernetics*, 3(6):610–621, 1973.

[109] Hu Ming Kuei. Visual pattern recognition by moment invariants. *IRE Transactions on Information Theory*, 8(2):179–187, 1962.

[110] Jiliang Tang, Salem Alelyani, and Huan Liu. Feature selection for classification: A review. In *Data Classification: Algorithms and Applications*, number 28, pages 37–64. 2014.

[111] Jain Divya and Singh Vijendra. Feature selection and classification systems for chronic disease prediction: A review, 2018.

[112] Cossetin Marcelo J., Nievola Julio C., and Koerich Alessandro L. Facial expression recognition using a pairwise feature selection and classification approach. In *Proceedings of the International Joint Conference on Neural Networks*, pages 5149–5155, 2016.

[113] Popescu Madalina Cosmina and Sasu Lucian Mircea. Feature extraction, feature selection and machine learning for image classification: A case study. In *2014 International Conference on Optimization of Electrical and Electronic Equipment, OPTIM 2014*, pages 968–973, 2014.

[114] Silla Carlos N. and Freitas Alex A. A survey of hierarchical classification across different application domains, 2011.

[115] Kiritchenko Svetlana, Matwin Stan, and Famili A. Fazel. Functional annotation of genes using hierarchical text categorization. *In Proc. of the BioLINK SIG: Linking Literature, Information and Knowledge for Biology (held at ISMB-05*, 2005.

[116] Krizhevsky Alex, Sutskever Ilya, and Hinton Geoffrey E. Imagenet classification with deep convolutional neural networks. In *Advances in Neural Information Processing Systems*, 2012.

[117] Simonyan Karen and Zisserman Andrew. Very deep convolutional networks for large-scale image recognition. In *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition*, 2015.

[118] Kaiming He, Xiangyu Zhang, Shaoqing Ren, and Jian Sun. Deep residual learning for image recognition. In *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition*, pages 770–778, 2016.

[119] Amirreza Mahbod, Gerald Schaefer, Rupert Ecker, and Isabella Ellinger. Pollen grain microscopic image classification using an ensemble of fine-tuned deep convolutional neural networks. In *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition*, 2020.

[120] David Gutierrez Arias, Marcos Vinicius Mussel Cirne, Josimar Edinson Chire, and Helio Pedrini. Classification of pollen grain images based on an ensemble of classifiers. In *Proceedings - 16th IEEE International Conference on Machine Learning and Applications, ICMLA 2017*, pages 234–240, 2017.

[121] Wanbin Hu, Leonie van Steijn, Chen Li, Fons J. Verbeek, Lu Cao, Roeland M. H. Merks, and Herman P. Spaink. A novel function of tlr2 and myd88 in the regulation of leukocyte cell migration behavior during wounding in zebrafish larvae. *Frontiers in Cell and Developmental Biology*, 9, 2021.

[122] Junya Hayashida and Ryoma Bise. Cell tracking with deep learning for cell detection and motion estimation in low-frame-rate. In *International Conference on Medical Image Computing and Computer-Assisted Intervention–MICCAI 2019*, volume 11764, pages 397–405, 2019.

[123] Akram Saad Ullah, Kannala Juho, Eklund Lauri, and Heikkilä Janne. Joint cell segmentation and tracking using cell proposals. In *2016 IEEE 13th International Symposium on Biomedical Imaging (ISBI)*, pages 920–924, 2016.

[124] Hernandez David E., Chen Steven W., Hunter Elizabeth E., Steager Edward B., and Vijay Kumar. Cell tracking with deep learning and the viterbi algorithm. In *2018 International Conference on Manipulation, Automation and Robotics at Small Scales (MARSS)*, pages 1–6, 2018.

[125] Tim Scherr, Katharina Löffler, Moritz Böhland, and Ralf Mikut. Cell segmentation and tracking using cnn-based distance predictions and a graph-based matching strategy. *Plos One*, 15(12):e0243219, 2020.

[126] Kuan Yan, Fons J. Verbeek, Sylvia Le Devedec, and Bob van de Water. Cell tracking and data analysis of in vitro tumour cells from time-lapse image sequences. In *Proceedings of the Fourth International Conference on Computer Vision Theory and Applications–VISAPP 2009*, volume 1, 2009.

[127] Jean-Baptiste Lugagne, Haonan Lin, and Mary J. Dunlop. Delta: Automated cell segmentation, tracking, and lineage reconstruction using deep learning. *Plos Computational Biology*, 16(4):e1007673, 2020.

[128] Assaf Arbelle, Jose Reyes, Jia-Yun Chen, Galit Lahav, and Tammy Riklin Raviv. A probabilistic approach to joint cell tracking and segmentation in high-throughput microscopy videos. *Medical Image Analysis*, 47:140–152, 2018.

[129] Yousef Al-Kofahi, Alla Zaltsman, Robert Graves, Will Marshall, and Mirabela Rusu. A deep learning-based algorithm for 2-d cell segmentation in microscopy images. *BMC Bioinformatics*, 19(365), 2018.

[130] Tomas Vicar, Jan Balvan, Josef Jaros, Florian Jug, Radim Kolar, Michal Masarik, and Jaromir Gumulec. Cell segmentation methods for label-free contrast microscopy: review and comprehensive comparison. *BMC Bioinformatics*, 20(360), 2019.

[131] Fuyong Xing and Lin Yang. Robust nucleus/cell detection and segmentation in digital pathology and microscopy images: A comprehensive review. *IEEE Reviews in Biomedical Engineering*, 9:234–263, 2016.

[132] Otsu Nobuyuki. A threshold selection method from gray-level histograms. *IEEE Transactions on Systems, Man, and Cybernetics*, 9(1):62–66, 1979.

[133] Vincent Luc and Soille Pierre. Watersheds in digital spaces: An efficient algorithm based on immersion simulations. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, 13:583–598, 1991.

[134] Olaf Ronneberger, Philipp Fischer, and Thomas Brox. U-net: Convolutional networks for biomedical image segmentation. In *Proceedings of the IEEE conference on computer vision and pattern Recognition (CVPR), arXiv:1505.04597*, 2015.

