*Int. J. Morphol.,*
*43(4)*:1421-1428, 2025.

# Methods for Detecting Neutrophil Extracellular Trap Formation: From Microscopy to High-Throughput Technologies

## Métodos para la Detección de la Formación de Trampas Extracelulares de Neutrófilos: Desde la Microscopía hasta Tecnologías de Alto Rendimiento

**Fabiola Zambrano[1,2]; Pamela Uribe[1,3]; Mabel Schulz[1,2]; Carlos Hermosilla[4]; Anja Taubert[4] & Raúl Sánchez[1,2]**

**ZAMBRANO, F.; URIBE, P.; SCHULZ, M.; HERMOSILLA, C.; TAUBERT, A. & SÁNCHEZ, R.** Methods for detecting neutrophil extracellular trap formation: from microscopy to high-throughput technologies. *Int. J. Morphol., 43(4)*:1421-1428, 2025.

**SUMMARY:** Neutrophil extracellular traps (NETs) are chromatin-based structures released during NETosis, a specialized form of neutrophil cell death with essential roles in host defense and pathogenesis. While NETs aid in trapping pathogens, their dysregulation contributes to autoimmune, inflammatory, and thrombotic disorders. Given their dual nature, the accurate detection of NETs is critical for both basic and clinical research. This review provides a comprehensive overview of the current and emerging methodologies used to detect NET formation, ranging from traditional microscopy and fluorescent DNA dyes to advanced flow cytometry, omics technologies, and machine learning-assisted platforms. Microscopy-based methods offer visual confirmation but are labor-intensive, whereas flow cytometry and automated imaging enable high-throughput quantification. Omics approaches, including proteomics and transcriptomics, reveal molecular signatures and regulatory pathways of NETosis across disease contexts. Despite these advances, challenges remain regarding marker specificity, sample preparation artifacts, and the standardization of protocols. Future research should focus on integrating multi-modal techniques and establishing robust, validated detection strategies suitable for in vivo and clinical applications. This will be key for leveraging NETs as biomarkers and therapeutic targets in infection, cancer, and immune-mediated diseases.

**KEY WORDS: NETosis; Neutrophil extracellular traps; Detection methods.**

### 1. Overview of NETosis and Its Biological Significance

NETosis is a unique form of programmed neutrophil death marked by the release of neutrophil extracellular traps (NETs), web-like structures of decondensed chromatin decorated with antimicrobial proteins such as myeloperoxidase (MPO) and neutrophil elastase (NE) (Fuchs *et al*., 2007). These structures play a dual role in innate immunity and disease pathogenesis (Huang *et al*., 2022). Two distinct pathways of NETosis have been identified: suicidal (or classical) NETosis, which is triggered by stimuli like phorbol esters, LPS, or immune complexes and involves NADPH oxidase (NOX)-derived ROS production, PAD4-mediated histone citrullination, and subsequent membrane rupture; and vital NETosis, initiated by live pathogens or activated platelets, in which chromatin is released via vesicles while neutrophils remain viable (Vorobjeva & Chernyak, 2020; Chen *et al*., 2021). NETosis can be triggered by various stimuli including pathogens (e.g., Staphylococcus aureus, Candida albicans), pro-inflammatory cytokines (IL-8, TNF-a), physicochemical agents (PMA, uric acid crystals), and autoimmune factors like ANCAs (Yipp & Kubes, 2013; Vorobjeva & Chernyak, 2020; Chen *et al*., 2021). Unlike apoptosis or necrosis, NETosis is characterized by ROS and PAD4 dependency and results in inflammatory outcomes due to chromatin and granule protein release (Kenny *et al*., 2017; Elsherif *et al*., 2019; Stoimenou *et al*., 2022). Functionally, NETs entrap and neutralize microbes, and impaired NETosis, as seen in chronic granulomatous disease, leads to increased susceptibility to infections. However, excessive, or dysregulated NETosis contributes to autoimmune diseases by providing autoantigens, induces endothelial damage, and activates complement pathways (Vorobjeva & Chernyak, 2020). In cancer, NETs facilitate

[1] Center of Excellence in Translational Medicine, Scientific and Technological Bioresource Nucleus CEMT, BIOREN, Faculty of Medicine, Universidad de La Frontera, Temuco, Chile.
[2] Department of Preclinical Sciences, Faculty of Medicine, Universidad de La Frontera, Temuco, Chile.
[3] Department of Internal Medicine, Faculty of Medicine, Universidad de La Frontera, Temuco, Chile.
[4] Institute of Parasitology, Justus Liebig University Giessen, 35392 Giessen, Germany.
FUNDED. This research was funded partially by the Universidad de La Frontera, for sponsoring teachers' support, grant PP24– 0008.

