DIGITAL IMAGE ANALYSIS RESULTS SHOW HIGH REPRODUCIBILITY AND AGREEMENT WITH HUMAN INTERPRETATION ON HEp-2 CELLS

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INTRODUCTION

The term "anti-nuclear antibody" (ANA) describes a variety of autoantibodies that react with constituents of cell nuclei including DNA, RNA, proteins and riboproteins.[1] The detection of ANA in human serum is an important tool for diagnosing connective tissue diseases, especially systemic lupus erythematosus (SLE).[1-3] Indirect imunofluorescence (IFA) is the reference method for ANA testing which detects a wide range of autoantibodies to nuclear and cytoplasmic antigens.[1,2] A negative test virtually rules out SLE.[3] "Currently, the ACR ANA task force believes the IFA test is the best screening ANA. For SLE, in conjunction with the history and physical, it recognizes almost all patients with this disorder (sensitivity over 95%)." [4]

Lack of standardization for IFA ANA testing still remains a concern.[5] Sources of variability include, but are not limited to, the microscope and the interpretation by the operator. The introduction of automation can eliminate these sources of variability as it provides an objective output.[6,7] The NOVA View® automated system contains an Olympus microscope with an automated stage, a CCD digital camera, a LED light source and software that controls the motorized stage, takes digital images, archives the images and preliminarily categorizes the samples as positive or negative. It is followed by human visual interpretation of the archived images that allows review and user confirmation of the automated results. In addition, the archived images facilitate training and allows for the exchange of results between labs and clinicians.[7] The NOVA View reduces variability and provides an approach to standardize ANA interpretation.
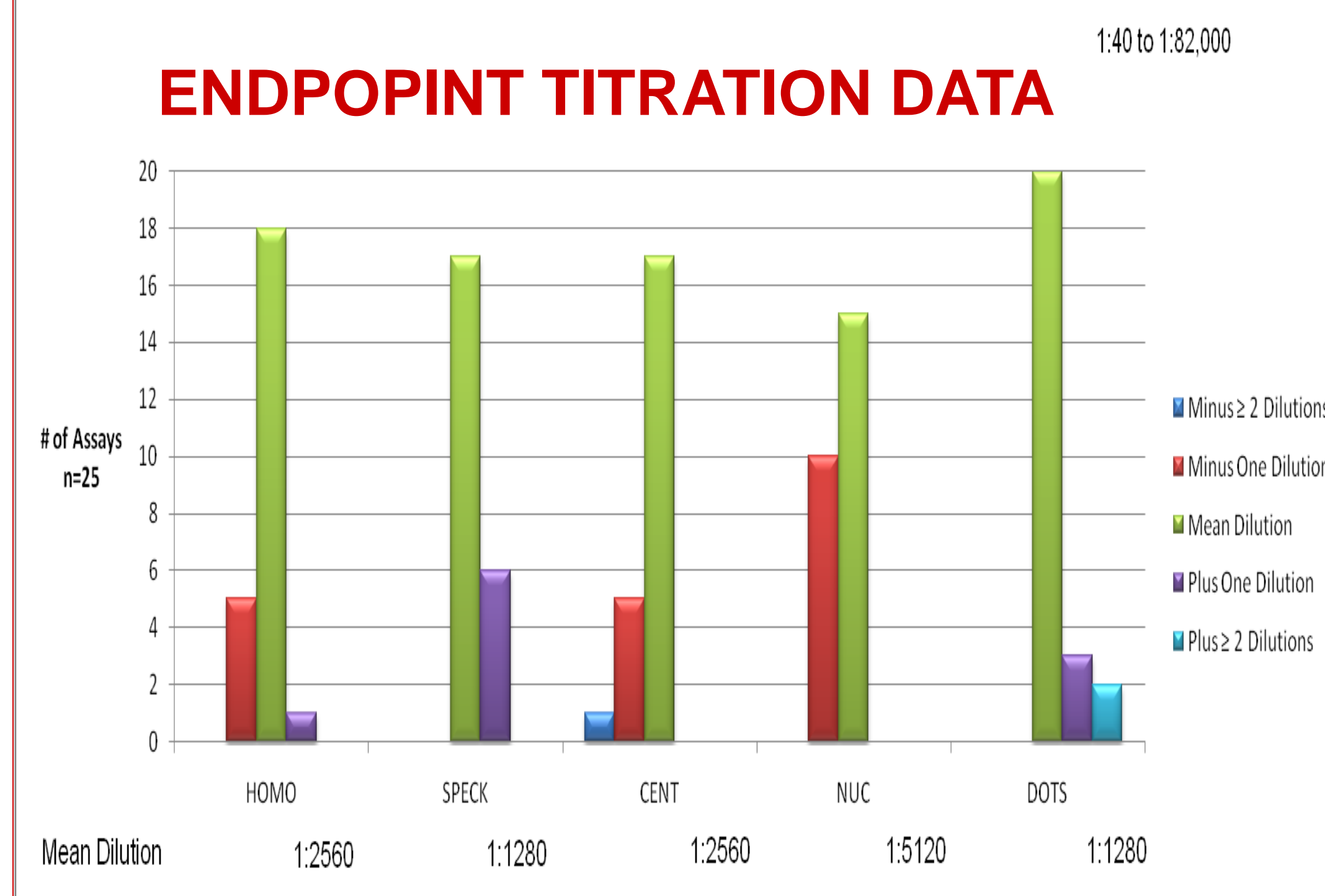
OBJECTIVES

