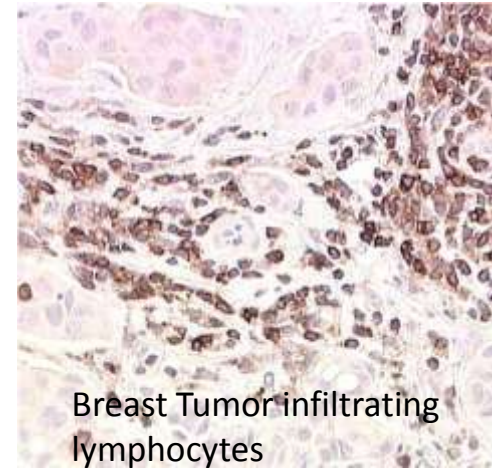
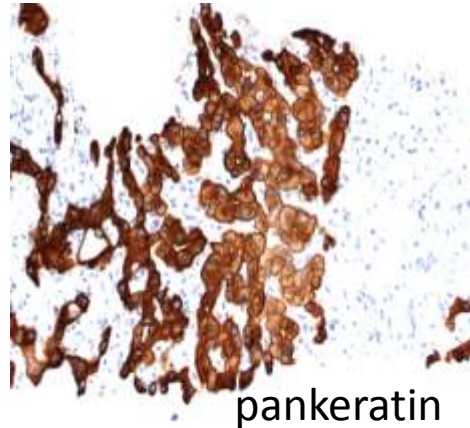
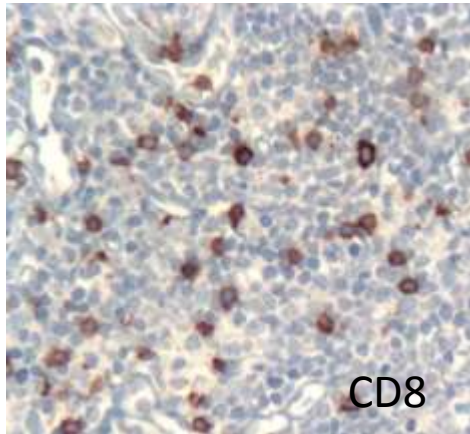


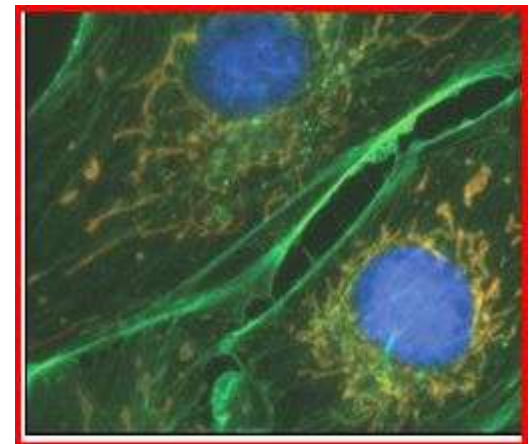
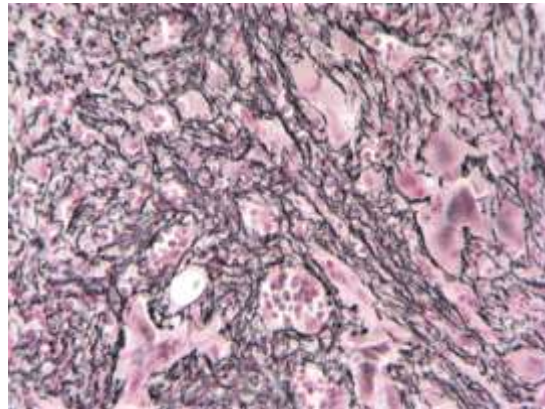
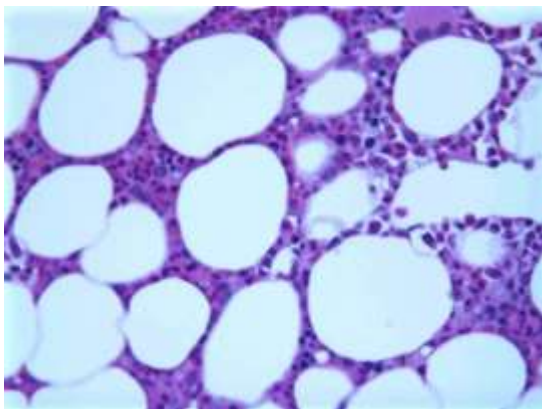
# **Application of Biomedical Digital Solutions: Virtual Flow Cytometry and Hematometrics in Pathology and Research**

**Hernani D Cualing  
IHCFLOW Inc  
Lutz, FL, USA**

Can these diagnostic images become data instead of just pictures? Can we go beyond eyeballs or estimate?



Brightfield and fluorescent Models: Routine HE tissue stain of bone marrow, reticulin fibers stain for fibrosis in bone marrow and fluorescent probes in cells



# Future of healthcare



Virtual Medicine & Patient Trust

# Approach to Segmentation Goals

A good segmentation is typically one which satisfies the user requirements, fits the model system for wide generalization, fast implementation and applicability:

App -fits my workflow as pathologist, really fast

- adapt to the heterogeneity of images by using the common thread from these images, no matter how different

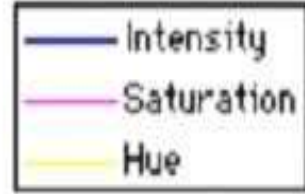
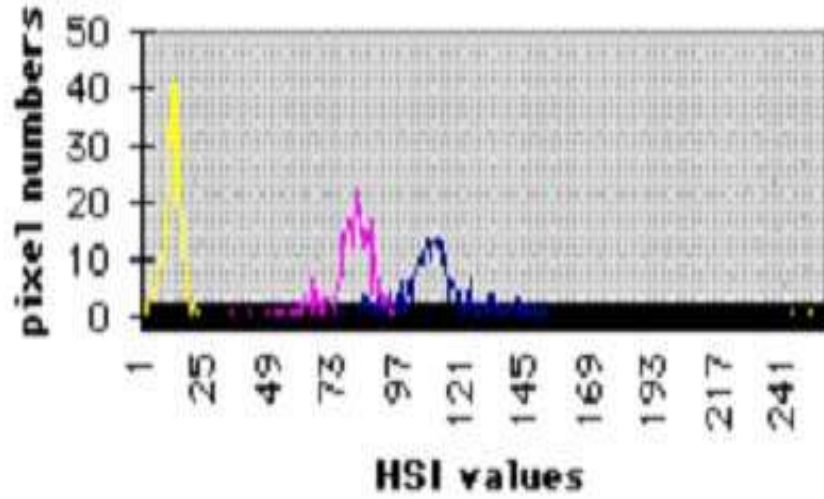
- should be multidisciplinary, as collaboration between scientists, medical persons, and computer engineers.

# Image Data: common thread?

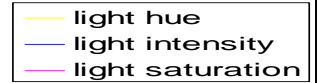
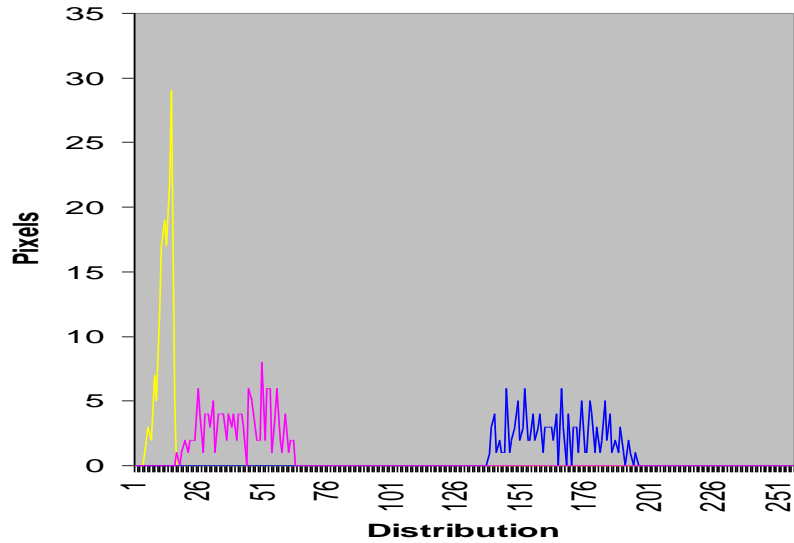
- Red, Blue, Green channels contain all the information on objects.
- Hue, saturation and Brightness channels also have useful information on all these objects
- Combination of above channels allow application in a wide variety of tissue of different tissue stains using standard segmentation bioengineering tools in a defined ordered sequence



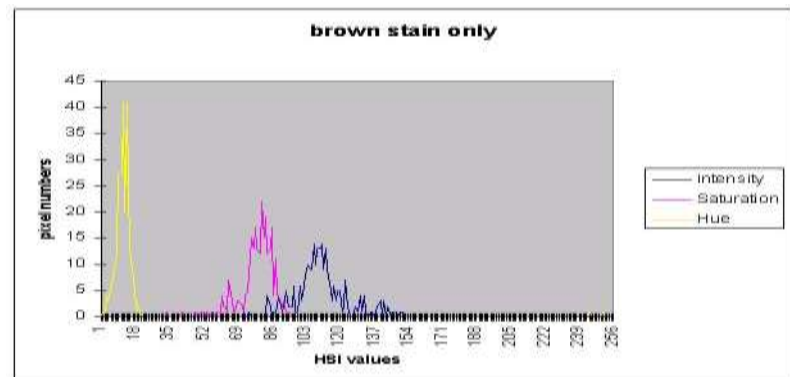
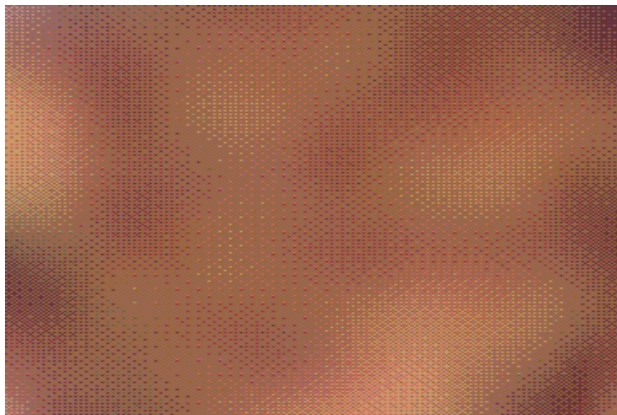
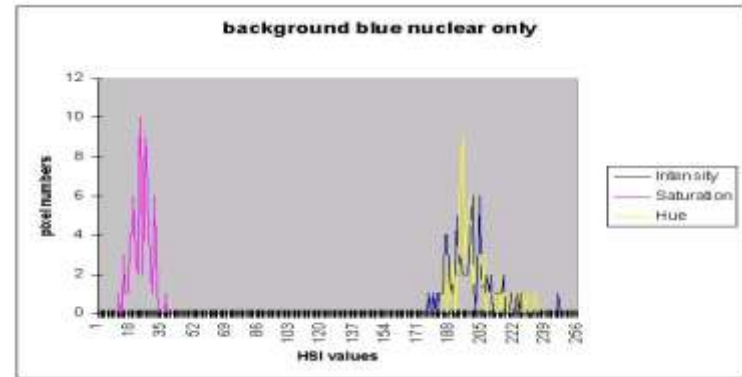
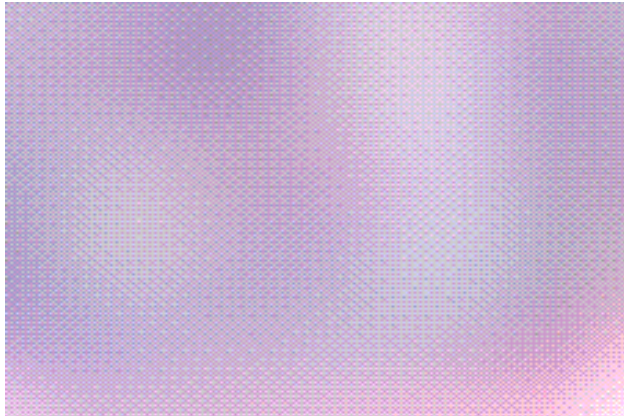
**brown stain only**



