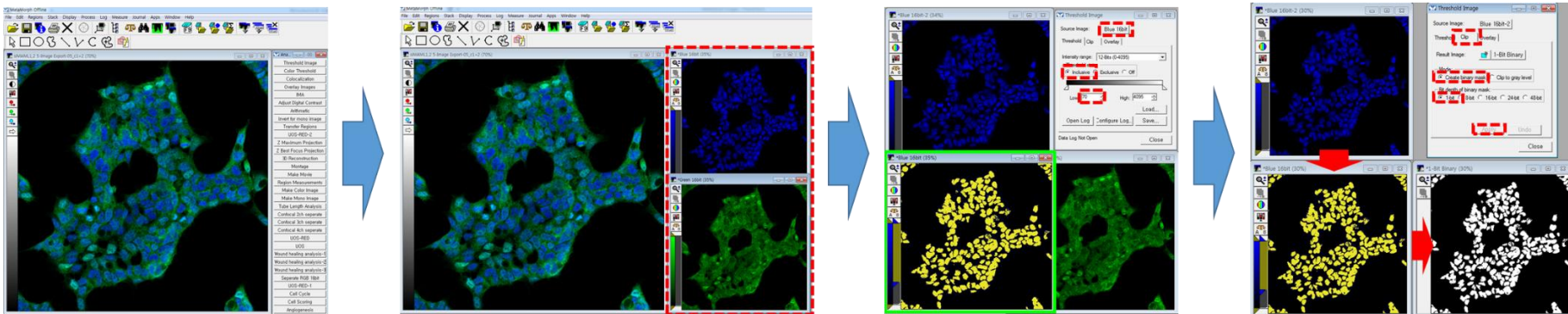## 핵(nucleus)으로 이동한 target protein 정량 (MetaMorph V.7.8.1)

세포체학실험실에 보유 중인 MetaMorph image analysis software (Molecular Devices, CA, USA)는 immunofluorescence (IF)로 염색된 images의 channel별 분리 및 세포(cell) 전체영역에서 핵(nucleus)만 오려내어 핵 내에 위치하는 target protein의 양을 정량하거나, 핵을 제외한 cytosol부분만 filtering하여 cytosol에 존재하는 target protein을 정량화 할 수 있습니다. 또한, region of interest (ROI) 영역 설정을 통해 개별 세포, 세포질, 핵의 면적, 형광 intensity (total, average, mean value), XY localization, shape factor (0 to 1) 등을 계산하여 excel file로 export할 수 있으며, 현재 보유중인 추가적인 options (Angiogenesis, Cell Cycle, Count Nuclei, Cell Scoring, Granularity, Monopole Detection, Wound healing analysis)을 통해 다양한 분석이 가능합니다. ImageJ로 분석 algorithm 또는 building block을 직접 제작하기 어려운 경우, MetaMorph를 사용하여 분석한다면, 손쉽게 도움을 받을 수 있습니다.
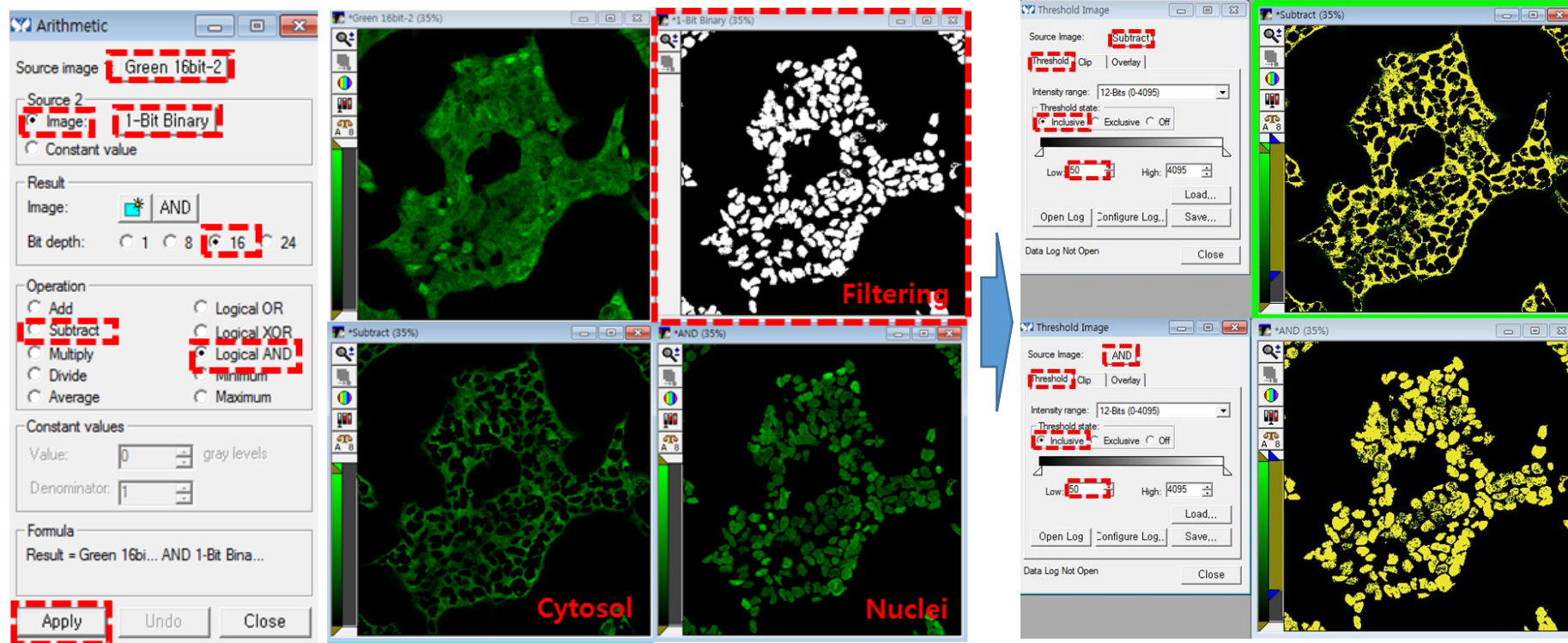
분석 algorithm:

