To the Editor
Journal of Visual Experiments

Lund, 2018

Dear Jaydev Upponi, Ph.D, Science Editor,

Please consider the enclosed manuscript "***Automated image-based quantification of neutrophil extracellular traps using NETQUANT"*** by Pontus Nordenfelt, and myself for publication as an article in the *Journal of Visual Experiments*.

Neutrophils are known to produce neutrophil extracellular traps (NETs) in response to a wide range of infectious and sterile agents. NETs have been implicated in several diseases including infections, cancer and autoimmune disorders[1]. This has resulted in an increase in interest, spanning across multiple fields and disciplines to study NETs. Detection of NETs by immunofluorescence microscopy and subsequent image-based quantification remains a prevalent choice among researchers to assess NET formation. However, manual image-based quantification methods have drawbacks in being either subjective and error prone, especially for non-trained users. Currently available software rooted options are either semi-automatic or require training prior to operation. Hence, there is a genuine need for developing novel, easy-to-use and reproducible software/approaches to quantify NET formation.

In the manuscript, we demonstrate the use of a fully automated immunofluorescence-based image quantification method to quantify NET formation called NETQUANT[2]. NETQUANT has been designed as a freely available app for MATLAB. It is easy to use, with programmable parameters and a user friendly graphic user interface (GUI). The quantification is based on biologically relevant parameters such as an increase in surface area and DNA:NET marker protein ratio, and loss of nuclear morphology to stringently distinguish between extruded NETs from cells that have only underwent nuclear decondensation. Furthermore, NETQUANT also performs analysis and quantification at a single-cell level, which is the highest achievable resolution.

NETQUANT can be potentially used for rapidly quantifying NET formation by various stimuli and to screen for NET enhancing/inhibiting drugs in a high throughput fashion, while also allowing for single quantification and analysis. Based on these points, we believe that the software solution presented in the manuscript represents a significant advancement in immunofluorescence image-based NET quantification and therefore could be of interest to the broad readership of *Journal of Visual Experiments*.

Yours sincerely,

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References

1. Papayannopoulos, V. Neutrophil extracellular traps in immunity and disease. *Nat Rev Immunol* (2017).
2. Mohanty, T., Sorensen, O.E. & Nordenfelt, P. NETQUANT: Automated Quantification of Neutrophil Extracellular Traps. *Front Immunol* **8**, 1999 (2017).