**Light Stain Histograms**



# Basic color of tissue stains and their H, S, I histograms

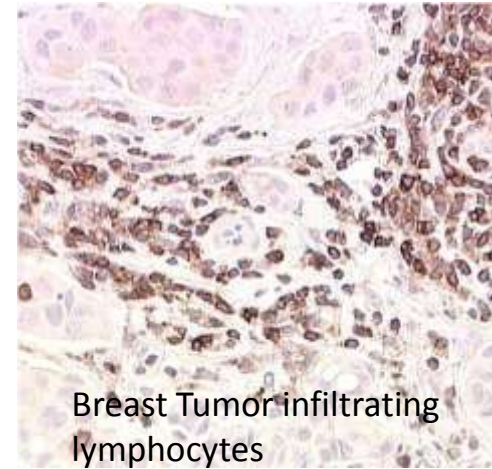
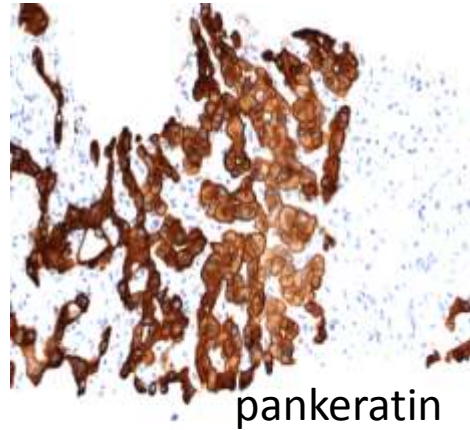
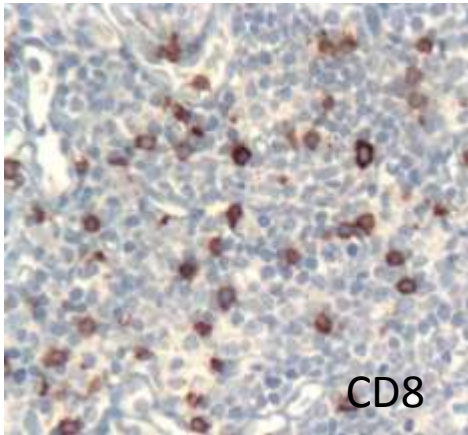


# Workshop paradigm

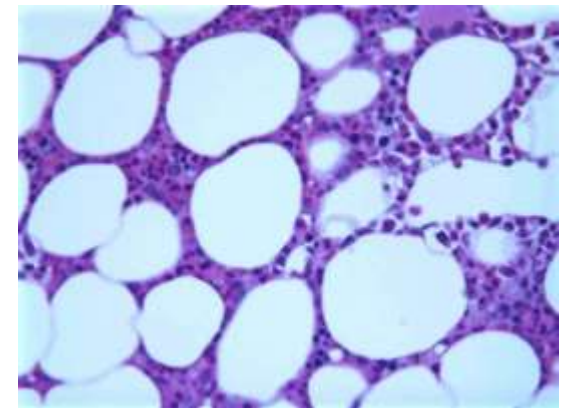
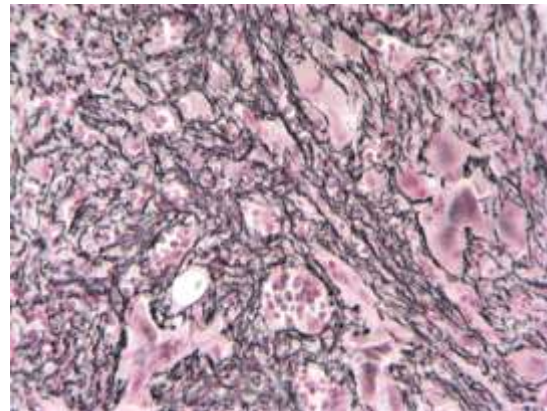
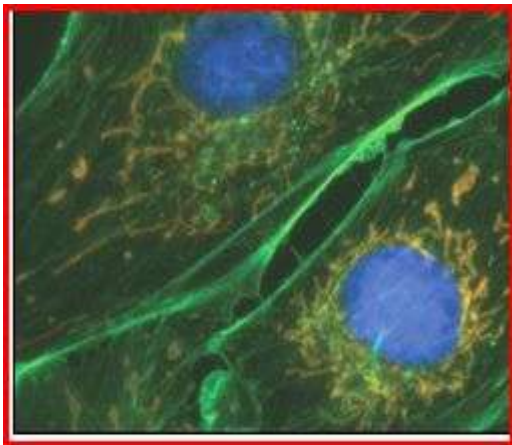
- We created a **workshop paradigm : set of tools in the workshop used in a defined sequence, ie best chisels, planes, tools**, as analogy for example, to convert Planks of Honduras Mahogany to a Queen Ann table.
- We premise a strategy of using a pipeline of primitive, familiar, but reliable segmentation thresholding and object partitioning tools using the workshop model: thresholding histogram by intermeans or max entropy, bit or grayscale masks, Watershed, Voronoi tessellations, Centroids, Math morphology, image math
- Will lead to robust APPS in any number of target antigens or tissue (bone marrow, stroma or any biomarkers,etc )
- And handle brightfield or fluorescent images