[135] Xiao Xiao, Shen Lian, Zhiming Luo, and Shaozi Li. Weighted res-unet for high-quality retina vessel segmentation. In *2018 9th International Conference on Information Technology in Medicine and Education (ITME)*, pages 327–331, 2018.

[136] Steven Guan, Amir A. Khan, Siddhartha Sikdar, , and Parag V. Chitnis. Fully dense unet for 2-d sparse photoacoustic tomography artifact removal. *IEEE Journal of Biomedical and Health Informatics*, 2(2):568–576, 2020.

[137] Tao He, Hua Mao, Jixiang Guo, and Zhang Yi. Cell tracking using deep neural networks with multi-task learning. *Image and Vision Computing*, 60:142–153, 2017.

[138] Engin Turetken, Xinchao Wang, Carlos J. Becker, Carsten Haubold, and Pascal Fua. Network flow integer programming to track elliptical cells in time-lapse sequences. *IEEE Transaction on Medical Imaging*, 36(4):942–951, 2017.

[139] Magnusson Klas E.G. and Jaldén Joakim. A batch algorithm using iterative application of the viterbi algorithm to track cells and construct cell lineages. In *2012 9th IEEE International Symposium on Biomedical Imaging (ISBI)*, pages 382–385, 2012.

[140] Magnusson Klas E.G., Jalden Joakim, Gilbert Penney M., and Blau Helen M. Global linking of cell tracks using the viterbi algorithm. *IEEE Transaction on Medical Imaging*, 34(4):911–929, 2015.

[141] Christian Payer, Darko Štern, Marlies Feiner, Horst Bischof, and Martin Urschler. Segmenting and tracking cell instances with cosine embeddings and recurrent hourglass networks. *Medical Image Analysis*, 57:106–119, 2019.

[142] Marzieh R. Moghadam and Yi-Ping Phoebe Chen. Tracking neutrophil migration in zebrafish model using multi-channel feature learning. *IEEE Journal of Biomedical Health Informatics*, 25(4):1197–1205, 2021.

[143] Erick Moen, Dylan Bannon, Takamasa Kudo, William Graf, Markus Covert, and David Van Valen. Deep learning for cellular image analysis. *Nature Methods*, 16:1233–1246, 2019.

[144] Jean-Yves Tinevez, Nick Perry, Johannes Schindelin, Genevieve M. Hoopes, Gregory D. Reynolds, Emmanuel Laplantine, and et al. Trackmate: An open and extensible platform for single-particle tracking. *Methods*, 115:80–90, 2017.

[145] Katherine M. Henry, Luke Pase, Carlos Fernando Ramos-Lopez, Graham J. Lieschke, Stephen A. Renshaw, and Constantino Carlos Reyes-Aldasoro. Phagosight: An open-source matlab package for the analysis of fluorescent neutrophil and macrophage migration in a zebrafish model. *Plos One*, 8(8):e72636, 2013.

[146] J.A. Cornwell, J. Li, S. Mahadevan, J.S. Draper, G.L. Joun, H. Zoellner, and et al. Trackpad: Software for semi-automated single-cell tracking andlineage annotation. *SoftwareX*, 11, 2020.

[147] Anne E. Carpenter, Thouis R. Jones, Michael R. Lamprecht, Colin Clarke, In Han Kang, Ola Friman, and et al. Cellprofiler: image analysis software for identifying and quantifying cell phenotypes. *Genome Biology*, 7(10), 2006.

[148] Tashita Atsuki, Kobashi Syoji, Mori Yuki, Morimoto Masakazu, Aikawa Satoru, Yoshioka Yoshichika, and et al. Macrophage tracking using the hungarian algorithm in time lapse mr images. In *2015 7th International Conference on Emerging Trends in Engineering Technology (ICETET)*, pages 169–173, 2015.

[149] Réka Hollandi, Ákos Diósdi, Gábor Hollandi, Nikita Moshkov, and Péter Horváth. Annotatorj: an imagej plugin to ease hand annotation of cellular compartments. *Molecular Biology of the Cell*, 31(20):2179–2186, 2020.

[150] Michael Yeung, Evis Sala, Carola-Bibiane Schönlieb, and Leonardo Rundo. A mixed focal loss function for handling class imbalanced medical image segmentation. *ArXiv*, abs/2102.04525, 2021.

[151] Carole H Sudre, Wenqi Li, Tom Vercauteren, Sébastien Ourselin, and M. Jorge Cardoso. Generalised dice overlap as a deep learning loss function for highly unbalanced segmentations. In *Proceedings of the IEEE conference on computer vision and pattern Recognition (CVPR)*, 2017.

[152] Kuan Yan and Fons J. Verbeek. Segmentation for high-throughput image analysis: Watershed masked clustering. *Leveraging Applications of Formal Methods, Verification and Validation. Applications and Case Studies*, 7610:25–41, 2012.

[153] Juan C. Caicedo, Jonathan Roth, Allen Goodman, Tim Becker, Kyle W. Karhohs, and Matthieu Broisin. Evaluation of deep learning strategies for nucleus segmentation in fluorescence images. *Cytometry Part A: the Journal of the International Society for Analytical Cytology*, 95(9):953–965, 2019.

[154] Kevin Smith, Daniel Gatica-Perez, Jean-Marc Odobez, and Sileye Ba. Evaluating multi-object tracking. In *2005 IEEE Computer Society Conference on Computer Vision and Pattern Recognition (CVPR'05) - Workshops*, pages 36–36, 2005.

[155] Martin Maška, Vladimír Ulman, David Svoboda, Pavel Matula, Petr Matula, Cristina Ederra, and et al. A benchmark for comparison of cell tracking algorithms. *Bioinformatics*, 30:1609–1617, 2014.

[156] Wanbin Hu, Shuxin Yang, Yasuhito Shimada, Magnus Münch, Rubén Marín-Juez, Annemarie H. Meijer, and Herman P. Spaink. Infection and rna-seq analysis of a zebrafish tlr2 mutant shows a broad function of this toll-like receptor in transcriptional and metabolic control and defense to mycobacterium marinum infection. *BMC Genomics*, 20:1–18, 2019.

[157] Marzieh R. Moghadam and Yi-Ping Phoebe Chen. Tracking leukocytes in intravital time lapse images using 3d cell association learning network. *Artificial Intelligence in Medicine*, 118, 2021.