Received: 2025-04-14 Accepted: 2025-05-14

metastasis and thrombosis, while in COVID-19, elevated NET formation correlates with disease severity and thromboembolic complications (Matta *et al*., 2022; Jaboury *et al*., 2023). Given its diverse roles, accurate detection of NETosis remains a challenge due to its overlap with other cell death pathways and the complexity of biological samples, underscoring the need for robust and specific assays in both research and clinical settings. This review aims to provide a comprehensive overview the biological relevance of NETosis and critically assess current and emerging methods for detecting neutrophil extracellular traps, highlighting their strengths, limitations, and potential for research and clinical application.

## 2. Microscopy-Based Methods of NETosis detection

Microscopy-based techniques are central to the detection and analysis of NETs, providing critical insights into the morphological and kinetic features of NETosis. Among these, immunofluorescence microscopy remains the gold standard, leveraging DNA-binding dyes such as DAPI or SYTOX Green in combination with antibodies targeting NET-associated proteins (e.g., MPO, NE, and citrullinated histone H3 [CitH3]) to visualize chromatin decondensation and protein colocalization (Stoimenou *et al*., 2022; Schöenfeld *et al*., 2023). This approach enables the distinction between NETosis, and other forms of cell death based on nuclear morphology but is inherently labor-intensive and susceptible to observer bias (Gupta *et al*., 2018). Innovations such as automated quantification tools (e.g., NETQUANT2) and confocal laser scanning microscopy (CLSM) have improved objectivity and resolution, particularly for 3D imaging (Gupta *et al*., 2018; Kumra Ahnlide *et al*., 2024). Electron microscopy techniques, including scanning EM (SEM) and transmission EM (TEM), provide nanoscale detail of NET ultrastructure (e.g., DNA-protein fibers) with high resolution, though their use is limited by sample preparation complexity and the inability to yield functional insights (Stoimenou *et al*., 2022). Conversely, live-cell imaging platforms, including IncuCyte ZOOM, offer real-time assessment of NET formation using fluorescent dyes, enabling automated quantification of NETotic events and suitability for high-throughput drug screening (Gupta *et al*., 2018; Zukas *et al*., 2024). The integration of machine learning (ML) and artificial intelligence (AI), such as convolutional neural networks (CNNs), enhances classification accuracy (>94%) and scalability in NET detection, particularly when paired with tools like NETQUANT2 for batch image analysis (Kumra Ahnlide *et al*., 2024).

TissueFAXS with StrataQuest software offers groundbreaking capabilities for NET formation assessment. This powerful integrated system enables precise identification of neutrophil extracellular traps through sophisticated multiplex fluorescence imaging. The platform excels at detecting extranuclear SytoxOrange® or DAPI areas indicative of NETs while simultaneously quantifying critical NET markers including MPO, NE, and CitH3 (Klinge *et al*., 2022; Rivera-Concha *et al*., 2023). StrataQuest's advanced segmentation algorithms differentiate NETs from intact neutrophils with remarkable accuracy. The software further enhances analysis through spatial relationship mapping between NETs and surrounding immune cells. For researchers investigating NETosis in inflammatory conditions or autoimmune diseases, TissueFAXS with StrataQuest provides unparalleled insights into NET formation dynamics, composition, and tissue distribution with exceptional reproducibility and quantitative precision (Klinge *et al*., 2022).

Emerging approaches include high-content imaging that merges immunofluorescence with automated analysis, and whole-blood assays that bypass neutrophil isolation by detecting SYTOX Green fluorescence in plasma (Ginley *et al*., 2017; Zukas *et al*., 2024). Each method (Fig. 1) presents unique advantages and limitations: while immunofluorescence excels in specificity, it is time-consuming; electron microscopy offers structural detail but is static and artifact-prone; live-cell imaging provides kinetic data but is constrained to *in vitro* systems; and AI-based analysis facilitates rapid, unbiased assessment but depends on well-curated training datasets (more details see Table I).