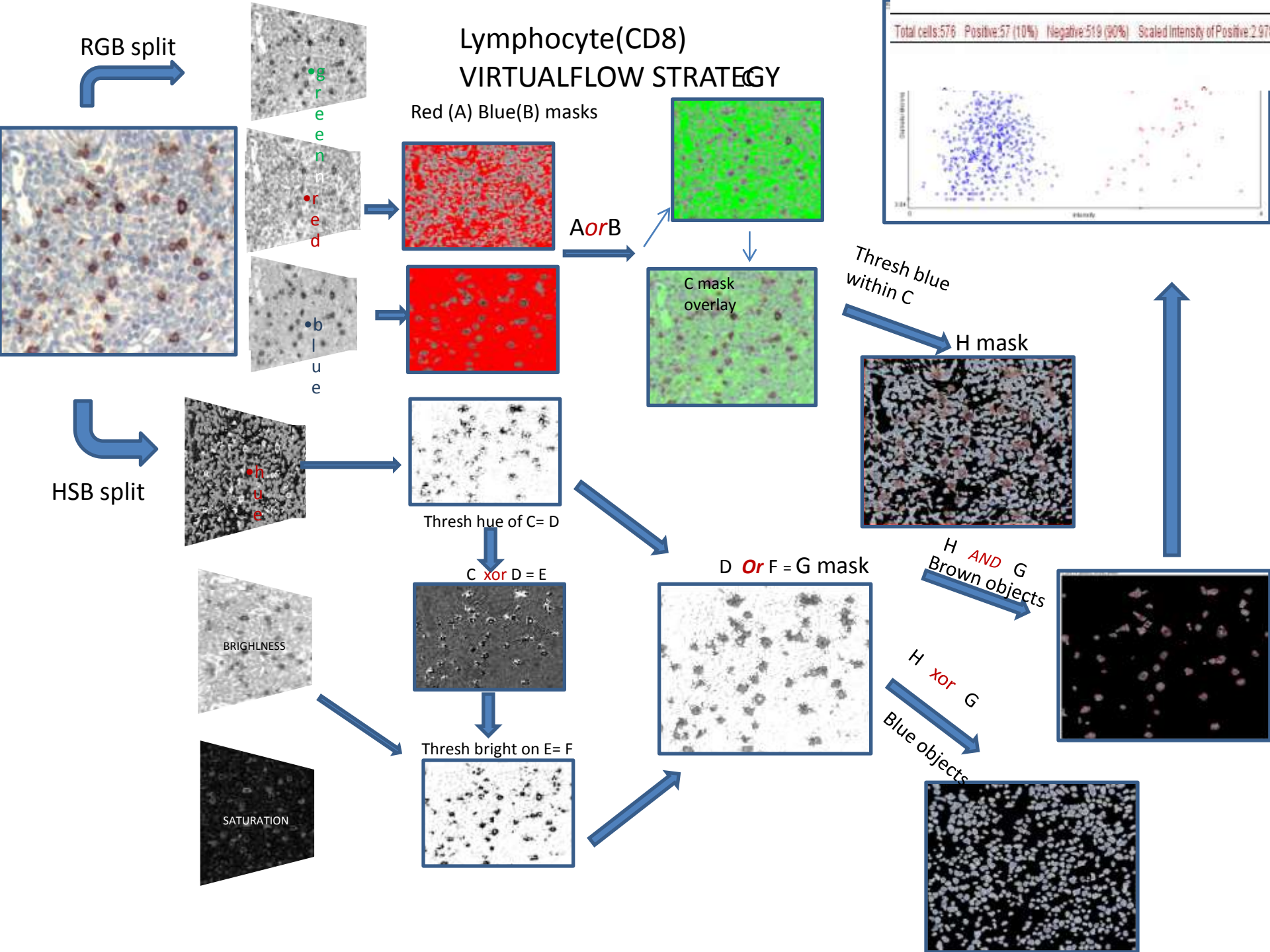


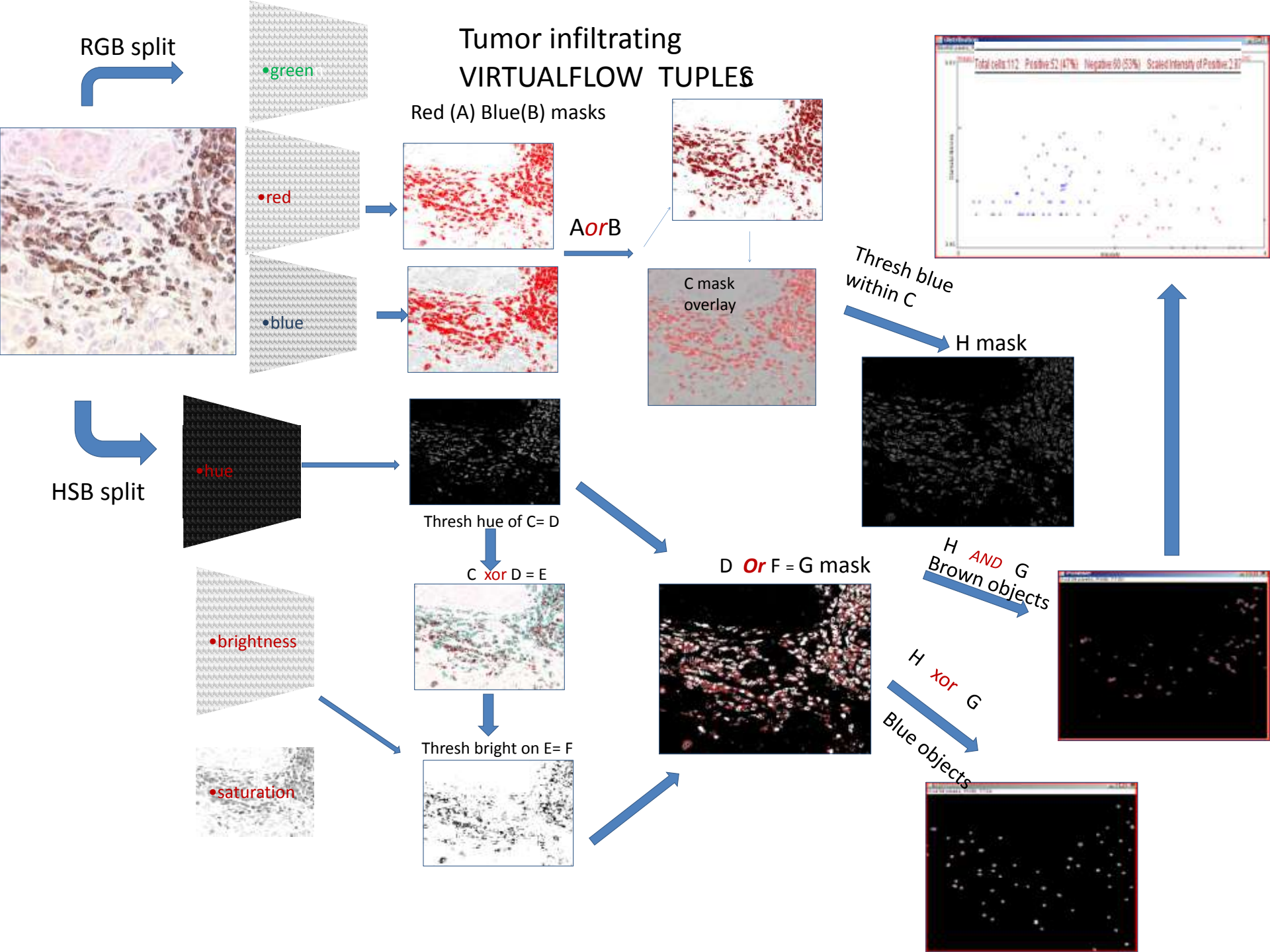
An app should be versatile, and based on a workshop paradigm: we called hematometrics, by using only a limited set of good segmentation tools applied to widely different tissue materials to create customized final segmentation results .

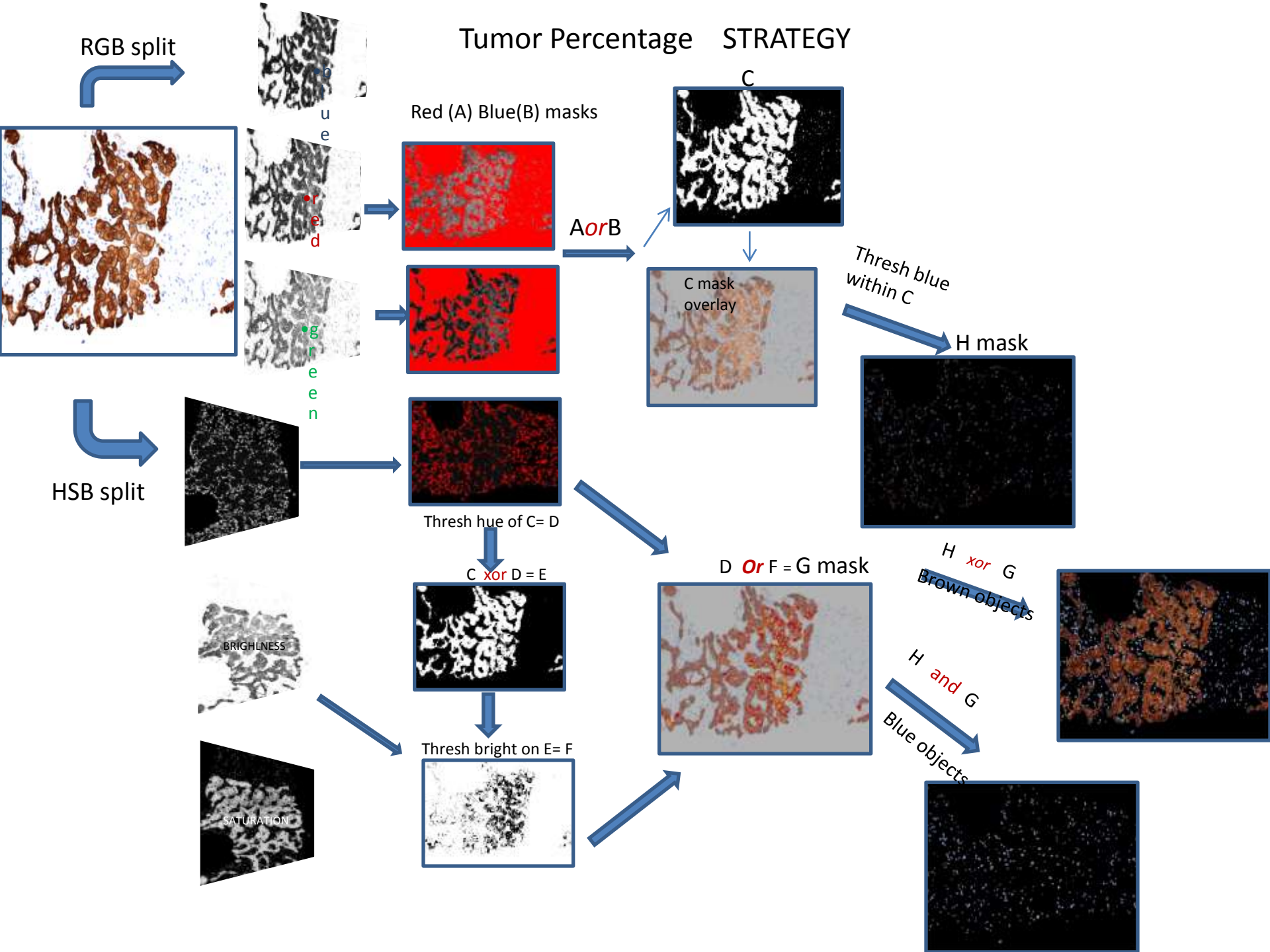


Brightfield and fluorescent Models: Routine HE tissue stain of bone marrow, reticulin fibers stain for fibrosis in bone marrow and fluorescent probes in cells

