Harnessing AI in immunofluorescence microscopy

AI-enhanced software provides accurate classification of autoantibody patterns in immunofluorescence diagnostics.

By Dr. Jacqueline Gosink

The indirect immunofluorescence assay (IFA) is a bedrock of autoimmune diagnostics, enabling highly sensitive, specific and broad screening of autoantibodies in many different diseases. The traditional microscopic evaluation of the cell and tissue substrates is, however, time-consuming and prone to variability. Furthermore, a shortage of technicians or specialists with sufficient experience in evaluating the fluorescence patterns may limit IFA testing capacity. Many laboratories are therefore turning to automated pattern classification systems to increase efficiency and standardisation in routine diagnostics.

AI-enhanced evaluation software

Software for pattern classification in IFA must be rigorously trained to interpret the complex microscopic images. Artificial intelligence (AI) processes based on deep learning have proven to be the best tool for developing the prediction models required. One such software is the EUROPattern Classifier from EUROIMMUN, which generates result proposals in IFA for a wide range of autoantibodies. Using defined data sets, the software has been taught during development to recognise specific features and patterns and to apply this knowledge to new data. The artificial networks are organised into multiple layers known as deep convolutional neural networks, which enable the evaluation of complex data. The software is thus able to accurately distinguish positive and negative IFA results, classify fluorescence patterns and determine titers. The automatically generated result proposals must nevertheless always be approved by a specialist before release. The EUROPattern Classifier software is already established for many cell-based applications in autoimmune diagnostics and has recently been extended to include more complex tissue substrates.

ANA and ANCA

One of the most performed analyses in autoimmune diagnostics is detection of anti-nuclear antibodies (ANA) on human epithelial cells (HEp-2) cells. This serves as an important diagnostic criterion in autoimmune rheumatic diseases. The fluorescence patterns of the autoantibodies are classified according to criteria from the International Consensus on Antinuclear Antibody Patterns (ICAP). The EUROPattern Classifier recognises nine fluorescence patterns defined by ICAP, as well as mixed patterns, which occur when more than one autoantibody is present. It generates titer suggestions with confidence values from the fluorescence intensities of the incubated dilutions. The software also supports evaluation of autoantibodies against double-stranded DNA on the substrate Crithidia luciliae by identifying the specific organelle fluorescence of the protozoan.

Another core application in autoimmune diagnostics is the detection of anti-neutrophil cytoplasm antibodies (ANCA). These are important markers for autoimmune vasculitides but also occur in chronic inflammatory bowel diseases. ANCA are screened by IFA using ethanol-fixed and formalin-fixed granulocytes, which generate different staining patterns depending on the target antigen. The classifier software distinguishes positive and negative reactions and recognises the main patterns of cytoplasmic and perinuclear ANCA, as well as atypical ANCA. An additional substrate consisting of HEp-2 cells coated with granulocytes allows immediate differentiation between ANCA

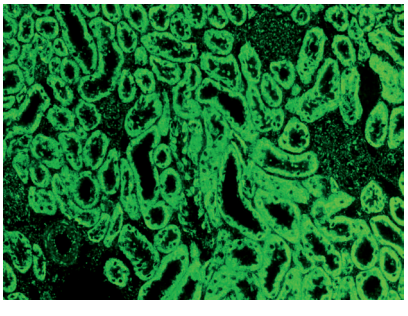
and ANA. Microdot substrates of purified proteins can be analysed in parallel to confirm the specific target antigen of the ANCA.

Cell-based assays

Cell-based assays utilise transfected cells expressing defined antigens for monospecific antibody detection. The technique is especially useful for detecting autoantibodies against fragile, conformation-dependent antigens. Cell-based assays represent an important technique for detection of diagnostically critical autoantibodies, for example in autoimmune encephalitis, neuromyelitis optica spectrum disorders or primary membranous nephropathy. The EUROPattern Classifier supports the evaluation of results for these applications.

Tissue substrates

Tissue substrates can be difficult to evaluate manually due to their complex structures. Automating the pattern recognition is also challenging, since the small structures relevant for classification are only present in certain parts of the tissues. Standard deep neural networks are not suitable for processing these images due to limitations in computer memory and the number of available training images. The deep neural networks used in the EUROPattern Classifier were adapted to the interpretation of complex tissue substrates by incorporating segmentation of relevant regions into the prediction process. This helps to focus the attention of the classification networks to the crucial regions. The developed algorithms provide accurate identification of fluorescence patterns on various tissue substrates.



Positive reaction for anti-mitochondrial antibodies on rat kidney tissue

UNIQO 160 for all-in-one automation of IFA

The software recognises, for example, anti-endomysium antibodies (EmA, IgA) on the tissue substrates liver or oesophagus. This analysis is an important confirmatory test in diagnostics of coeliac disease. The Classifier also supports evaluation of autoantibodies that occur in autoimmune liver diseases on tissue sections of rat liver, kidney and stomach. The classification includes relevant ANA, anti-mitochondrial antibodies (AMA), anti-smooth muscle antibodies (ASMA), and anti-liver kidney microsome (LKM) pattern. These analyses can aid in the differential diagnostics of autoimmune hepatitis types 1 and 2 and primary biliary cholangitis. The software has also recently been applied in a research capacity to the detection of autoantibodies in autoimmune skin diseases on oesophagus and salt-split skin substrates. The developed algorithms provide accurate pattern classification on these substrates. This application is, however, not yet available commercially.

Complete IFA automation

The EUROPattern Classifier forms an integral part of EUROIMMUN's solutions for automated IFA in the diagnostic laboratory. It is used in the EUROPattern series of automated microscopes, as well as in the UNIQO 160, an all-in-one instrument for complete IFA automation from primary sample to result proposal. The EUROPattern Classifier is connected to the EUROLabOffice 4.0 laboratory management software, which provides complete and fully traceable administration of all patient data, analyses and results.

Outlook

Deep learning has evolved into a state-of-the-art method for computer-aided evaluation in immunofluorescence microscopy. Pattern classification using neural networks is less subjective than traditional visual evaluation and can increase the standardisation and efficiency of laboratory diagnostics. Even very complex microscopy images with tissue layers that are difficult for professionals to evaluate can be reliably interpreted via computer-aided algorithms.

The EUROPattern Classifier is a useful extension of the screening methods for autoimmune diseases and can reduce the workload of professionals when reading cell and tissue substrates in IFA testing. Future developments of the software will focus on classification of autoantibody patterns on further tissue substrates to support the diagnostics of other organ-specific autoimmune diseases. The regulatory status and availability of EUROPattern Classifier applications is dependent on the instrument and country of use; laboratories are advised to check the status for their individual jurisdiction. ●

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