## 3. Quantification of NET Components

Quantifying extracellular DNA is a fundamental approach for evaluating NET formation. Among the most widely used techniques is the PicoGreen assay, which utilizes a fluorescent dye that selectively binds to double-stranded DNA (dsDNA). With a detection threshold as low as 25 pg/mL, PicoGreen offers high sensitivity and reproducibility, making it particularly advantageous for analyzing NET release in complex systems such as neutrophil-spermatozoa co-cultures (Zambrano *et al*., 2016). This method enables accurate quantification of the DNA backbone of NETs, even at minimal extracellular concentrations, and is ideal for studies exploring NETosis dynamics in reproductive immunology (Tong & Abrahams, 2021). Complementarily, Sytox Green assays employ a cell-impermeant dye that stains nucleic acids only in cells with compromised plasma membranes. This selective permeability is critical for differentiating between DNA from NETs and intracellular DNA, thereby minimizing false positives due to necrotic or apoptotic cells (Masuda *et al*., 2017). Additionally, DNA release measurements based on spectrophotometric or

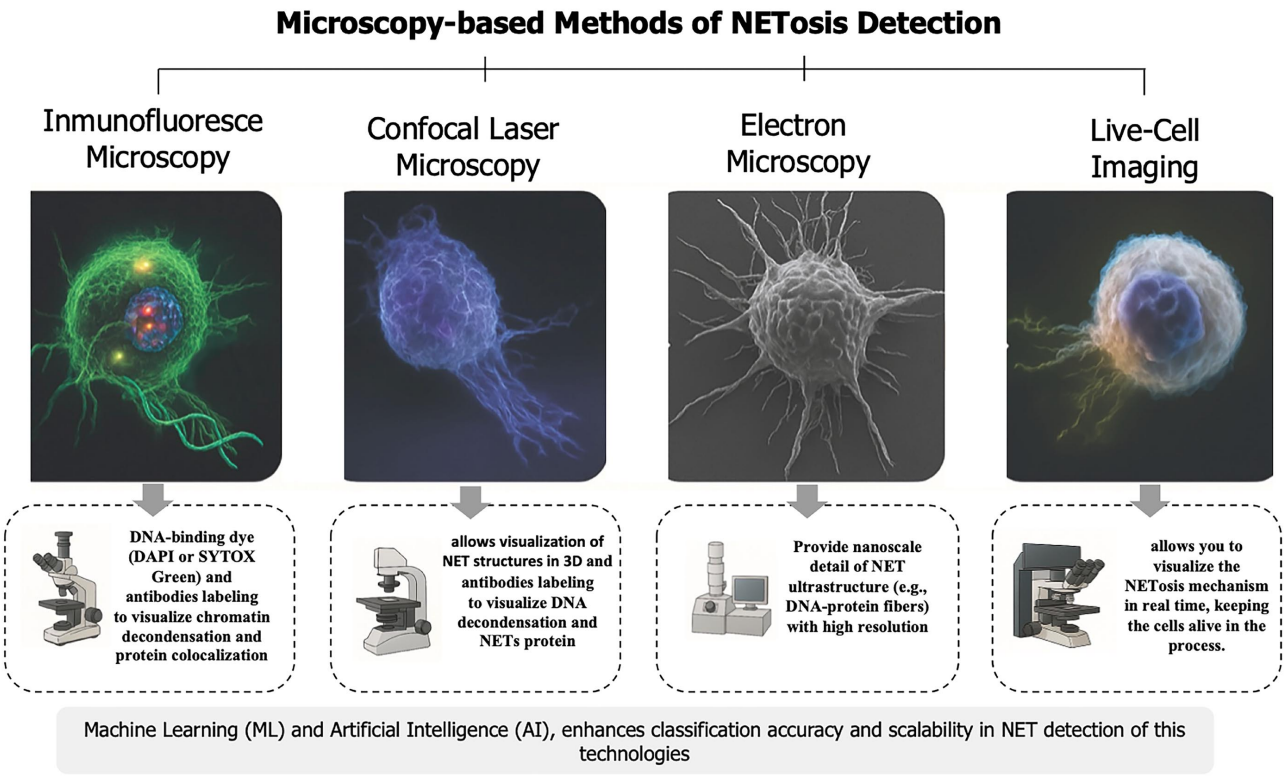


Fig. 1. Microscopy-Based Methods of NETosis Detection. Schematic overview of key techniques used to study NET formation. Immunofluorescence microscopy remains the gold standard, allowing specific visualization of chromatin and NET-associated proteins. Electron microscopy provides high-resolution imaging of NET ultrastructure, while live-cell imaging enables real-time analysis of NETosis kinetics. Machine learning and AI approaches facilitate automated, unbiased quantification in large datasets. Each method offers unique strengths and limitations in terms of resolution, throughput, and applicability to *in vitro* or *in vivo* settings.

fluorometric detection of nucleic acids in culture supernatants provide a broader perspective on NET kinetics, this method offers several advantages, including its non-destructive nature, the absence of a need for additional reagents, and the ability to obtain rapid measurements within seconds. These measurements are widely used in time-course experiments, allowing researchers to monitor NET formation over time and assess the effects of various stimuli or inhibitors on NET release (Tong & Abrahams, 2021). Taken together, these methods (Fig. 2) offer complementary insights into the extent and dynamics of NETosis and are instrumental for advancing our understanding of NET-mediated pathophysiology.