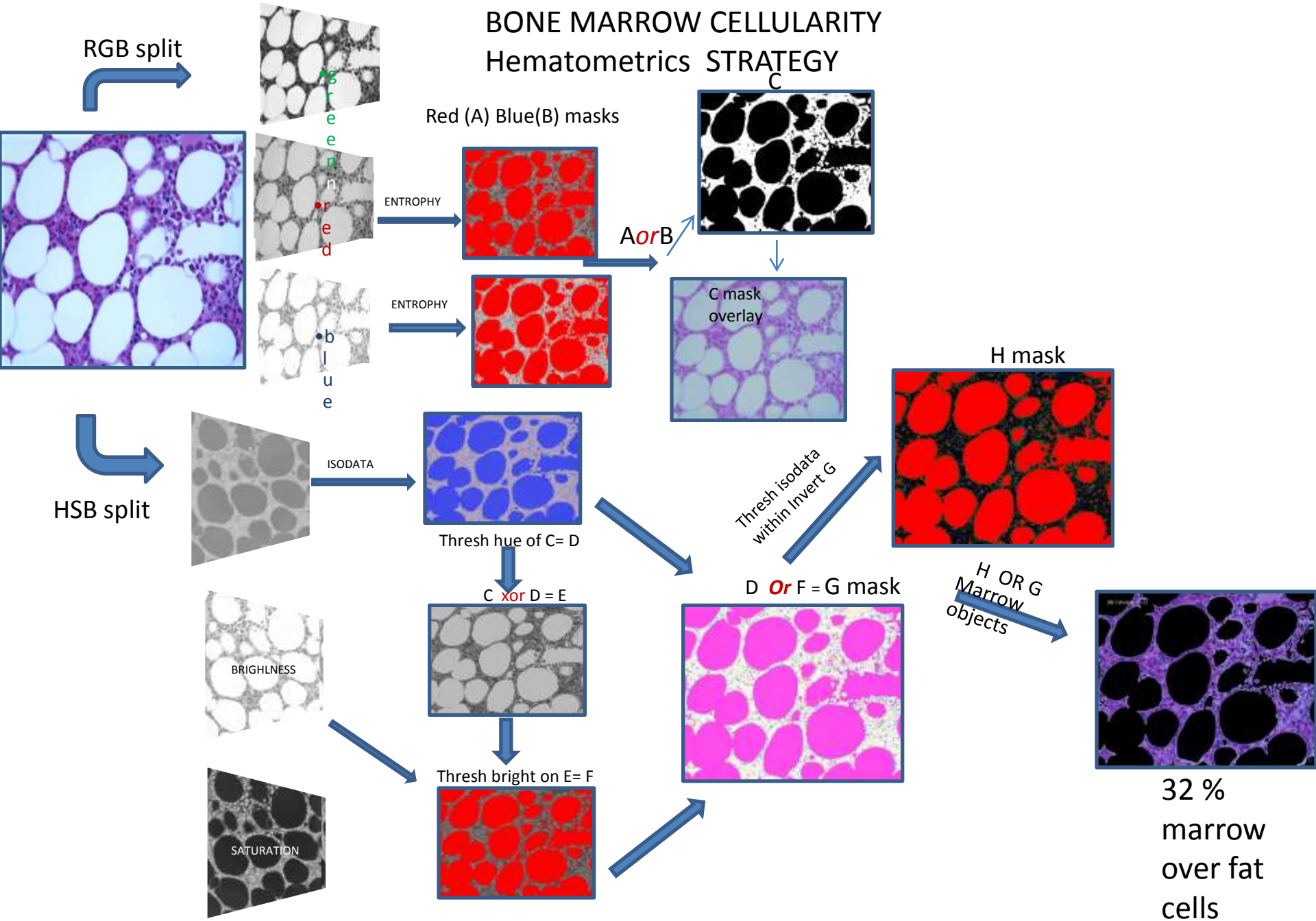




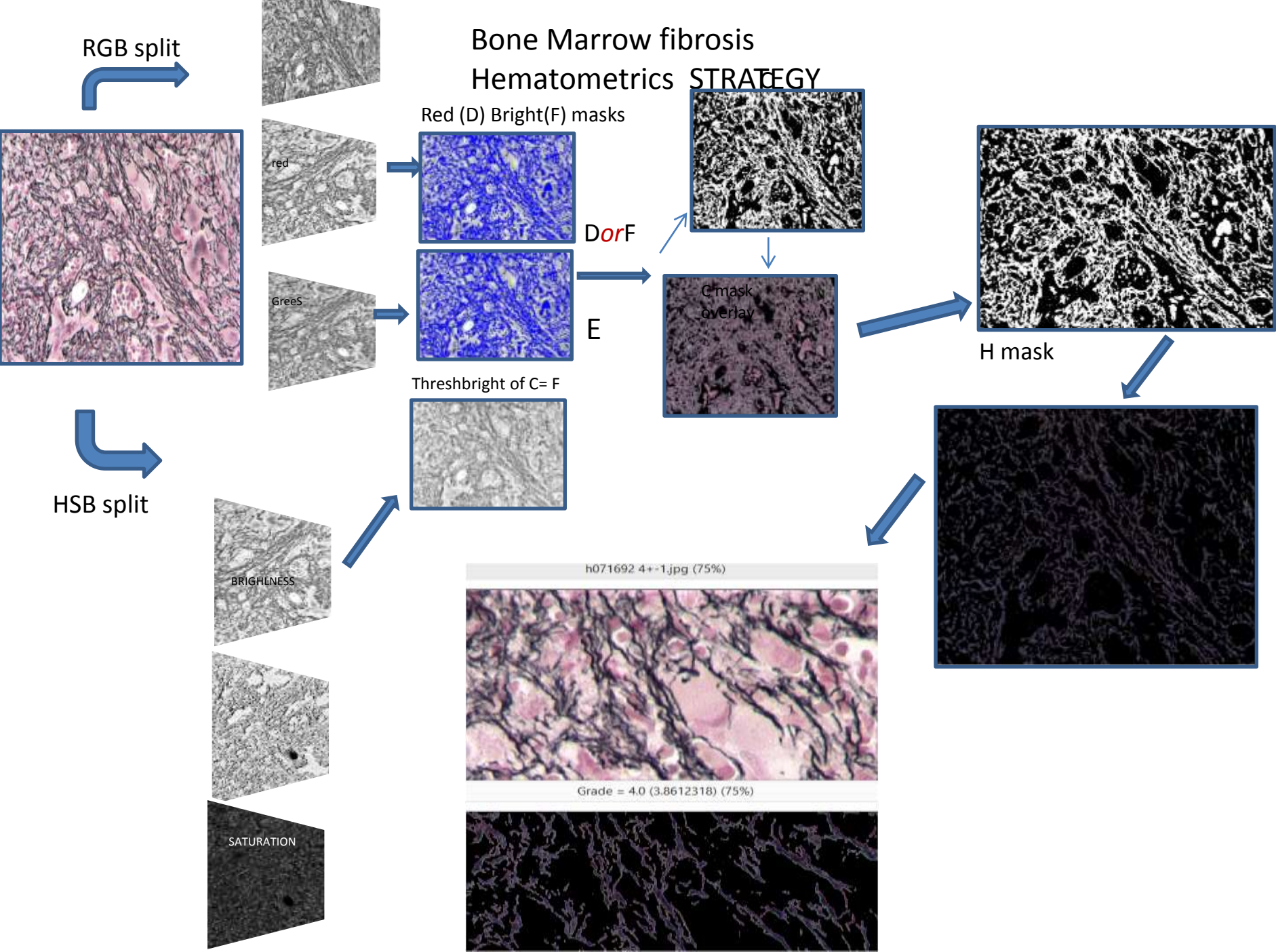


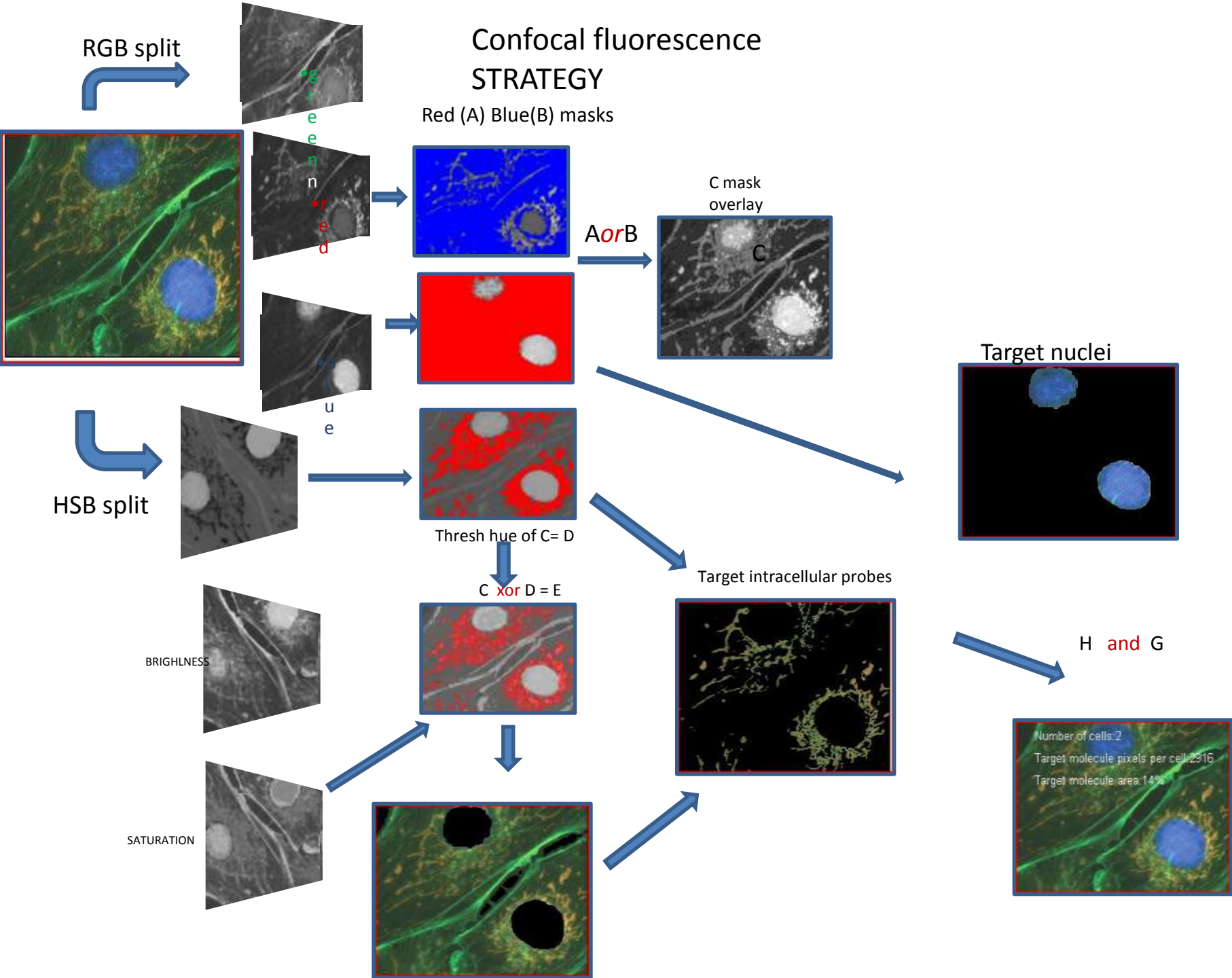


# BONE MARROW CELLULARITY Hematometrics STRATEGY

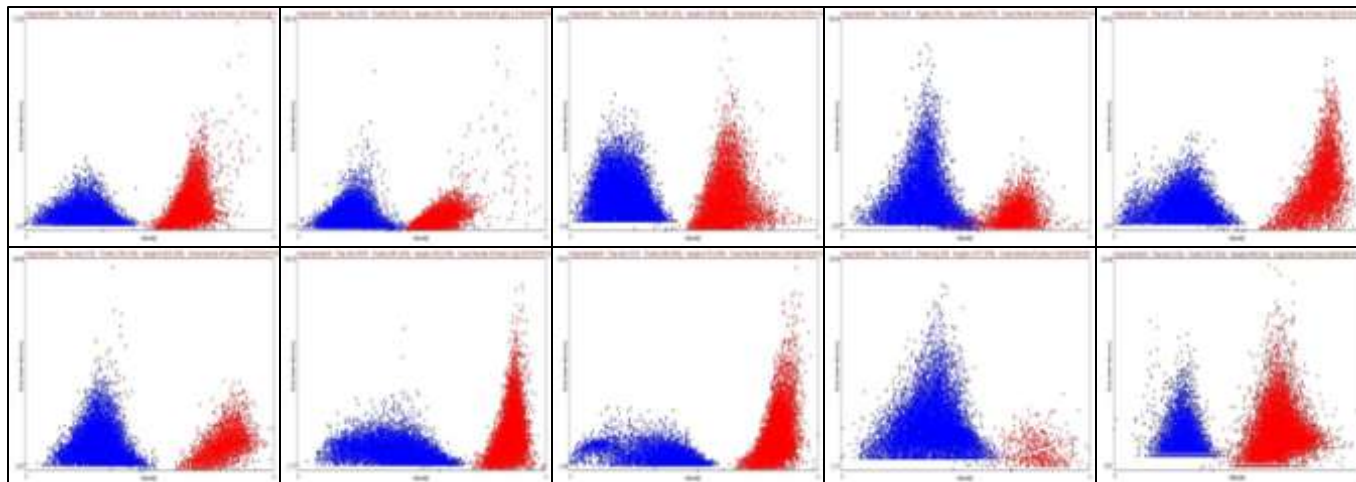
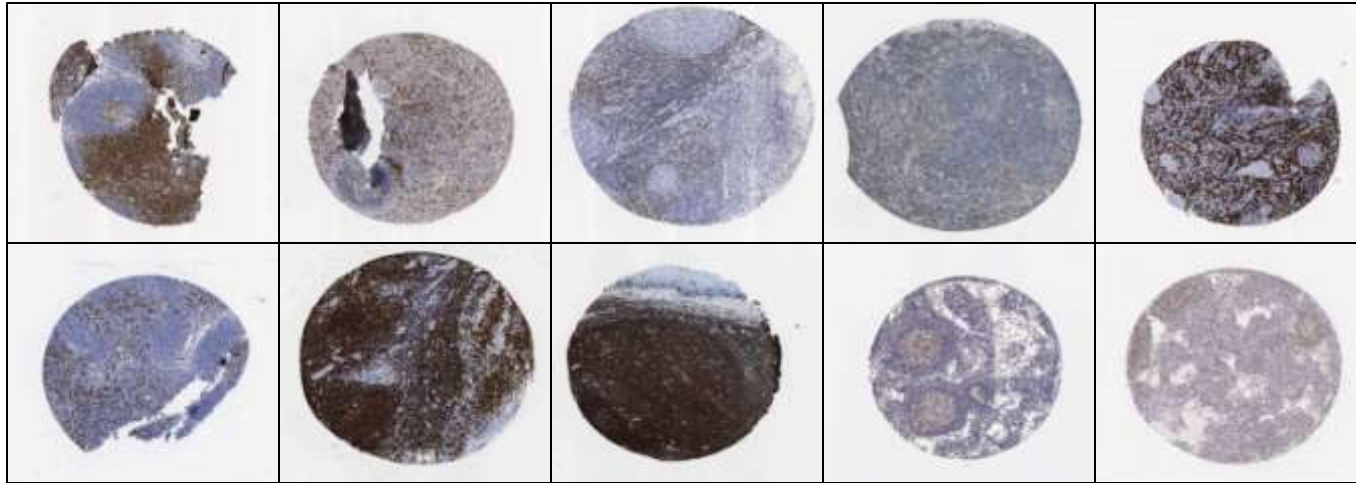