Open file (IF형광 image) -> Separate RGB -> Adjustment of inclusive threshold of "DAPI image" by input of low threshold -> Move to the next tap "Clip" and check "Create binary mask", "1-bit depth" and "Apply" -> 1bit black & white image가 생성 됨.
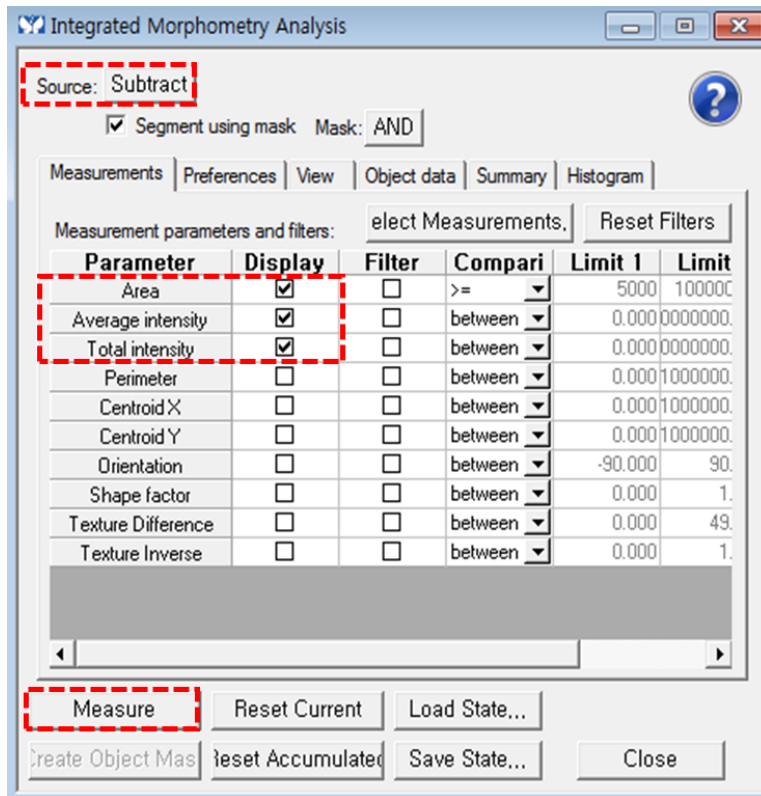
Arithmetic:

Go to "Arithmetic" -> Source image 1: 세포(cell) image 선택 -> Source image 2: 1bit black & white image 선택 -> Image mode: AND -> Bit depth: 16bit -> Operation: "Logical AND (핵 부위 오려내기) or Subtract (세포질 부위 오려내기)" -> Apply

Threshold Adjustment:

Go to "Threshold Image" -> Select source image by clicking "Cytosol" or "Nuclei" image -> Inclusive threshold -> Decide "Low" threshold by controlling a sliding bar. Tip: in order to make reliable comparison of batch images, input a fixed number onto the Low threshold box in inclusive threshold mode.

Cell_Green intensity

	Area	Average intensity	Total intensity
Average	441.475	58.87	35795.125
Std. Dev.	12835	6.245	1032934.313
Minimum	2	50	100
Maximum	410724	133.538	33048396
Total	452070	60283.148	36654208

Nucleus_Green intensity   N/C ratio %

	Area	Average intensity	Total intensity	
Average	632.692	95.93	62475.703	
Std. Dev.	1308.21	39.028	131943.281	
Minimum	2	50.5	101	
Maximum	13895	241	1373481	
Total	205625	31177.412	20304604	55.395

Integrated Morphometry Analysis:

Go to IMA (Integrated Morphometry Analysis) -> Select source image -> Check segment using mask and select the image for masking (Masking is a useful tool to extract specific region for its analysis. If you mask your image with DAPI, only the image region which covered by DAPI signal will be analyzed) -> Decide parameters desired (check display boxes) -> For more specific selections, check "Filter" and give Limit1 and Limit2 numbers. -> Measure -> Confirm object data and summary -> Data export.

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