[158] Duncan Carradice and Graham J Lieschke. Zebrafish in hematology: sushi or science? *Blood*, 111(7):3331–3342, 2008.

[159] Hsieh-Fu Tsai, Joanna Gajda, Tyler F.W. Sloan, Andrei Rares, and Amy Q. Shen. Usiigaci: Instance-aware cell tracking in stain-free phase contrast microscopy enabled by machine learning. *SoftwareX*, 9:230–237, 2019.

[160] Claire Mitchell, Lauryanne Caroff, Jose Alonso Solis-Lemus, Constantino Carlos Reyes-Aldasoro, Alessandra Vigilante, Fiona Warburton, and et al. Cell tracking profiler – a user-driven analysis framework for evaluating 4d live-cell imaging data. *Journal of Cell Science*, 133(22), 2020.

[161] Austin E.Y.T. Lefebvre, Dennis Ma, Kai Kessenbrock, Devon A. Lawson, and Michelle A. Digman. Automated segmentation and tracking of mitochondria in live-cell time-lapse images. *Nature Methods*, 18:1091–1102, 2021.

[162] Seifedine Kadry, Venkatesan Rajinikanth, David Taniar, Robertas Damaševičius, and Xiomara Patricia Blanco Valencia. Automated segmentation of leukocyte from hematological images—a study using various cnn schemes. *The Journal of Supercomputing*, 78:6974–6994, 2022.

[163] Özgün Çiçek, Ahmed Abdulkadir, Soeren S. Lienkamp, Thomas Brox, and Olaf Ronneberger. 3d u-net: Learning dense volumetric segmentation from sparse annotation, arxiv:1606.06650. In *Proceedings of the IEEE conference on computer vision and pattern Recognition (CVPR)*, volume 9901, pages 424–432, 2016.

[164] Chentao Wen, Takuya Miura, Venkatakaushik Voleti, Kazushi Yamaguchi, Motosuke Tsutsumi, Kei Yamamoto, and et al. 3deecelltracker, a deep learning-based pipeline for segmenting and tracking cells in 3d time lapse images. *Computational and Systems Biology, Neuroscience*, pages 1–36, 2021.

[165] S. Shailja, Jiaxiang Jiang, and B.S. Manjunath. Semi supervised segmentation and graph-based tracking of 3d nuclei in time-lapse microscopy. In *Proceedings of the IEEE conference on computer vision and pattern Recognition (CVPR), arXiv:2010.13343*, pages 385–389, 2021.

[166] Petit Manuel, Cerutti Guillaume, Godin Christophe, and Malandain Grégoire. Robust plant cell tracking in fluorescence microscopy 3d+t series. In *2022 IEEE 19th International Symposium on Biomedical Imaging (ISBI)*, pages 1–4, 2022.

[167] Huaizu Jiang, Deqing Sun, Varun Jampani, Ming-Hsuan Yang, Erik Learned-Miller, and Jan Kautz. Super slomo: High quality estimation of multiple intermediate frames for video interpolation. In *Proceedings of the IEEE conference on computer vision and pattern Recognition (CVPR), arXiv:1712.00080*, 2018.

[168] David Borland, Carolyn M. McCormick, Niyanta K. Patel, Oleh Krupa, Jessica T. Mory, Alvaro A. Beltran, and et al. Segmentor: a tool for manual refinement of 3d microscopy annotations. *BMC Bioinformatics*, 22:1–12, 2021.

[169] H.W. Kuhn. The hungarian method for the assigment problem. *Naval Research Logistics Quarterly*, 2(1-2):83–97, 1995.

[170] Johannes Schindelin, Ignacio Arganda-Carreras, Erwin Frise, Verena Kaynig, Mark Longair, Tobias Pietzsch, and et al. Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9:676–682, 2012.

Appendix A

Supplementary Materials in Chapter 2

Supplementary Table S1

Locations of all Urticaceae specimens and number of images. NL = the Netherlands, SP = Spain and PO = Portugal. *collected in 2018 and 2019, deposited in the Naturalis Biodiversity Center herbarium.

Species (n=total images)	**Geographical origin**	**Collection date**	**No. of images used**	**Deposition number**
Parietaria	Montejaque (SP)	17/10/2011	54	WAG.1186948
judaica L.	Leiden, Stationsweg (NL)	19/11/2019	168	L.3993376*
(n = 1670)	Huizen (NL)	20/09/2014	174	L.4303913
	Leiden, Robijnstraat (NL)	23/07/2012	139	L.2071680
	Den Haag (NL)	05/10/2018	392	L.3993377*
	Leiden, Paterstraatje	09/10/2018	250	L.3993378*
	Sassenplaat (NL)	03/07/2013	233	L.4304093
	Rotterdam, Hartelkanaal (NL)	27/09/2014	260	L.4304136
Parietaria	Middelburg (NL)	26/06/2014	234	L.3974371
officinalis L.	Haarlem (NL)	13/07/2013	191	L.2073373
(n = 1359)	Wageningse Polder (NL)	19/07/2012	64	WAG.1186992
	Leiden (NL)	07/2012	369	L.3963901
	Den Haag, Escamplaan (NL)	12/10/2018	383	L.3993379*
	Den Haag, Bosjes van Poot (NL)	01/08/2012	248	L.2071818
Urtica dioica L.	Leiden, Hogeschool 1 (NL)	06/11/2019	316	L.3993380*
(n = 1055)	Leiden, Hogeschool 2 (NL)	07/11/2019	299	L.3993381*
	Den Haag (NL)	17/11/2019	182	L.3993382*

	Leiden, Sandifortdreef (NL)	15/11/2019	191	L.3993383*
	Arnhem (NL)	29/05/2001	67	WAG.1188104
Urtica membranacea Poir. ex Savigny (n = 1118)	Amsterdam (NL)	11/2018	521	L.3993384*
	Overloon (NL)	17/06/2014	135	L.3959964
	Cape st. Vincent (PO)	03/1995	87	L.1629741
	Leiden, Sandifortdreef (NL)	15/11/2019	191	L.3993383*
	Den Haag (NL)	06/03/2019	375	L.3993385*
Urtica urens L. (n = 1270)	Leiden (NL)	01/11/2019	128	L.3993386*
	Castilla-la-Mancha (SP)	27/05/2016	165	WAG.1962413
	Zandvoort (NL)	05/08/2012	201	L.2071917
	Meijendel (NL)	12/08/2011	140	L.2074446
	Zwolle (NL)	29/04/2005	134	L.4271105
	Wassenaar (NL)	15/09/2002	219	L.4233917
	Den Haag (NL)	13/03/2020	283	L.3993387*