# Bone Marrow fibrosis Hematometrics STRATEGY



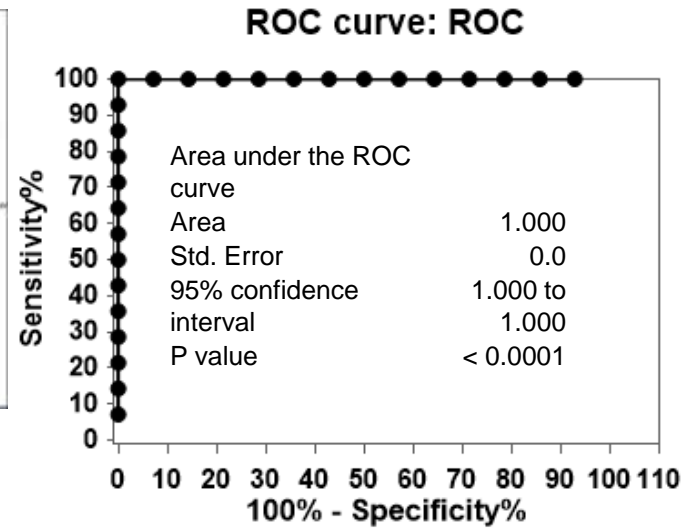
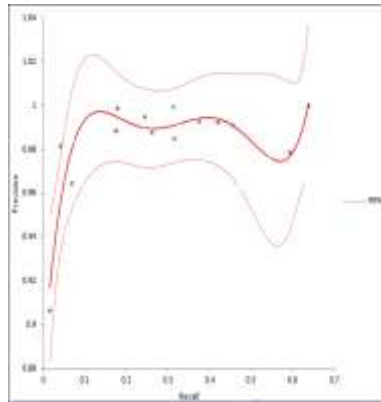
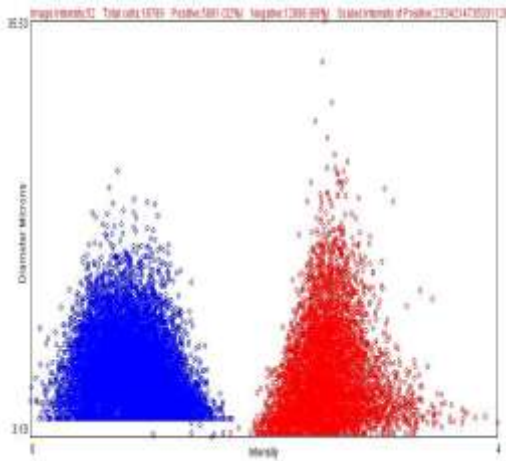


# Tissue microarray virtual flowHigh throughput analysis



Corresponding virtualflow tissue cytomics





— Linear Regression  
 — 95% Confidence Interval

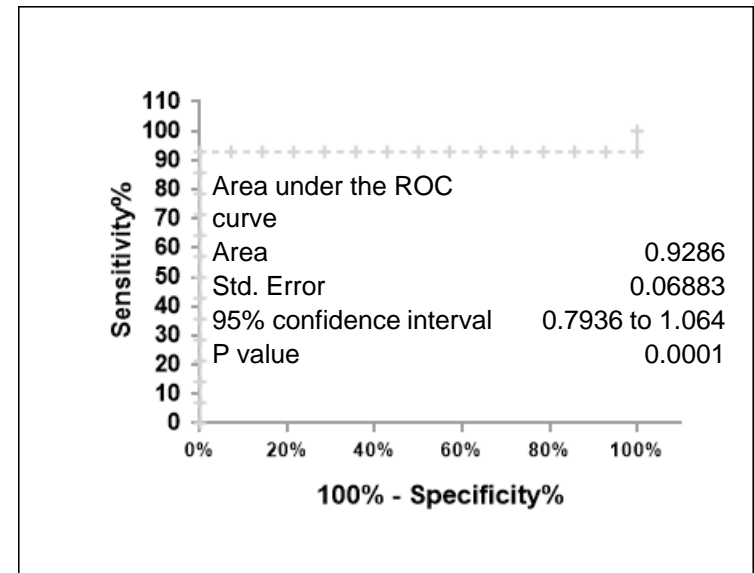
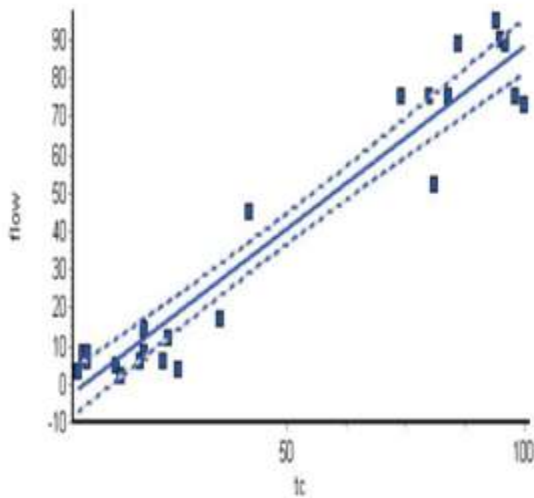
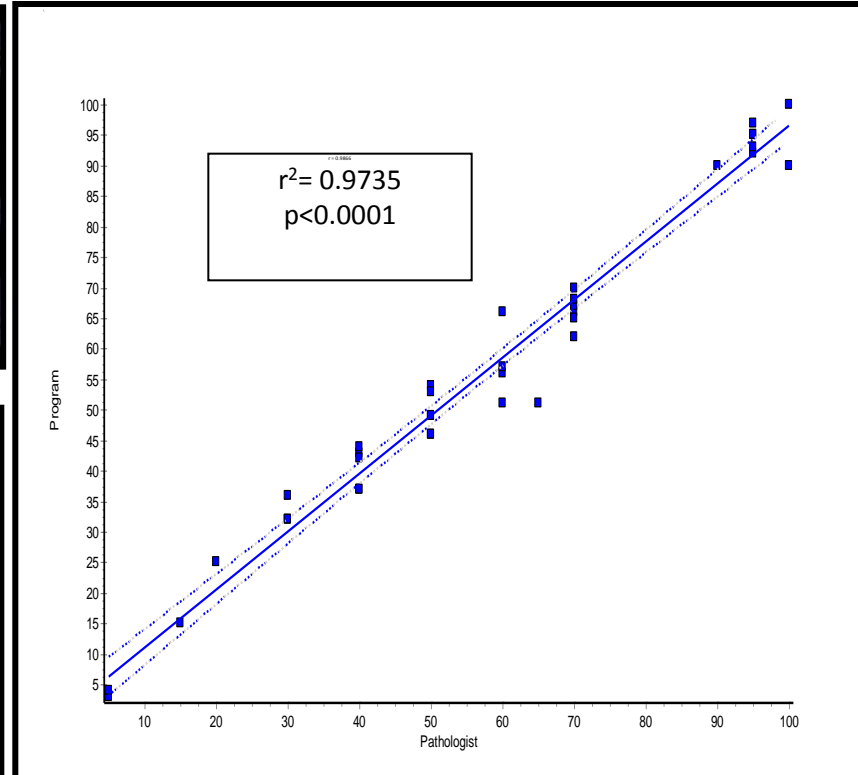
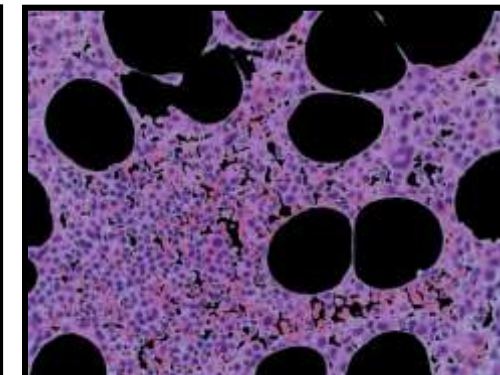
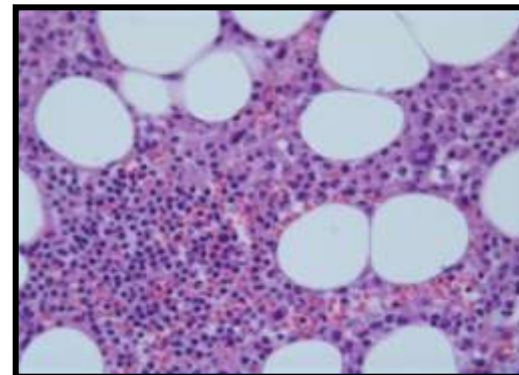
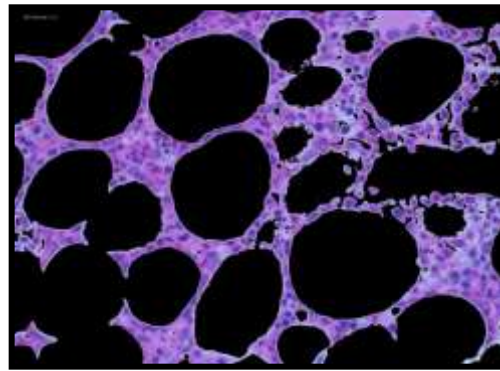
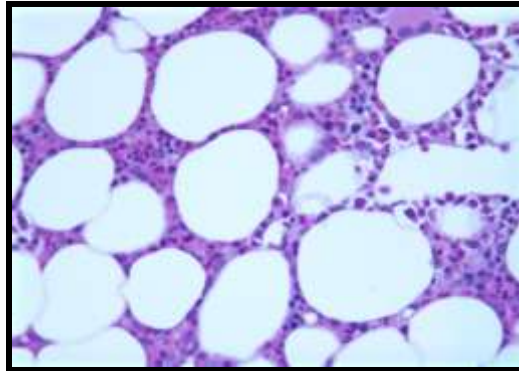
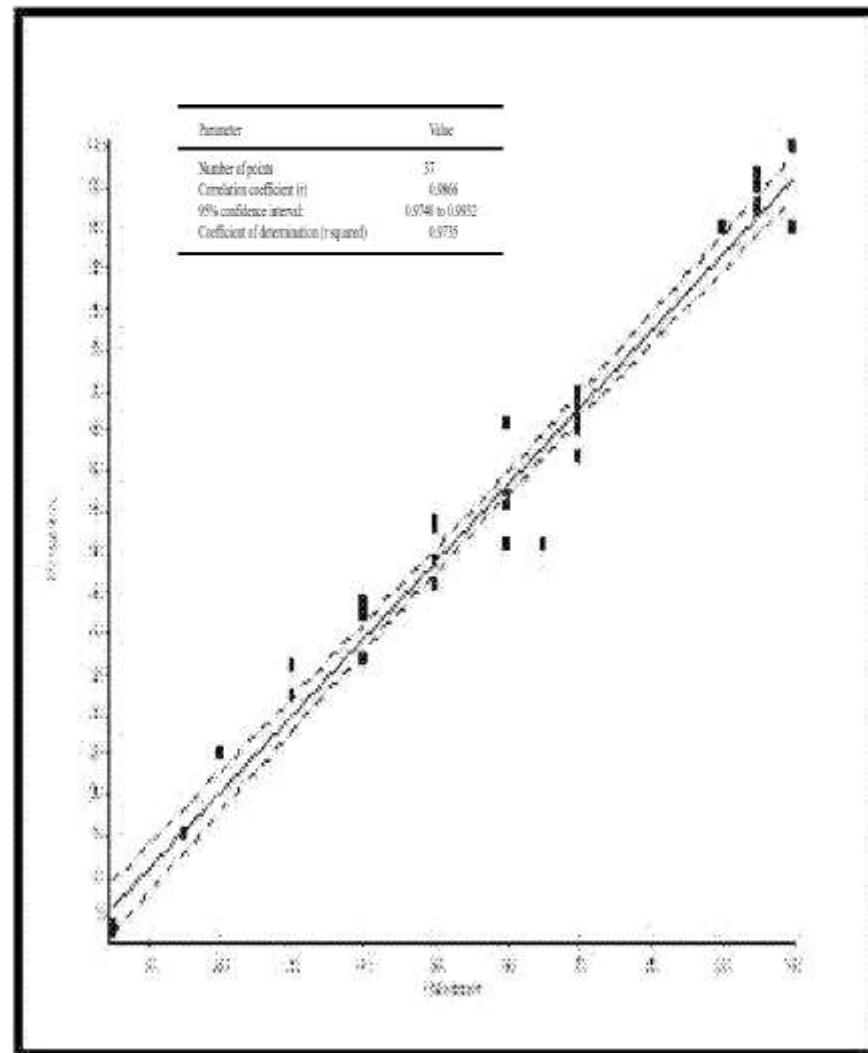
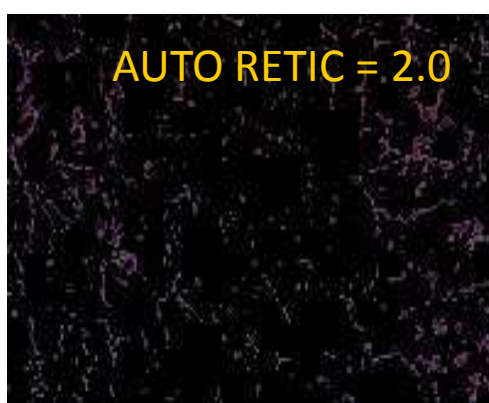
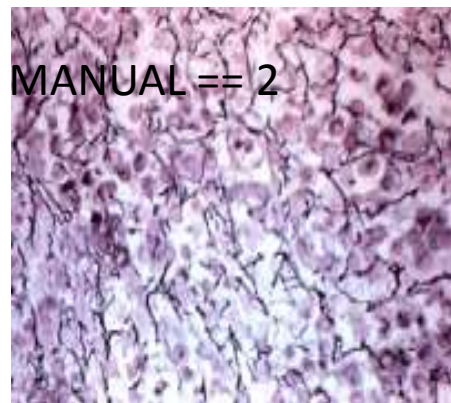
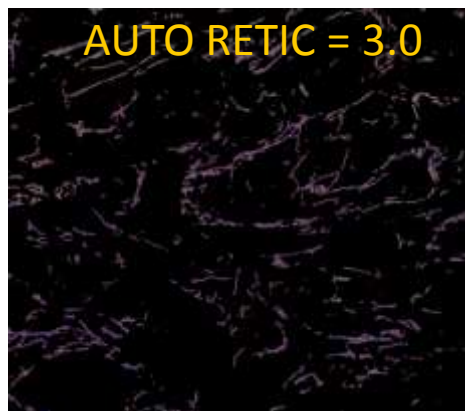
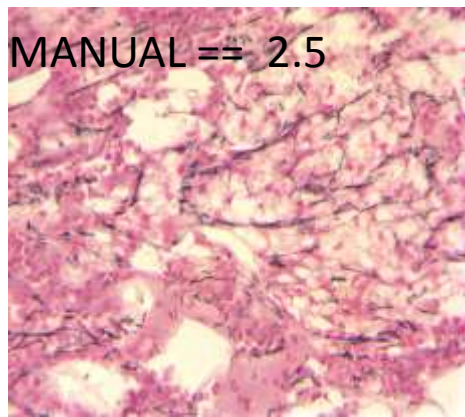