▪ Evaluate the precision (repeatability and reproducibility) of the NOVA View automated digital microscope system for anti-nuclear antibody (ANA) testing on HEp-2 cells based on nuclear light intensity units (LIU) and endpoint titration data.

▪ Compare the NOVA View output generated on 204 clinically defined sera to the visual human interpretation of the same image captured by the NOVA View and archived as a digital image.

METHODS

▪Intra-assay variability was determined running five controls with five different patterns 36 times each within one run.

▪ Total variability was determined by running five controls 45 times. The 45 individually run assays integrated two lots of HEp-2 slides, two lots of conjugates and three operators.

▪ Endpoint titration studies were performed by diluting five sera from 1:40 to 1:81,920 in PBS for 25 separate runs.

▪To evaluate positive, negative and total % agreement, 204 clinically defined sera were analyzed. The output of the NOVA View was compared to the visual human interpretation of the archived images.

CONCLUSION

Our results showing low intra- and total assay variation suggest that NOVA View results are highly reproducible and precise. The analysis of the endpoint titration data and results from 204 clinically defined sera demonstrated the capability of the NOVA View to reliably discriminate between positive and negative.

In addition, the archived images can be stored, reviewed and shared at any time. The NOVA View provides the capability to quantify results with nuclear LIU values which can provide the basis for objective ANA interpretation and facilitate the establishment of ANA standardization.

% AGREEMENT BETWEEN ARCHIVED IMAGES & NOVA View INTERPRETATION

	Archived Image Positive	Archived Image Negative	Total
* NOVA View Positive	90	13	103
* NOVA View Negative	3	98	101
Total	93	111	204
% AGREEMENT	POSITIVE = 97 %	NEGATIVE = 88 %	TOTAL = 92 %

*Based on NOVA View nuclear LIU cut-off of 100

ENDPOPINT TITRATION DATA

1:40 to 1:82,000

# of Assays n=25

Legend: Minus ≥ 2 Dilutions, Minus One Dilution, Mean Dilution, Plus One Dilution, Plus ≥ 2 Dilutions

	HOMO	SPECK	CENT	NUC	DOTS
Mean Dilution	1:2560	1:1280	1:2560	1:5120	1:1280

INTRA-ASSAY VARIABILITY

Pattern	Average Nuclear LIU	%CV
Homogeneous	486.2	12.3
Speckled	421.6	16.9
Centromere	343.1	14.5
Nucleolar	451.6	9.0
Nuclear dot	422.1	13.1