Supplementary Table S2

Probability scores for Urticaceae pollen grains scanned from aerobiological samples using the pre-trained VGG16 model with 5-fold cross-validation. *U. mem* = *Urtica membranacea*

Lleida (16-06-2019), n=63

Image No.	**Probability *Parietaria***	**Probability *Urtica***	**Probability *U.mem***	**Final ID Threshold 0.6**	**Final ID Threshold 0.7**
1	0.95	0.05	0.00	*Parietaria*	*Parietaria*
2	0.98	0.02	0.00	*Parietaria*	*Parietaria*
3	0.29	0.70	0.01	*Urtica*	*Urtica*
4	0.98	0.02	0.00	*Parietaria*	*Parietaria*
5	0.24	0.76	0.00	*Urtica*	*Urtica*
6	1.00	0.00	0.00	*Parietaria*	*Parietaria*
7	0.94	0.06	0.00	*Parietaria*	*Parietaria*
8	0.99	0.01	0.00	*Parietaria*	*Parietaria*
9	0.12	0.88	0.00	*Urtica*	*Urtica*
10	0.99	0.01	0.00	*Parietaria*	*Parietaria*

11	0.99	0.01	0.00	*Parietaria*	*Parietaria*
12	0.96	0.04	0.00	*Parietaria*	*Parietaria*
13	1.00	0.00	0.00	*Parietaria*	*Parietaria*
14	0.96	0.04	0.00	*Parietaria*	*Parietaria*
15	1.00	0.00	0.00	*Parietaria*	*Parietaria*
16	0.90	0.09	0.01	*Parietaria*	*Parietaria*
17	0.90	0.01	0.09	*Parietaria*	*Parietaria*
18	0.73	0.16	0.10	*Parietaria*	*Parietaria*
19	0.95	0.04	0.00	*Parietaria*	*Parietaria*
20	0.98	0.00	0.02	*Parietaria*	*Parietaria*
21	0.16	0.83	0.00	*Urtica*	*Urtica*
22	0.67	0.31	0.02	*Parietaria*	unknown
23	0.02	0.98	0.00	*Urtica*	*Urtica*
24	0.95	0.04	0.00	*Parietaria*	*Parietaria*
25	0.99	0.01	0.00	*Parietaria*	*Parietaria*
26	0.99	0.01	0.00	*Parietaria*	*Parietaria*
27	0.95	0.05	0.00	*Parietaria*	*Parietaria*
28	0.02	0.98	0.00	*Urtica*	*Urtica*
29	0.34	0.66	0.00	*Urtica*	unknown
30	1.00	0.00	0.00	*Parietaria*	*Parietaria*
31	0.98	0.02	0.00	*Parietaria*	*Parietaria*
32	1.00	0.00	0.00	*Parietaria*	*Parietaria*
33	0.99	0.01	0.00	*Parietaria*	*Parietaria*
34	0.92	0.02	0.06	*Parietaria*	*Parietaria*
35	0.57	0.41	0.02	unknown	unknown
36	1.00	0.00	0.00	*Parietaria*	*Parietaria*
37	0.87	0.13	0.00	*Parietaria*	*Parietaria*
38	0.99	0.00	0.00	*Parietaria*	*Parietaria*
39	0.97	0.03	0.00	*Parietaria*	*Parietaria*
40	0.58	0.41	0.01	unknown	unknown
41	0.98	0.02	0.00	*Parietaria*	*Parietaria*
42	0.70	0.29	0.00	*Parietaria*	*Parietaria*
43	0.84	0.16	0.00	*Parietaria*	*Parietaria*
44	0.97	0.02	0.01	*Parietaria*	*Parietaria*
45	0.83	0.17	0.00	*Parietaria*	*Parietaria*
46	0.99	0.00	0.00	*Parietaria*	*Parietaria*

47	1.00	0.00	0.00	*Parietaria*	*Parietaria*
48	0.99	0.00	0.00	*Parietaria*	*Parietaria*
49	0.96	0.04	0.01	*Parietaria*	*Parietaria*
50	0.00	1.00	0.00	*Urtica*	*Urtica*
51	0.99	0.01	0.00	*Parietaria*	*Parietaria*
52	0.99	0.00	0.01	*Parietaria*	*Parietaria*
53	0.91	0.04	0.05	*Parietaria*	*Parietaria*
54	0.95	0.04	0.00	*Parietaria*	*Parietaria*
55	0.90	0.10	0.00	*Parietaria*	*Parietaria*
56	0.95	0.05	0.00	*Parietaria*	*Parietaria*
57	0.99	0.01	0.00	*Parietaria*	*Parietaria*
58	1.00	0.00	0.00	*Parietaria*	*Parietaria*
59	0.99	0.01	0.00	*Parietaria*	*Parietaria*
60	0.17	0.82	0.00	*Urtica*	*Urtica*
61	0.41	0.56	0.02	unknown	unknown
62	0.98	0.02	0.00	*Parietaria*	*Parietaria*
63	0.76	0.21	0.03	*Parietaria*	*Parietaria*

Vielha (09-08-2019), n=26

Image No.	**Probability *Parietaria***	**Probability *Urtica***	**Probability *U.mem***	**Final ID Threshold 0.6**	**Final ID Threshold 0.7**
1	0.03	0.97	0.00	*Urtica*	*Urtica*
2	0.07	0.86	0.07	*Urtica*	*Urtica*
3	0.10	0.90	0.00	*Urtica*	*Urtica*
4	0.02	0.98	0.00	*Urtica*	*Urtica*
5	0.09	0.91	0.00	*Urtica*	*Urtica*
6	0.26	0.74	0.00	*Urtica*	*Urtica*
7	0.00	1.00	0.00	*Urtica*	*Urtica*
8	0.41	0.04	0.55	unknown	unknown
9	0.61	0.39	0.01	*Parietaria*	unknown
10	0.81	0.10	0.09	*Parietaria*	*Parietaria*
11	0.02	0.98	0.00	*Urtica*	*Urtica*
12	0.01	0.99	0.00	*Urtica*	*Urtica*
13	0.49	0.13	0.38	unknown	unknown