Fig. 3. The correlation coefficient and 95 % confidence interval by linear regression analysis yielded a high concordance between M ( $r = 0.93$ ), FC results ( $r = 0.9648$ ), and TC results,  $P < 0.002$ . [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

Results demonstrate positive strong correlation between the manual grading and computer grading using our test and control groups. The correlation was high with  $r$  0.9735 (nonparametric Spearman  $p < 0.0001$ ).



Results demonstrate positive strong correlation between the manual grading and computer grading using our test and control groups. In the test group, the mean reticulín manual grading is 1.8 (95% CI 1.58 - 2.07) vs AUTORETIC mean of 1.734 with 95% CI (1.47 - 1.99) with no significant difference between the mean (SD). The correlation was high with  $r$  0.98 (nonparametric Spearman  $p < 0.0001$ ).

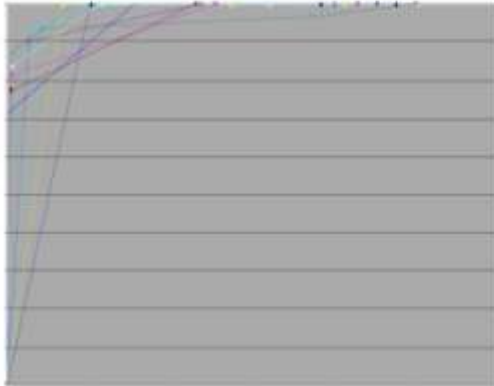


So in these limited examples,  
data could be obtained.

Is this a **better way than just eyeballing** and estimating metrics in everyday pathology work?

High disagreement without use of computerized image analysis.

ROC CURVE ANALYSIS OF 10 PATHOLOGISTS  
ASSESSING HERCEPTEST MANUALLY

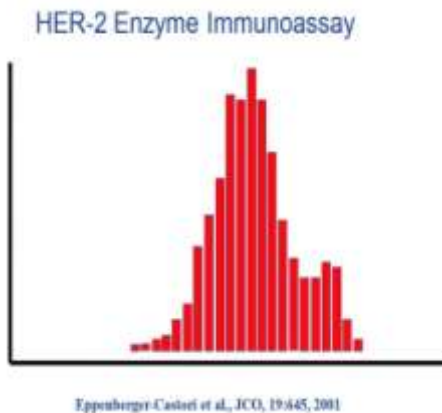


ROC CURVE ANALYSIS OF 10 PATHOLOGISTS  
UTILIZING AUTOMATED IMAGE ANALYSIS



Biomarker grading  
1+ to 4+ of same  
slides to 10  
pathologists  
without and with  
computer aid

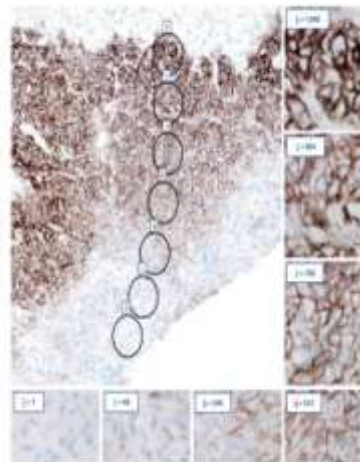
Gaussian in log x scale



Dynamic Range of DAB

Note the dynamic range of  
Integrated Brown / 10  $\mu\text{m}^2$ .