## 4. Flow Cytometry-Based NETosis Detection

Flow cytometry-based methods for the detection and quantification of NETs have evolved significantly, offering increasingly specific and high-throughput alternatives to traditional imaging. One of the most accessible and widely adopted techniques is the SYTOX Green method. This method is particularly useful for detecting NETosis in neutrophils stimulated with agents such as phorbol 12-myristate 13-acetate (PMA). Importantly, the proportion of SYTOX Green-positive cells increases in a time- and dose-dependent manner, reflecting the extent of NET formation (Masuda *et al*., 2017). A previous study validated this method by showing strong correlation with DAPI-based fluorescent microscopy ($R^2 = 0.7314$), supporting its utility as a reliable and simple quantification tool. However, while SYTOX Green provides an efficient means of screening, it lacks specificity for distinguishing between NET-associated and apoptotic or necrotic DNA release, underscoring the need for complementary approaches (Masuda *et al*., 2017).

To address this limitation, multi-component detection protocols, such as the one developed by Gavillet *et al*. (2015), have introduced greater specificity by simultaneously targeting multiple NET markers, including DNA, CitH3, and MPO. This approach has been successfully applied to both murine and human models, including genetically modified mice deficient in PAD4, which exhibit impaired NET formation. Its ability to detect both *in vitro* and *in vivo* NETosis makes it a robust and translationally relevant method, particularly in studies aiming to delineate NET-mediated pathophysiological mechanisms (Gavillet *et al*., 2015).

Table I. Comprehensive overview of NET detection methods and required equipment.