14	0.00	1.00	0.00	*Urtica*	*Urtica*
15	0.14	0.84	0.02	*Urtica*	*Urtica*
16	0.63	0.10	0.27	*Parietaria*	unknown
17	0.12	0.88	0.00	*Urtica*	*Urtica*
18	0.09	0.90	0.10	*Urtica*	*Urtica*
19	0.24	0.76	0.00	*Urtica*	*Urtica*
20	0.04	0.96	0.00	*Urtica*	*Urtica*
21	0.85	0.12	0.03	*Parietaria*	*Parietaria*
22	0.80	0.14	0.07	*Parietaria*	*Parietaria*
23	0.00	1.00	0.00	*Urtica*	*Urtica*
24	0.17	0.83	0.00	*Urtica*	*Urtica*
25	0.02	0.98	0.00	*Urtica*	*Urtica*
26	0.57	0.43	0.00	unknown	unknown

Leiden (23-08-2019), n=112

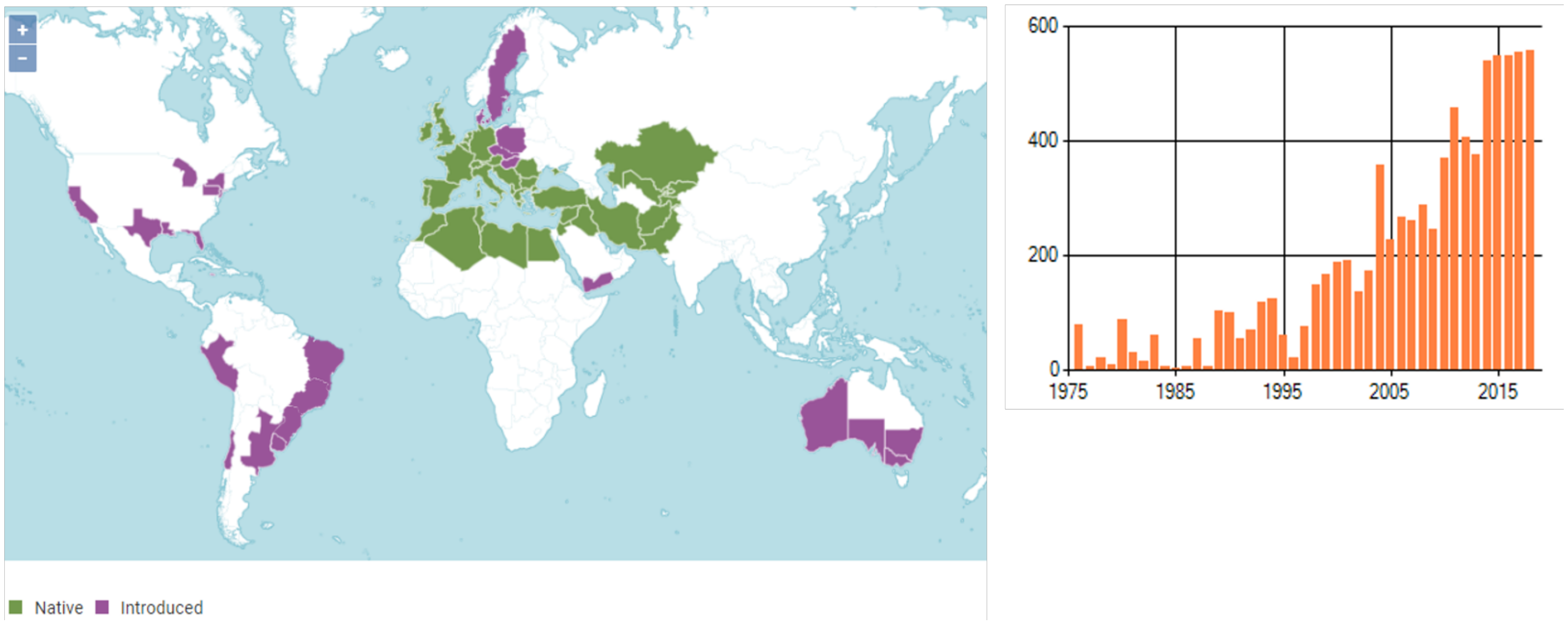
Image No.	**Probability *Parietaria***	**Probability *Urtica***	**Probability *U.mem***	**Final ID Threshold 0.6**	**Final ID Threshold 0.7**
1	0.04	0.96	0.00	*Urtica*	*Urtica*
2	0.01	0.99	0.00	*Urtica*	*Urtica*
3	0.07	0.93	0.00	*Urtica*	*Urtica*
4	0.16	0.83	0.00	*Urtica*	*Urtica*
5	0.19	0.81	0.00	*Urtica*	*Urtica*
6	0.02	0.98	0.00	*Urtica*	*Urtica*
7	0.00	1.00	0.00	*Urtica*	*Urtica*
8	0.28	0.72	0.00	*Urtica*	*Urtica*
9	0.11	0.89	0.00	*Urtica*	*Urtica*
10	0.34	0.66	0.00	*Urtica*	unknown
11	0.04	0.96	0.00	*Urtica*	*Urtica*
12	0.18	0.81	0.00	*Urtica*	*Urtica*
13	0.00	1.00	0.00	*Urtica*	*Urtica*
14	0.47	0.53	0.00	unknown	unknown
15	0.11	0.89	0.00	*Urtica*	*Urtica*
16	0.01	0.99	0.00	*Urtica*	*Urtica*
17	0.20	0.80	0.00	*Urtica*	*Urtica*