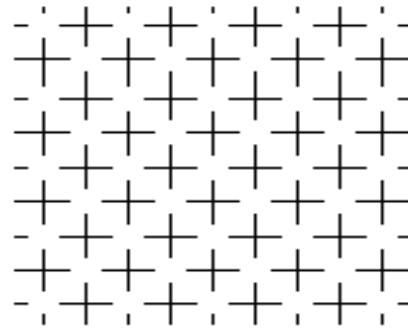
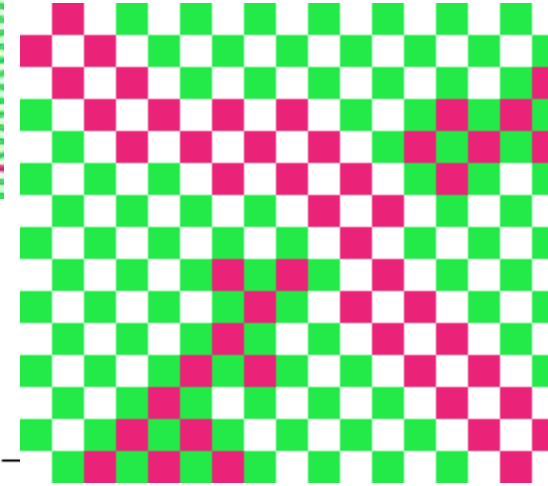
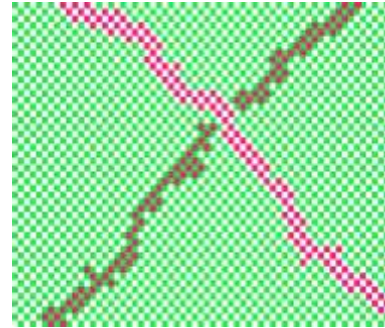
1	1068
2	864
3	764
4	633
5	246
6	48
7	1



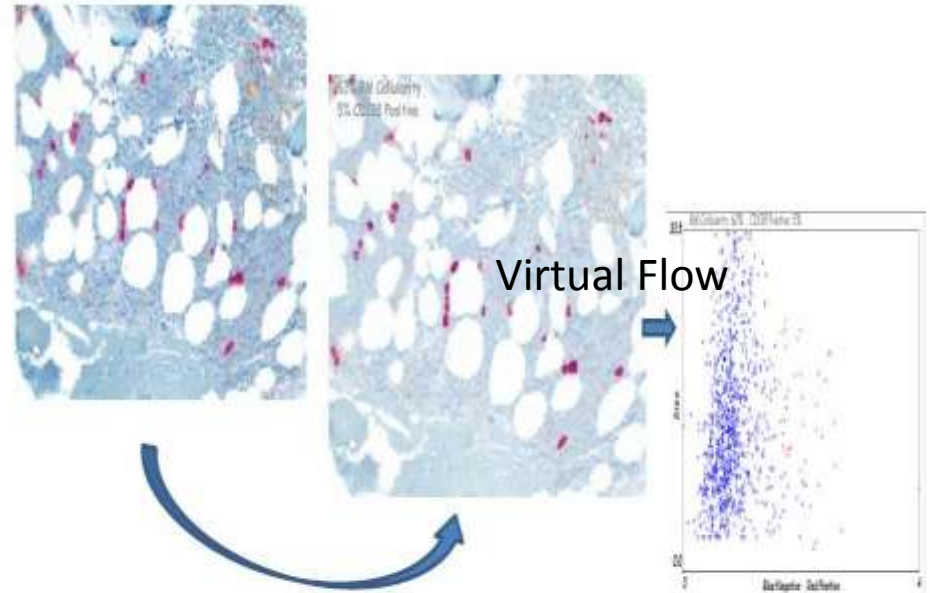
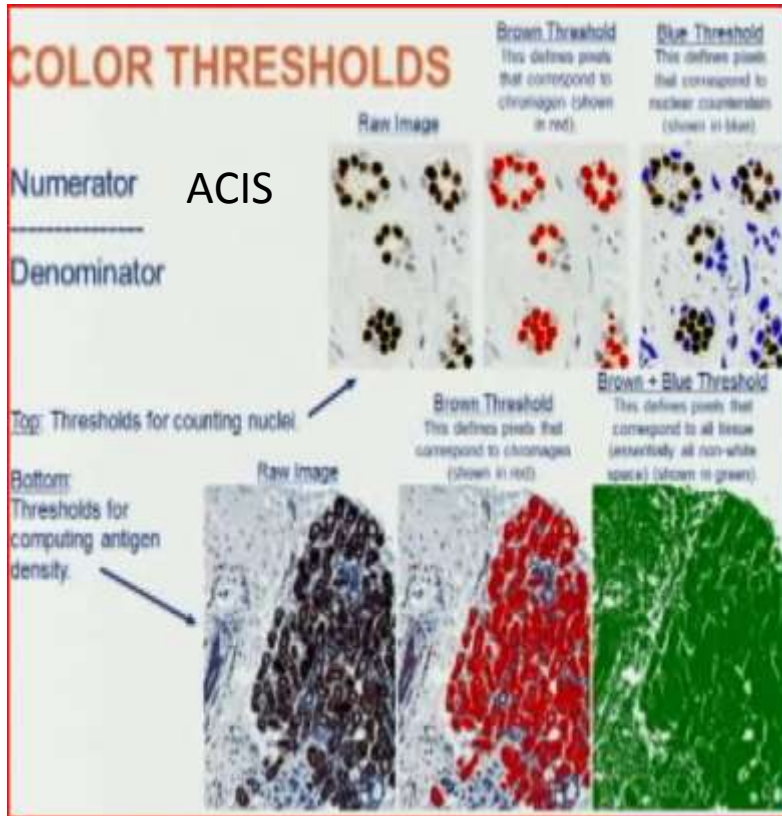
The low agreement  
is not the fault of  
pathologists, but lies  
from the limitation  
of our visual system  
We are good with  
linear scales but the  
brown staining are  
in logarithmic scale  
from 0 to 1 log

# Limitation of the human eye

1. Resolution is limited to 0.1 mm
2. Limited perception of intensity difference (can differentiate up to 40 grays ) in linear scale
3. Inability to separate hue from other components or susceptible to surround illusion
4. Perceive “false” contours and illusions



# How Virtual Flow/ Hematometrics differs from most commercially image analysis packages available?

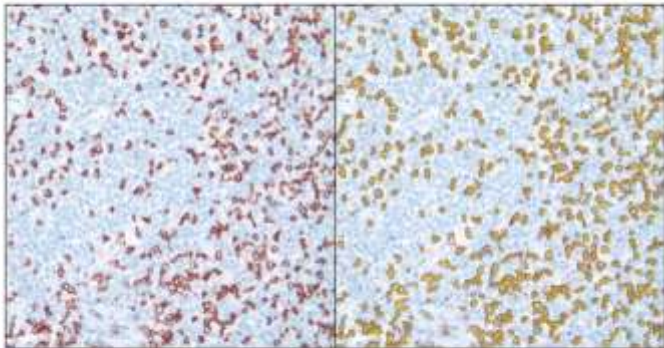


Most other systems are pixel and color ratio based, for antigen density

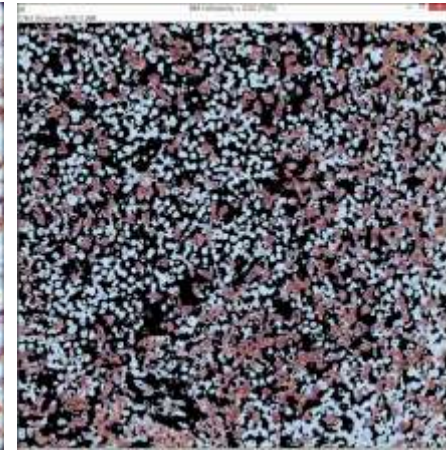
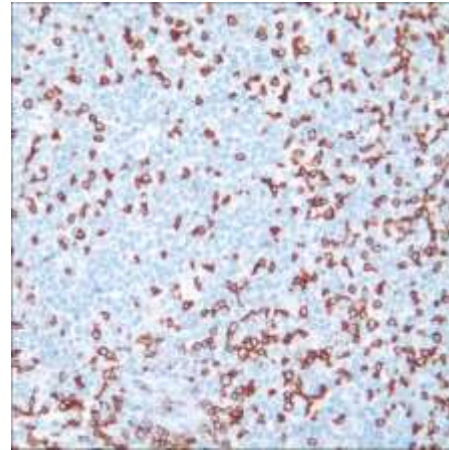
Virtual Flow/ Hematometrics is more suited to cell events counting, with ratios based on single cells or intracellular single cells.

# Comparison with area analysis

## CytoNuclear Module – CD8

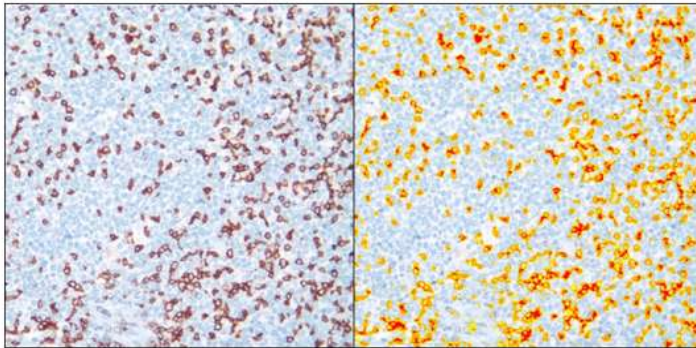


- Positive Cells shown in Yellow
- Outputs: Tissue Area, Positive Cells (weak, medium, strong), Average Nuclear Size (Average Cell Size in this example)
- 790 Positive Cells, 120,000um<sup>2</sup> Tissue Area



52%  
objects  
area

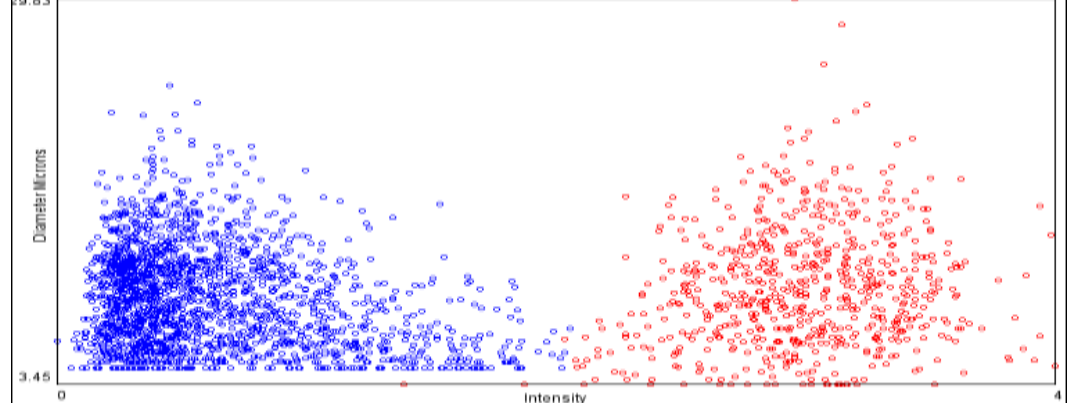
## Area Analysis Module – CD8



Positive Area indicated in yellow (weak), orange (medium), and red (strong)  
Outputs include: Total tissue area, Positive Area (weak, medium, strong), Average positive optical density  
21.7533% Positive Tissue Area



Image Intensity:31 Total cells:2659 Positive:673 (26%) Negative:1986 (74%) Scaled Intensity of Positive:2.989997148513794

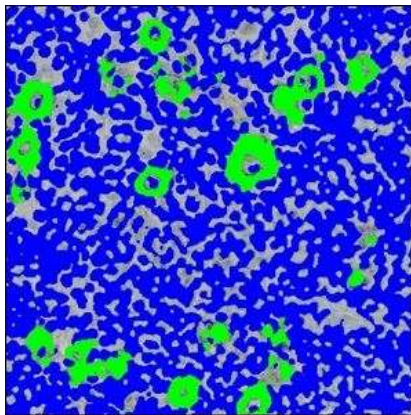
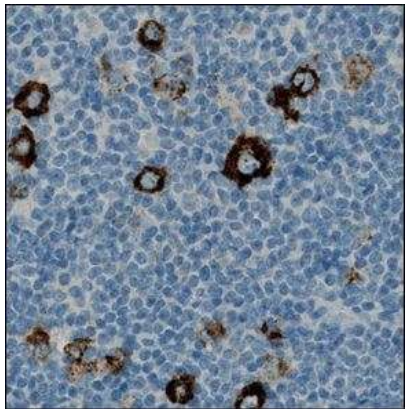