| Method | Equipment Required | Principle | Applications | Citation |
|---|---|---|---|---|
| **Fluorescent Dye-Based Methods** | | | | |
| SYTOX Green Assay | Fluorescence plate reader or microscope | Cell-impermeant DNA dye that fluoresces >500-fold upon binding to extracellular DNA | High-throughput screening, kinetic studies of NET formation | van Breda *et al.* (2019), Matta *et al.* (2022), Stoimenou *et al.* (2022) |
| PicoGreen/Qubit® Assays | Fluorescence plate reader | Selective binding to double-stranded DNA with minimal RNA interference | Quantification of NET DNA in solution, high sensitivity (25 pg/mL to 1 _g/mL) | van Breda *et al.* (2019) |
| Hoechst Dyes (33258, 33342) | Fluorescence microscope | Binds to AT-rich regions in DNA minor groove; used with cell-permeant variants | Nuclear staining in live-cell imaging, often paired with SYTOX dyes | Gupta *et al.* (2018) |
| SYBR Green | Fluorescence microscope or plate reader | Intercalates between DNA base pairs with >1000-fold fluorescence enhancement | DNA quantification in NET samples | van Breda *et al.* (2019) |
| **Microscopy-Based Methods** | | | | |
| Immunofluorescence Microscopy (IFM) | Fluorescence microscope, antibodies against NET markers (MPO, NE, CitH3), DNA dyes | Visual confirmation of NETs through co-localization of extracellular DNA with NET-specific proteins | Gold standard for NET visualization; most widely accepted technique | van Breda *et al.* (2019), Stoimenou *et al.* (2022) |
| Live-Cell Imaging | Automated widefield microscope with environmental chamber, membrane-permeable and impermeable dyes | Real-time visualization of NET formation process | Kinetic studies, drug screening, temporal resolution of NET formation | Silva *et al.* (2021) |
| Confocal Microscopy | Laser scanning or spinning disk confocal microscope, fluorophore-labeled antibodies, DNA dyes | High-resolution 3D imaging of NET structures | Detailed morphological analysis, co-localization studies | Silva *et al.* (2021) |
| Electron Microscopy (SEM/TEM) | Scanning or transmission electron microscope, specialized sample preparation | Ultra-high resolution imaging of NET ultrastructure | Detailed structural studies of NET components | van Breda *et al.* (2019) |
| **Flow Cytometry-Based Methods** | | | | |
| Flow Cytometry (FACS) | Flow cytometer, fluorescent antibodies against NET markers, DNA dyes | High-throughput quantification of NETosis at single-cell level | Screening large cell populations, may miss fully formed NETs | van Breda *et al.* (2019), Matta *et al.* (2022) |
| Microscopy Imaging Flow Cytometry (MIFC) | Imaging flow cytometer (e.g., ImageStream), antibodies, DNA dyes | Combines flow cytometry with microscopy imaging | Phenotyping cells undergoing NETosis, single-cell analysis | van Breda *et al.* (2019) |
| **Automated Analysis Systems** | | | | |
| StrataQuest® software v. 7.0 | TissueFAXS i Plus Cytometry | It allows the detection of NETotic cells by evaluating nuclear expansion. | Useful in reproduction by detecting the presence of NETotic cells in species such as bovine and canine | Rivera-Concha *et al.* (2023), León *et al.* (2024) |
| IncuCyte ZOOM System | IncuCyte ZOOM platform, dual-dye system (membrane permeable/impermeable), automated stage | Automated real-time imaging using two-color platform to distinguish NETosis from other cell death types | High-throughput screening, drug testing, kinetic analysis | Gupta *et al.* (2018) |
| NETQUANT Software | MATLAB software, fluorescence microscope with imaging capabilities | Software for NET quantification based on morphological parameters | Automated analysis of immunofluorescence images | Mohanty & Nordenfelt (2019) |
| NETQUANT2 Web-Based Software | Web browser, internet connection, digital microscopy images | Web-based NET quantification without need for proprietary software | Accessible image analysis for researchers without programming skills | Kumra Ahnlide *et al.* (2024) |
| ImageJ/Fiji-Based Analysis | Computer with ImageJ/Fiji software, digital microscopy images | Open-source image analysis of NET parameters (area, intensity) | Versatile image analysis with customizable workflows | Matta *et al.* (2022) |
| **Dual-Dye Approaches** | | | | |
| Membrane Permeability-Dependent Dual-Dye | Fluorescence microscope or live-cell imaging system, membrane-permeable nuclear dye, membrane-impermeable DNA dye | Distinguishes intact cells from those undergoing NETosis based on membrane integrity | Real-time monitoring of NETosis progression in live cells | Gupta *et al.* (2018), Nakabo *et al.* (2023) |
| **Spectrophotometric Methods** | | | | |
| UV Absorbance (260 nm) | Spectrophotometer (e.g., NanoDrop) | Nucleic acids absorb UV light at 260 nm | Basic DNA quantification, not NET-specific | Stoimenou *et al.* (2022) |
| NETosis Assay Kit (absorbance at 400-420 nm) | Spectrophotometer | Neutrophil Elastase activity | Detection of NETs in culture supernatants or biological fluids | Cell Signaling Technology (2023) |
| **Clinical Sample Analysis** | | | | |
| Plasma/Serum NET Detection | Fluorescence microscope, plate reader, antibodies for NET markers (MPO-DNA, CitH3) | Visualization or quantification of circulating NETs in patient samples | Biomarkers studies, disease association studies | Matta *et al.* (2022), Stoimenou *et al.* (2022) |
| ELISA-Based Methods | ELISA plate reader, antibodies against NET components | Quantification of NET-associated proteins (MPO-DNA, CitH3-DNA complexes) | Clinical samples, biomarker studies | Stoimenou *et al.* (2022) |