TOTAL ASSAY VARIABILITY

Pattern	Average Nuclear LIU	%CV
Homogeneous	1054.3	19.1
Speckled	1859.4	13.1
Centromere	672.2	13.8
Nucleolar	625.6	16.7
Nuclear dot	877.3	14.8

DAPI — FITC — DAPI/FITC Overlay
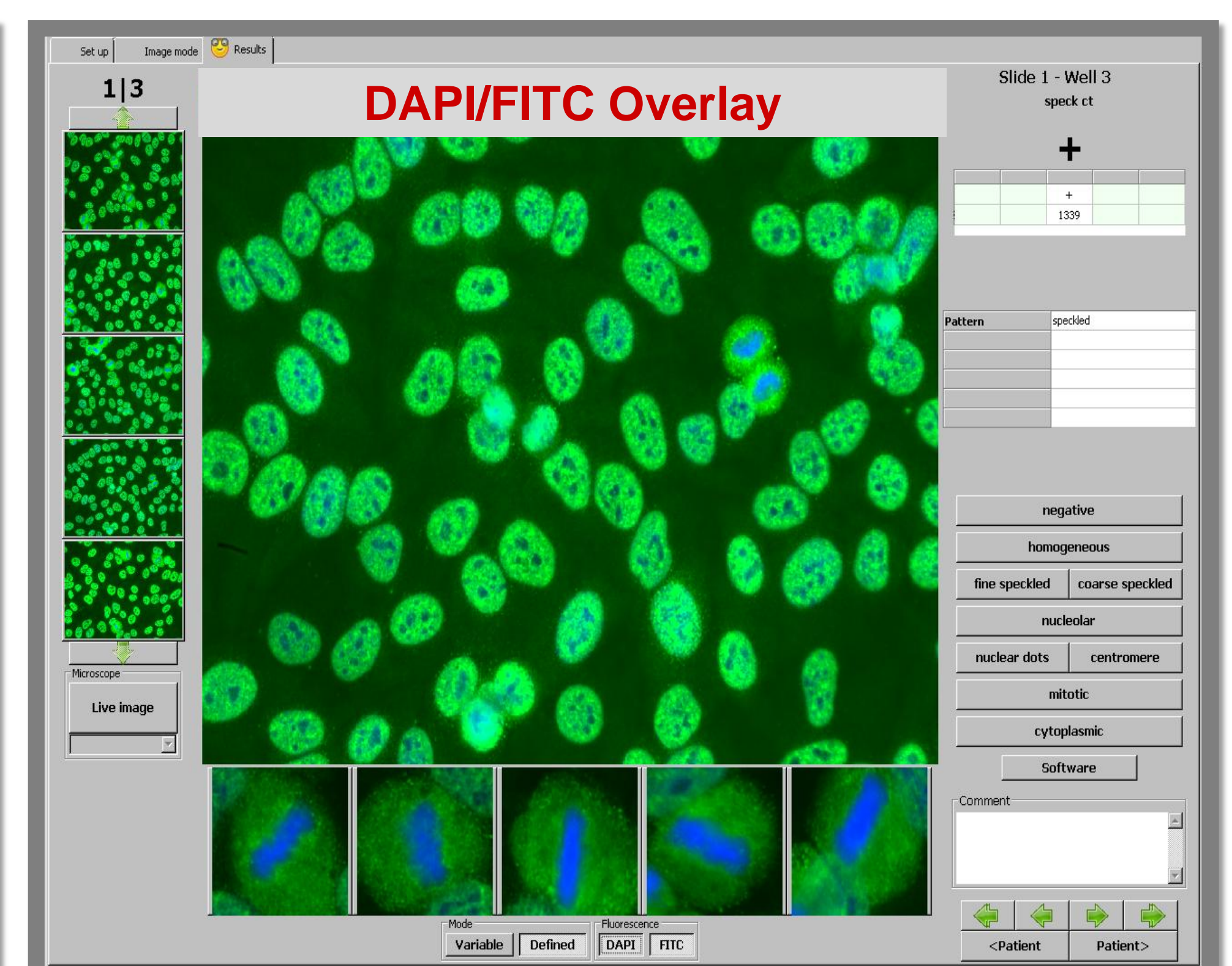