18	0.00	1.00	0.00	*Urtica*	*Urtica*
19	0.00	1.00	0.00	*Urtica*	*Urtica*
20	0.01	0.99	0.00	*Urtica*	*Urtica*
21	0.75	0.25	0.00	*Parietaria*	*Parietaria*
22	0.00	1.00	0.00	*Urtica*	*Urtica*
23	0.03	0.97	0.00	*Urtica*	*Urtica*
24	0.01	0.99	0.00	*Urtica*	*Urtica*
25	0.69	0.31	0.00	*Parietaria*	unknown
26	0.11	0.89	0.00	*Urtica*	*Urtica*
27	0.12	0.88	0.00	*Urtica*	*Urtica*
28	0.17	0.83	0.00	*Urtica*	*Urtica*
29	0.09	0.91	0.00	*Urtica*	*Urtica*
30	0.00	1.00	0.00	*Urtica*	*Urtica*
31	0.48	0.52	0.00	unknown	unknown
32	0.24	0.76	0.00	*Urtica*	*Urtica*
33	0.06	0.94	0.00	*Urtica*	*Urtica*
34	0.29	0.71	0.00	*Urtica*	*Urtica*
35	0.14	0.86	0.00	*Urtica*	*Urtica*
36	0.38	0.62	0.00	*Urtica*	unknown
37	0.06	0.94	0.00	*Urtica*	*Urtica*
38	0.55	0.45	0.00	unknown	unknown
39	0.01	0.99	0.00	*Urtica*	*Urtica*
40	0.00	1.00	0.00	*Urtica*	*Urtica*
41	0.00	1.00	0.00	*Urtica*	*Urtica*
42	0.02	0.98	0.00	*Urtica*	*Urtica*
43	0.03	0.97	0.00	*Urtica*	*Urtica*
44	0.21	0.79	0.00	*Urtica*	*Urtica*
45	0.02	0.98	0.00	*Urtica*	*Urtica*
46	0.00	1.00	0.00	*Urtica*	*Urtica*
47	0.01	0.99	0.00	*Urtica*	*Urtica*
48	0.79	0.20	0.07	*Parietaria*	*Parietaria*
49	0.54	0.46	0.00	unknown	unknown
50	0.01	0.99	0.00	*Urtica*	*Urtica*
51	0.00	1.00	0.00	*Urtica*	*Urtica*
52	0.01	0.99	0.00	*Urtica*	*Urtica*
53	0.01	0.99	0.00	*Urtica*	*Urtica*

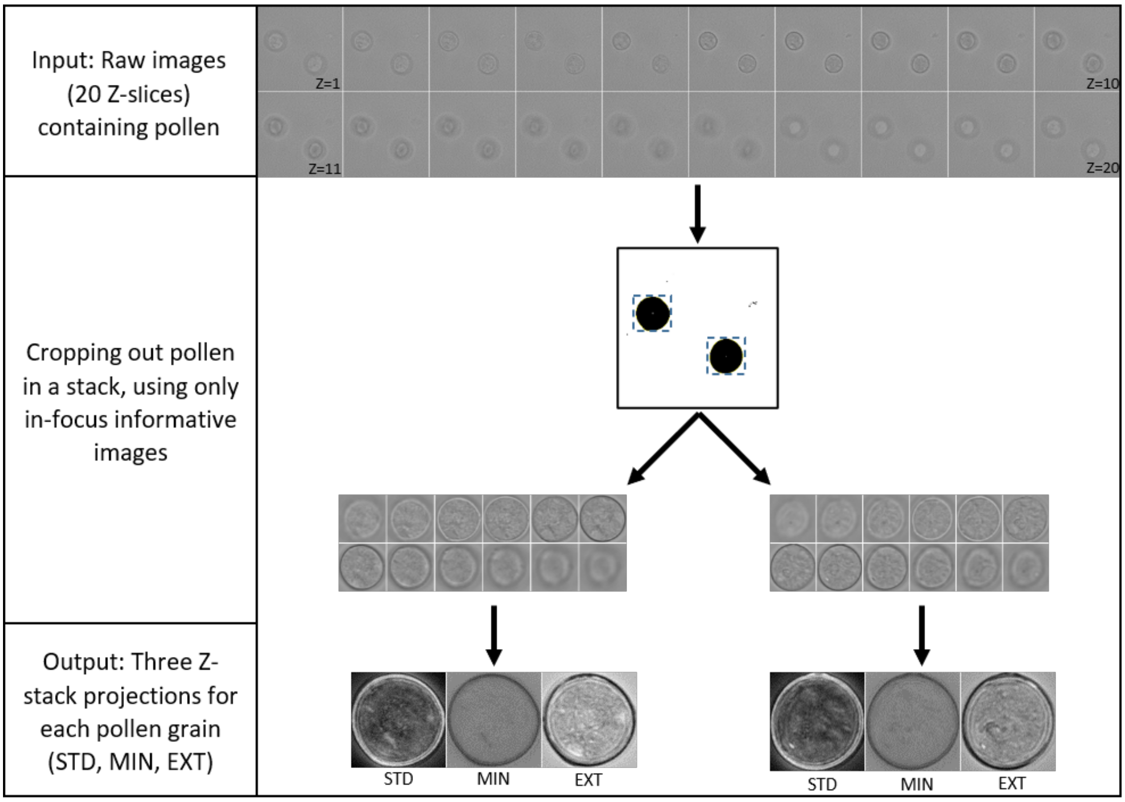
54	0.00	1.00	0.00	*Urtica*	*Urtica*
55	0.00	1.00	0.00	*Urtica*	*Urtica*
56	0.00	1.00	0.00	*Urtica*	*Urtica*
57	0.02	0.98	0.00	*Urtica*	*Urtica*
58	0.00	1.00	0.00	*Urtica*	*Urtica*
59	0.54	0.46	0.00	unknown	unknown
60	0.45	0.55	0.00	unknown	unknown
61	0.09	0.91	0.00	*Urtica*	*Urtica*
62	0.00	1.00	0.00	*Urtica*	*Urtica*
63	0.00	1.00	0.00	*Urtica*	*Urtica*
64	0.00	1.00	0.00	*Urtica*	*Urtica*
65	0.06	0.94	0.00	*Urtica*	*Urtica*
66	0.05	0.95	0.00	*Urtica*	*Urtica*
67	0.01	0.99	0.00	*Urtica*	*Urtica*
68	0.23	0.77	0.00	*Urtica*	*Urtica*
69	0.21	0.79	0.00	*Urtica*	*Urtica*
70	0.72	0.28	0.00	*Parietaria*	*Parietaria*
71	0.49	0.51	0.00	unknown	unknown
72	0.06	0.94	0.00	*Urtica*	*Urtica*
73	0.33	0.67	0.00	*Urtica*	unknown
74	0.00	1.00	0.00	*Urtica*	*Urtica*
75	0.28	0.72	0.00	*Urtica*	*Urtica*
76	0.00	1.00	0.00	*Urtica*	*Urtica*
77	0.03	0.97	0.00	*Urtica*	*Urtica*
78	0.05	0.95	0.00	*Urtica*	*Urtica*
79	0.21	0.79	0.00	*Urtica*	*Urtica*
80	0.00	1.00	0.00	*Urtica*	*Urtica*
81	0.00	1.00	0.00	*Urtica*	*Urtica*
82	0.03	0.97	0.00	*Urtica*	*Urtica*
83	0.02	0.98	0.00	*Urtica*	*Urtica*
84	0.12	0.88	0.00	*Urtica*	*Urtica*
85	0.17	0.83	0.00	*Urtica*	*Urtica*
86	0.01	0.99	0.00	*Urtica*	*Urtica*
87	0.90	0.10	0.00	*Parietaria*	*Parietaria*
88	0.11	0.89	0.00	*Urtica*	*Urtica*
89	0.02	0.98	0.00	*Urtica*	*Urtica*