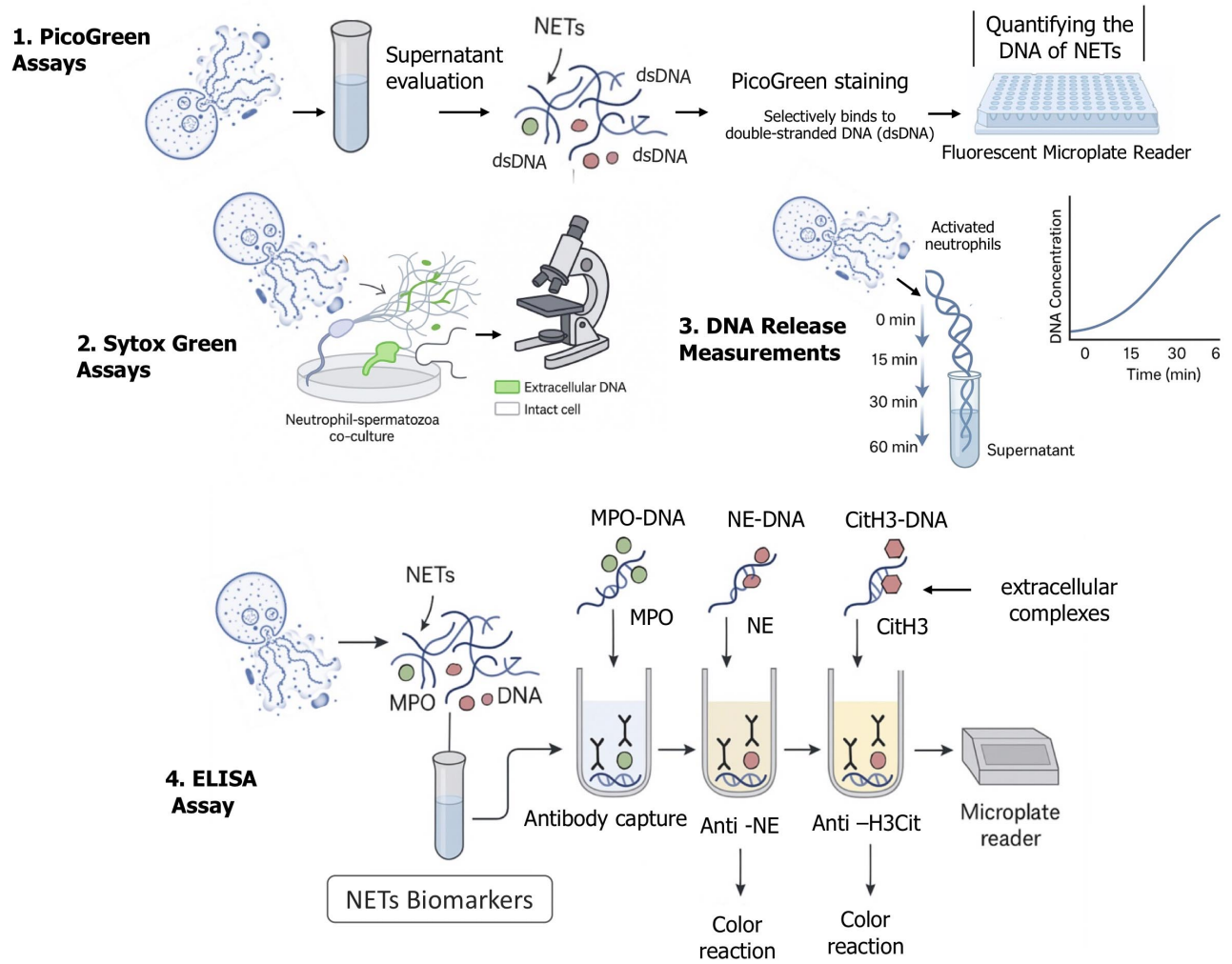


Fig. 2. Overview of the main methodologies used for the quantification of neutrophil extracellular trap (NET) components. (1) PicoGreen Assays involve staining cell-free supernatants with PicoGreen, a fluorescent dye that selectively binds double-stranded DNA (dsDNA), enabling sensitive quantification using a fluorescence microplate reader. (2) Sytox Green Assays utilize a cell-impermeant DNA dye that stains extracellular DNA in neutrophil-spermatozoa co-cultures, allowing discrimination between intact and NET-releasing cells via fluorescence microscopy. (3) DNA Release Measurements assess extracellular DNA in culture supernatants over time, typically using fluorometric detection to monitor NETosis kinetics. (4) ELISA Assays detect circulating NET biomarkers, such as MPO–DNA, NE–DNA, and CitH3–DNA complexes. These are captured using specific antibodies immobilized on microplates and detected via HRP-conjugated anti-DNA antibodies, with colorimetric readout reflecting NET abundance.

For researchers requiring both morphological insight and quantitative power, multi-spectral imaging flow cytometry presents a sophisticated option. As developed by Zhao *et al*. (2015), this method integrates nuclear morphometry with fluorescence intensity analysis, allowing for high throughput yet visually confirmable NET detection. By monitoring nuclear swelling and chromatin decondensation, it provides a powerful platform to track NETosis with high precision (Zhao *et al*., 2015; Dittrich *et al*., 2022), uses DNA-binding dyes (e.g., SYTOX Green) and antibodies against NET markers (MPO, CitH3) to identify extracellular DNA-protein complexes (Zhao *et al*., 2015; Tong & Abrahams, 2021). While this approach requires more advanced instrumentation and data analysis, its increased specificity and imaging capabilities offer considerable advantages in both basic and clinical research.