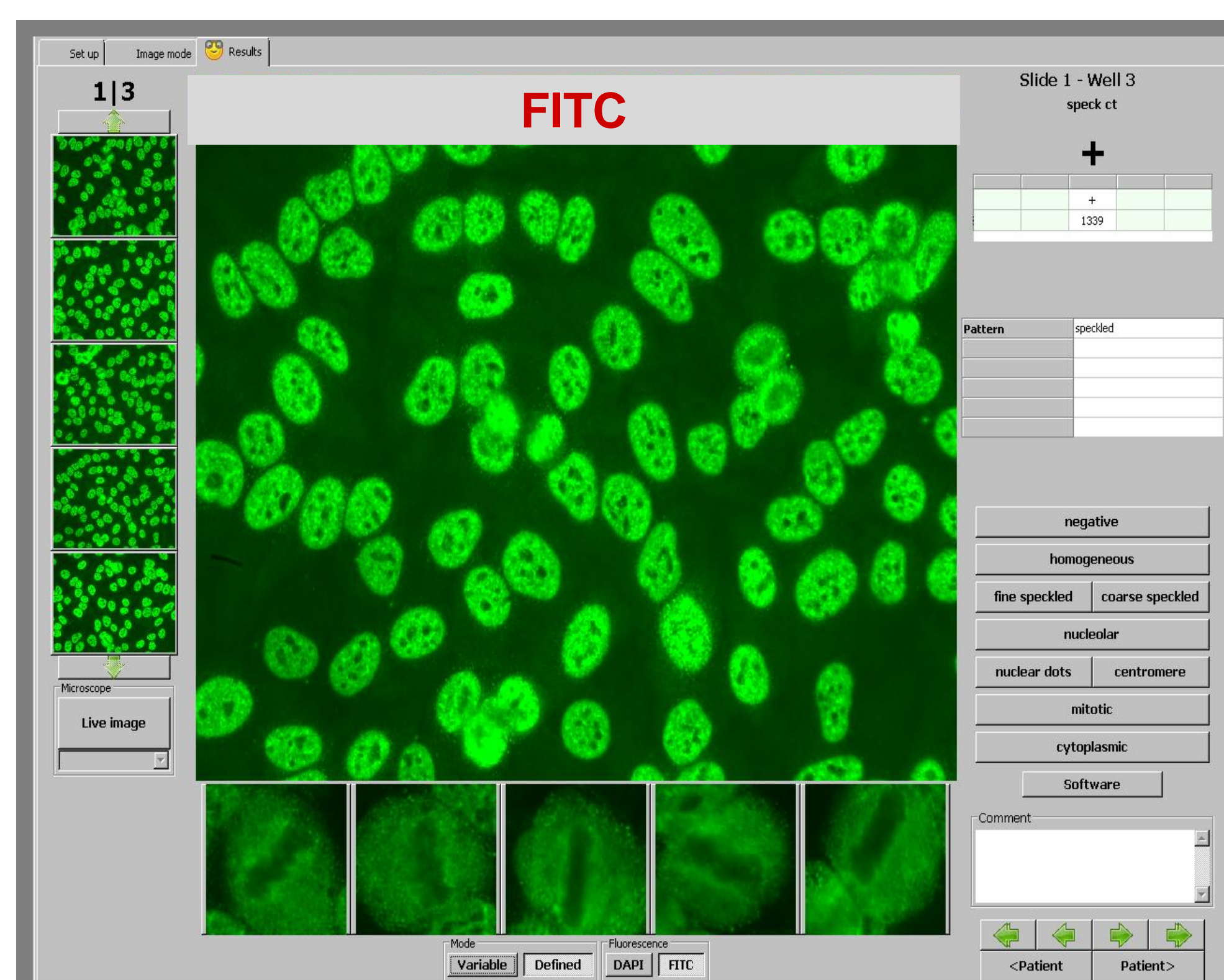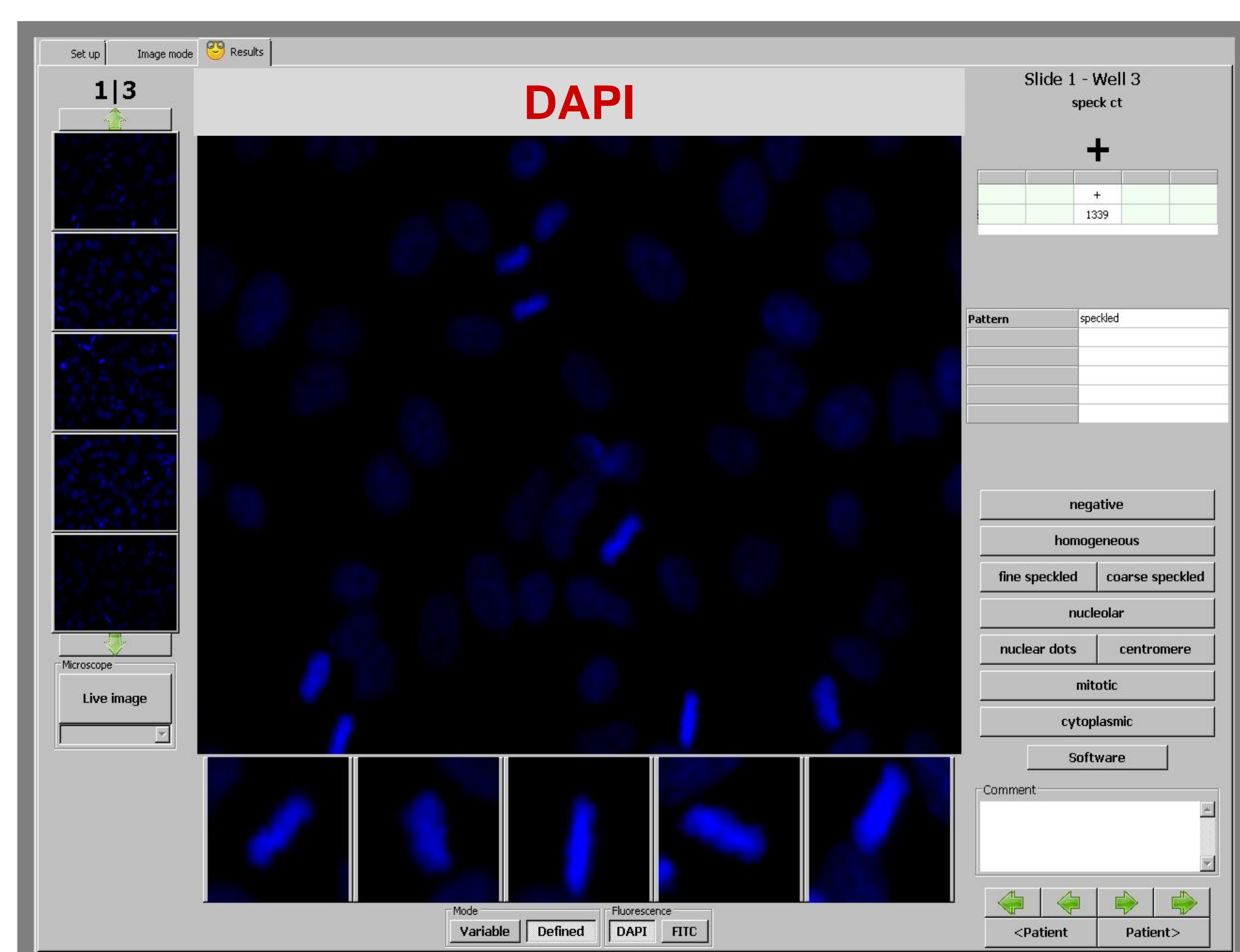
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