90	0.00	1.00	0.00	*Urtica*	*Urtica*
91	0.29	0.71	0.00	*Urtica*	*Urtica*
92	0.11	0.89	0.00	*Urtica*	*Urtica*
93	0.12	0.88	0.00	*Urtica*	*Urtica*
94	0.01	0.99	0.00	*Urtica*	*Urtica*
95	0.01	0.99	0.00	*Urtica*	*Urtica*
96	0.00	1.00	0.00	*Urtica*	*Urtica*
97	0.00	1.00	0.00	*Urtica*	*Urtica*
98	0.02	0.98	0.00	*Urtica*	*Urtica*
99	0.00	1.00	0.00	*Urtica*	*Urtica*
100	0.21	0.79	0.00	*Urtica*	*Urtica*
101	0.55	0.45	0.00	unknown	unknown
102	0.00	1.00	0.00	*Urtica*	*Urtica*
103	0.48	0.52	0.00	unknown	unknown
104	0.00	1.00	0.00	*Urtica*	*Urtica*
105	0.00	1.00	0.00	*Urtica*	*Urtica*
106	0.57	0.43	0.00	unknown	unknown
107	0.11	0.89	0.00	*Urtica*	*Urtica*
108	0.23	0.77	0.00	*Urtica*	*Urtica*
109	0.26	0.74	0.00	*Urtica*	*Urtica*
110	0.12	0.88	0.00	*Urtica*	*Urtica*
111	0.58	0.42	0.00	unknown	unknown
112	0.00	1.00	0.00	*Urtica*	*Urtica*

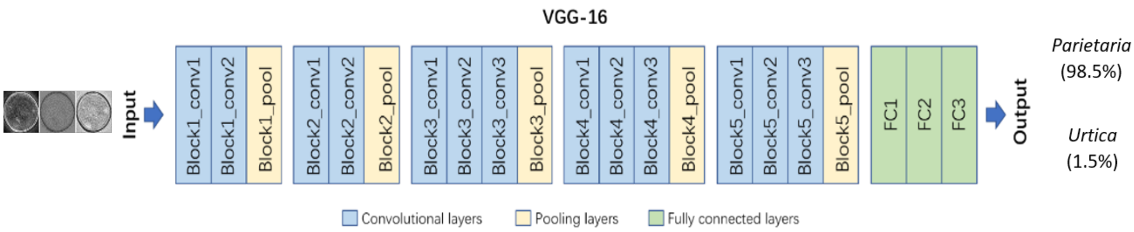
(a) (b)



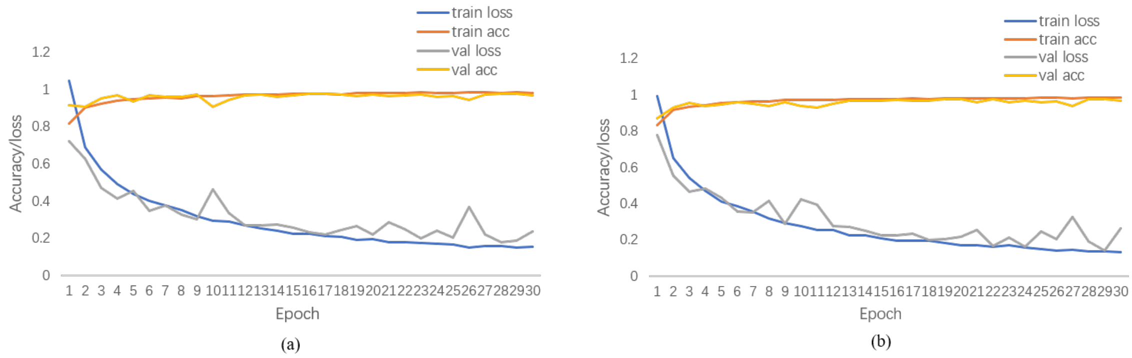
Supplementary Fig. S1. (a) Global native (green) and introduced (purple) distribution of *Parietaria judaica* and *P. officinalis* (POWO (2019). "Plants of the World Online. Map taken from the Royal Botanic Gardens, Kew. http://www.plantsoftheworldonline.org/ Retrieved 05 October 2020"). (b) Trend in Pellitory of the wall (*Parietaria judaica*) plant sightings per square kilometre in the Netherlands over the past 45 years. Index number = 100 for 1990 © NEM (CBS FLORON) 2019.