Several flow cytometry-based approaches have been

developed for the quantification of NETs (Table I). SYTOX Green staining remains a widely used method due to its simplicity, while multi-component assays incorporating H3cit and MPO enhance specificity. More advanced imaging flow cytometry techniques allow for the simultaneous acquisition of fluorescence and morphometric data, providing an integrated analysis of NETosis dynamics.

## 5. Omics Approaches for Identifying NETs: Proteomics and Transcriptomics Insights

The integration of omics technologies, especially proteomics and transcriptomics, has significantly expanded our understanding of neutrophil extracellular traps (NETs), enabling precise molecular dissection of their composition, regulatory mechanisms, and disease-specific signatures. These approaches provide a systems-level perspective that surpasses conventional detection techniques, offering not only descriptive but also functional insights into NET biology (Chapman *et al*., 2019).

In proteomics, shotgun proteomic approaches utilizing LC-MS/MS have uncovered a diverse array of NET-associated proteins, such as MPO and NE, which are critical for antimicrobial defense. Interestingly, these studies have demonstrated that the protein composition of NETs varies depending on the inducing stimulus, such as PMA or LPS, highlighting the context-specific nature of NETosis (Chapman *et al.*, 2019). Moreover, analysis of post-translational modifications (PTMs), such as methionine sulfoxidation and histone citrullination, has added a layer of mechanistic insight into the oxidative and epigenetic modulation of NET formation (Fang *et al*., 2024). However, a major limitation of proteomic analysis remains the interference caused by extracellular DNA, necessitating pre-treatment with enzymes like Benzonase, a technical hurdle that may impact sample integrity or recovery of DNA-associated proteins (Scieszka *et al*., 2022).

Targeted proteomics methods, such as Selected Reaction Monitoring (SRM), offer improved sensitivity for quantifying NET-specific markers, including citrullinated histones and disease-associated neoepitopes. These have proven particularly valuable in autoimmune diseases like rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), where distinct NET-derived antigens may serve as both biomarkers and drivers of pathology (Chapman *et al*., 2019). This targeted strategy holds promise for personalized diagnostics, yet it is inherently constrained by the need for prior marker identification.

On the transcriptomics front, bulk RNA sequencing has been instrumental in uncovering differentially expressed genes (DEGs) in NET-producing neutrophils, especially under pathological conditions such as diabetic retinopathy (DR) and sepsis (Hao *et al*., 2024). Notably, studies have reported the enrichment of mitochondrial genes and immune-modulatory transcripts (e.g., GBP2, P2RY12), suggesting a transcriptional reprogramming that primes neutrophils for NET release (Scieszka *et al*., 2022; Hao *et al*., 2024). The emergence of single-cell RNA sequencing (scRNA-seq) has further refined our understanding by capturing cell-type-specific NET-related gene expression, particularly in complex tissues affected by non-alcoholic fatty liver disease (NAFLD). Here, genes like CLIC3 and HOXA1 have been linked to NETosis signatures in monocytes and macrophages, bridging innate immune activation with tissue damage (Fang *et al*., 2024).

Finally, multi-omics integration, especially proteogenomic analyses, has enabled the simultaneous profiling of protein and gene expression signatures in NET-producing cells. When combined with machine learning algorithms (e.g., random forest, SVM, LASSO), these datasets have yielded predictive biomarkers such as GBP2 and PSAP, enhancing the diagnostic and prognostic power of NET-related studies.5. High-Throughput and Emerging Technologies (Fang *et al*., 2024; Hao *et al*., 2024).

## 6. Challenges, Artifacts, and Future Directions

Despite major advances in the field, the detection and quantification of NETs continue to face significant technical and conceptual challenges. One of the primary limitations lies in the lack of consensus on specific markers that definitively distinguish NETosis from other forms of cell death, such as necrosis or apoptosis. While markers like CitH3, MPO, NE and extracellular DNA are widely used, they can also be present in non-NET-related cellular processes, leading to false positives and overinterpretation of NET-associated pathology.

Additionally, methodological artifacts are a persistent concern. For instance, mechanical disruption during neutrophil isolation or slide preparation can result in artificial chromatin release, mimicking NET formation. Moreover, fluorescence-based assays such as SYTOX Green are unable to distinguish NET DNA from necrotic cell debris without complementary staining strategies. High-resolution imaging techniques, though highly informative, often suffer from low throughput, subjectivity, and variability in sample preparation and analysis.

In flow cytometry-based assays, while increased throughput and quantitative power are significant advantages, specificity remains a challenge unless multiple markers and morphometric validation are used. Even multi-

spectral imaging flow cytometry, though highly promising, requires expensive instrumentation and sophisticated data analysis pipelines, limiting its widespread adoption.

From an omics perspective, proteomic and transcriptomic methods have significantly expanded our understanding of NET biology, revealing stimulus-specific signatures and novel biomarkers. However, these approaches demand high technical expertise, rigorous controls, and often face limitations due to DNA-protein interactions that interfere with protein extraction. Furthermore, transcriptomic profiling of neutrophils is complicated by their short lifespan, low transcriptional activity, and heterogeneity across disease states.

Looking forward, future directions in NETosis research should prioritize the development of standardized protocols and validated reference markers to enable reproducibility across laboratories. Integrating multi-modal approaches, combining high-throughput imaging, omics profiling, and machine learning, may offer a more comprehensive and unbiased analysis of NET formation in diverse pathological contexts. Additionally, the application of single-cell technologies and spatial transcriptomics holds promise for delineating the contribution of NETosis in complex tissue microenvironments.

Finally, expanding NET detection methods to clinically relevant and physiologically representative models, including *in vivo* imaging and whole-blood assays, will be essential for translating basic research into diagnostic and therapeutic tools. Interdisciplinary efforts that bridge immunology, computational biology, and clinical research will be key to unlocking the full potential of NET biology in health and disease.

**ACKNOWLEDGMENTS.** We express our gratitude to ANID FONDEQUIP EQM200228 for providing access to the TissueFAXS i Plus Cytometer, which contributed valuable data and resources for the development of this review. Also, this research was funded partially by the Universidad de La Frontera, for sponsoring teachers' support, grant PP24– 0008.

**ZAMBRANO, F.; URIBE, P.; SCHULZ, M.; HERMOSILLA, C.; TAUBERT, A. & SÁNCHEZ, R.** Métodos para la detección de la formación de trampas extracelulares de neutrófilos: desde la microscopía hasta tecnologías de alto rendimiento. *Int. J. Morphol., 43(4)*:1421-1428, 2025.

**RESUMEN:** Las trampas extracelulares de neutrófilos (NETs, por sus siglas en inglés) son estructuras formadas por cromatina que se liberan durante la NETosis, una forma especializada de muerte celular de los neutrófilos con funciones esenciales en la defensa del huésped y en la patogénesis. Si bien las NETs ayudan a atrapar patógenos, su desregulación contribuye a trastornos autoinmunes, inflamatorios y trombóticos. Dada su naturaleza dual, la detección precisa de las NETs es crucial tanto para la investigación básica como clínica. Esta revisión ofrece una visión general exhaustiva de las metodologías actuales y emergentes empleadas para detectar la formación de NETs, que abarcan desde técnicas tradicionales de microscopía y colorantes fluorescentes para ADN hasta citometría de flujo avanzada, tecnologías ómicas y plataformas asistidas por aprendizaje automático. Los métodos basados en microscopía permiten una confirmación visual, pero son intensivos en tiempo y trabajo, mientras que la citometría de flujo y la imagen automatizada permiten una cuantificación de alto rendimiento. Los enfoques ómicos, como la proteómica y la transcriptómica, revelan firmas moleculares y vías regulatorias de la NETosis en diferentes contextos patológicos. A pesar de estos avances, persisten desafíos en cuanto a la especificidad de los marcadores, los artefactos en la preparación de muestras y la estandarización de protocolos. Las investigaciones futuras deben centrarse en integrar técnicas multimodales y establecer estrategias de detección robustas y validadas, adecuadas para aplicaciones *in vivo* y clínicas. Esto será clave para aprovechar las NETs como biomarcadores y objetivos terapéuticos en infecciones, cáncer y enfermedades mediadas por el sistema inmune.

**PALABRAS CLAVE: NETosis; Trampas extracelulares de neutrófilos; Métodos de detección.**

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Corresponding author:
Fabiola Zambrano
Center of Excellence in Translational Medicine
CEMT, BIOREN
Faculty of Medicine
Universidad de La Frontera
Temuco
CHILE

E-mail: fabiola.zambrano@ufrontera.cl