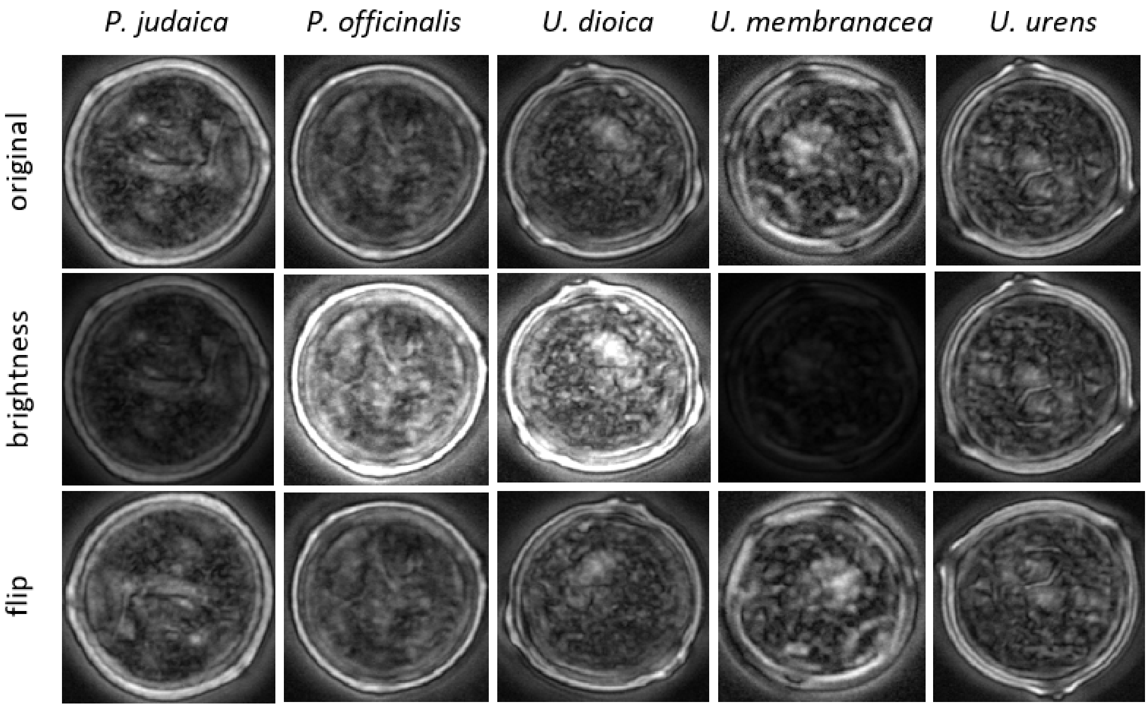
Supplementary Fig. S2. Pollen image acquisition and processing workflow carried out with in-house designed Pollen_Projector script. Once raw images are obtained at 20 different focal levels ('Z-slices'), subsequent steps involve cropping of whole individual pollen grains and producing three different projections from the Z-stacks. Abbreviations of projections: STD = Standard Deviation, MIN = Minimum Intensity and EXT = Extended Focus.



Supplementary Fig. S3. Schematic overview of the structure of VGG-16 with an example of three-channel input image of a *Parietaria judaica* pollen grain (known label) and the output generated, where it confidently identifies the images as *Parietaria* (98% probability). Adapted from Simonyan et al., (2014)[1]



Supplementary Fig. S4. Figures showing the accuracy/loss plots for the VGG16 model with 5- and 10-fold cross-validation.



Supplementary Fig. S5. Examples of data augmentation on the Standard Deviation Projection (STD) of selected pollen grains of all Urticaceae pollen species used in this study.

Appendix B

Supplementary Materials in Chapter 3

Supplementary Table S1 The classification performance of deep learning models on 5-species dataset. Standard deviation, training each model three times, is given in brackets.

	Precision	**Recall**	**F1 score**
VGG16	0.978(±0.001)	0.977(±0.001)	0.977(±0.001)
VGG19	0.974(±0.002)	0.973(±0.002)	0.973(±0.002)
ResNet50	0.980(±0.001)	0.979(±0.001)	0.979(±0.001)
MobileNet V1	0.958(±0.003)	0.957(±0.003)	0.957(±0.003)
MobileNet V2	0.970(±0.006)	0.970(±0.006)	0.970(±0.006)

Supplementary Table S2 The details of the dataset used in our study.

Classes	**Species**	**The number of images**	**The number of individual plants**
Parietaria	*Parietaria Judaica*	1670	8
	Parietaria officinalis	1359	6
Urtica	*Urtica dioica*	1055	5
	Urtica urens	1270	7
Urtica membranacea	*Urtica membranacea*	1118	4
Total images/individual plants were analyzed		6472	30

Supplementary Table S3 The final size of feature vector after feature selection/reduction.

Classifier	**Feature selection/reduction**	**The final size after feature selection/reduction**
SVM	PCA (0.8)	179×1
RF	SelectFromModel (mean)	2064×1
MLP	PCA (0.85)	337×1
Adaboost	Mutual information (2000)	2000×1

Supplementary Table S4 The number of images of predicted results for Urticaceae pollen grains scanned from aerobiological samples using the VGG16 and ResNet50 model with 5-fold cross-validation. *U. mem* = *Urtica membranacea*.

Location	**Leiden, the Netherlands**				**Lleida, Spain**			
Class labels	Label 1: *Parietaria*	Label 2: *Urtica*	Label 3: *U. mem*	unknown	Label 1: *Parietaria*	Label 2: *Urtica*	Label 3: *U. mem*	unknown
VGG16	5	96	0	11	51	9	0	3
ResNet50	8	97	0	7	60	3	0	0

Supplementary Table S5 Average performance comparison of different models on 5-fold and 10-fold cross-validation subset selection, respectively. Standard deviation of five/ten subsets, is given in brackets.

	VGG16	VGG19	ResNet50	Flat model	Hierarchical model
Average performance of 5-fold cross-validation selection with Standard Deviation	0.958 (±0.007)	0.958 (±0.007)	0.975 (±0.002)	0.867 (±0.012)	0.891 (±0.023)
Average performance of 10-fold cross-validation selection with Standard Deviation	0.940 (±0.008)	0.938 (±0.008)	0.955 (±0.010)	0.840 (±0.019)	0.873 (±0.030)

Supplementary Table S6 Ablation study with ResNet50. The average performance of ResNet50 based on ten (about) 500-images subsets via 10-fold cross-validation selection method is given. Standard deviation, of ten subsets, is given in brackets. Numbers in italics refer to training without transfer learning and data augmentation, respectively.

	Training from scratch	With/*without* transfer learning	With/*without* data augmentation	With hard voting
Accuracy	0.793(±0.034)	0.919(±0.019)	0.933(±0.011)	0.955(±0.010)
	0.793(±0.034)	0.919(±0.019)	*0.919(±0.019)*	0.939(±0.024)
	0.793(±0.034)	*0.793(±0.034)*	0.804(±0.032)	0.841(±0.033)