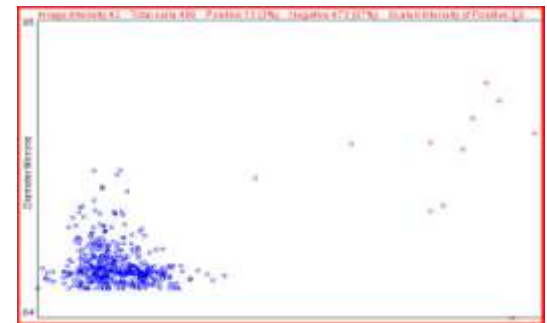
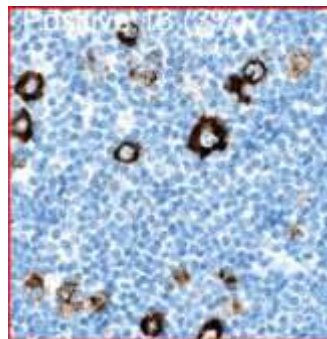
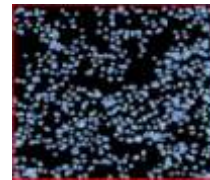
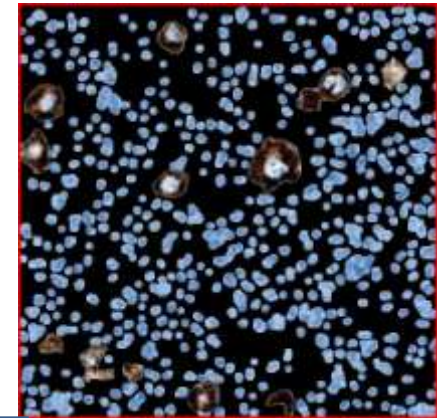
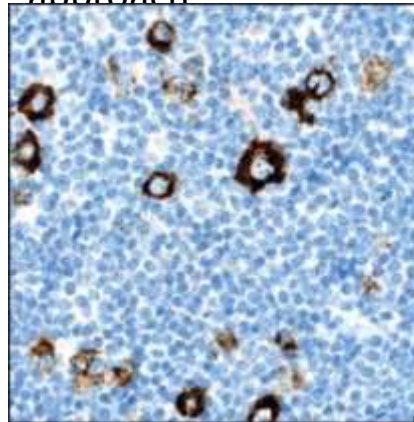
# Hodgkin lymphoma

## Area percent image analysis vs Positive and Negatively Stained Single cell Detection

Sample internet obtained image and area-based result (Company X)

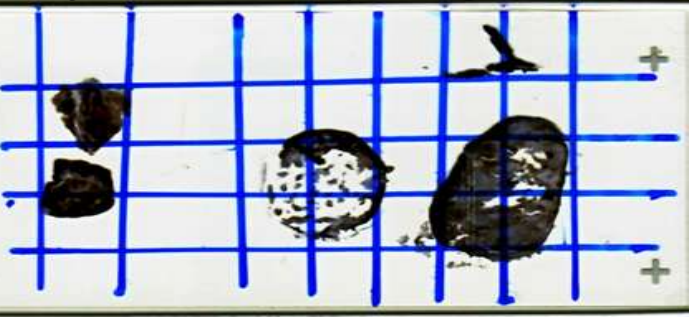


Comparison result using our cytomics approach

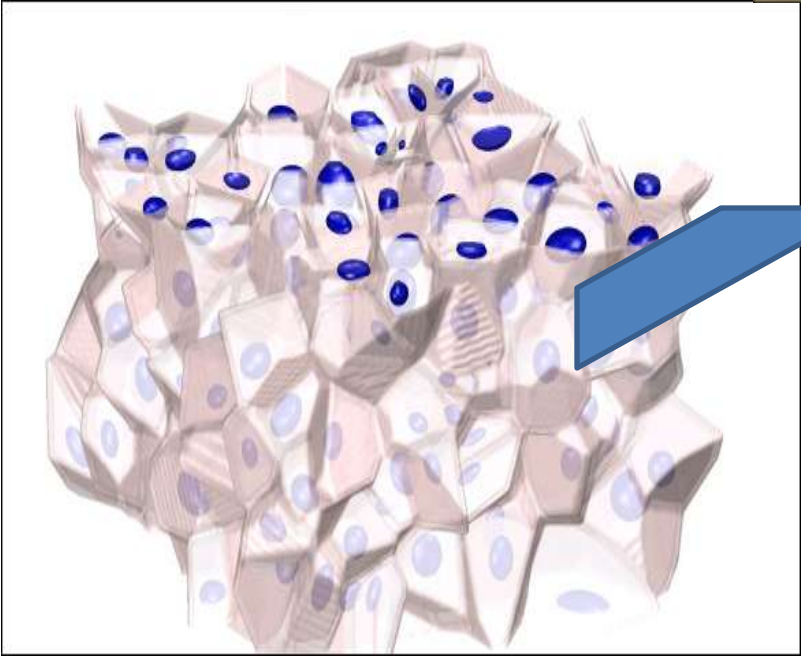
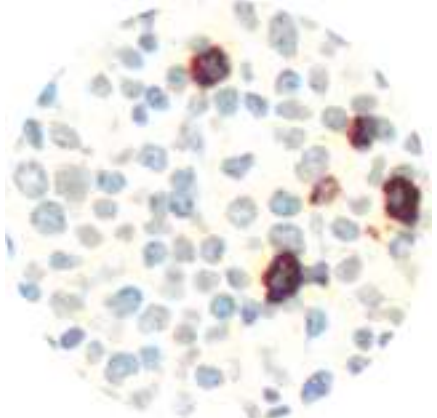
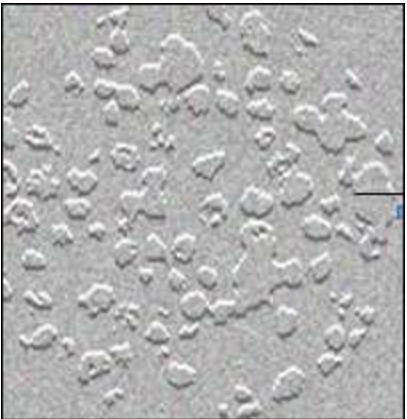


Virtual Flow Hematometrics will help in automating counting tissue markers or putting data on tissue as metrics. It is counting cell events, or subcellular objects or any target objects.

Lymph node marked with anti CD8 or T suppressor



5 um



Tissues are sections of cells that have 8 to 32 microns cell diameter

# DIGITAL PATHOLOGY AND VIRTUAL FLOW CYTOMETRY

WORKFLOW INTEGRATION WITH EASE  
EASY ACCESS TO AN APPLICATION  
LOW COST OF OWNERSHIP  
STANDARDIZED RESULTS AND UNIFIED EXPERIENCE

VFLOW + STORE+ SHARING + NETWORK + DATABASE =



APPS := VIRTUAL FLOW CYTOMETRY



SMART PHONE(sp) := WHOLE SLIDE IMAGING

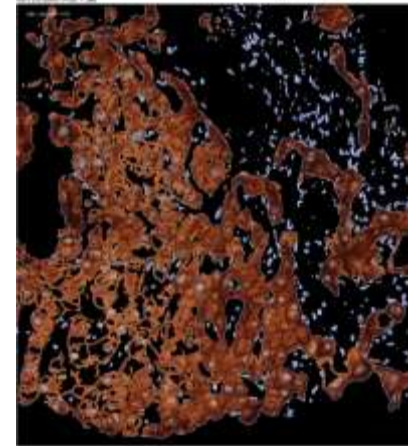
# App: Traditional vs Computational Molecular Pathology

Image analysis cancer %= 43

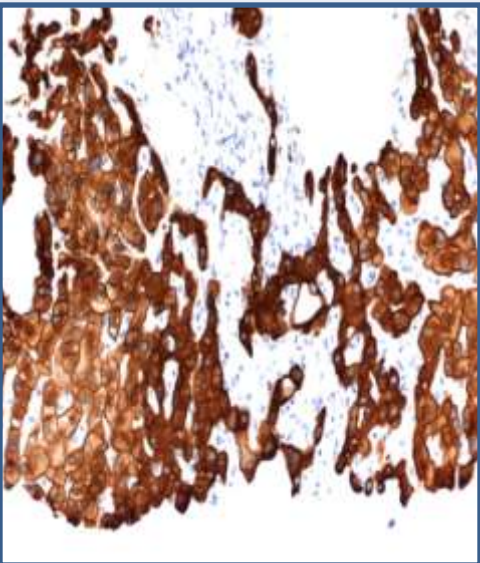
An 86-year-old woman presents with a lump in her right clavicular area. Relevant history includes prior bilateral breast cancer, a history of smoking, and a brother who died of colon cancer. She has no other symptoms, but has a suspicious skin nodule in her lower right quadrant. A PET/CT scan revealed activity in the retroperitoneum, left common iliac chain adenopathy, and nodular soft tissue along the left pelvic and adnexal regions.

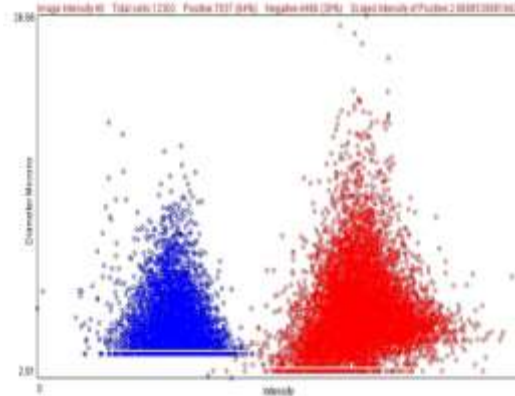
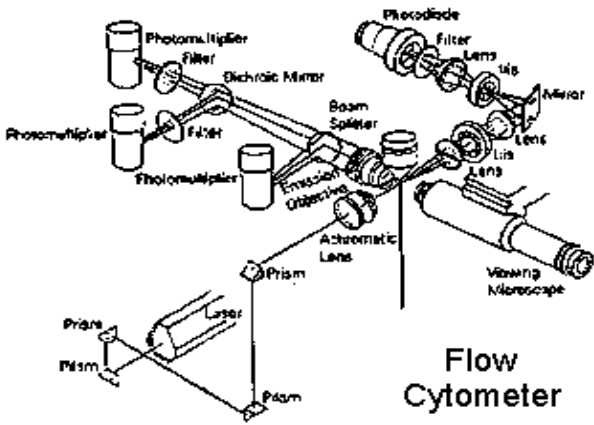
**DIAGNOSIS:** Metastatic squamous cell carcinoma; possible primaries include lung and breast.

CK7	Focally Positive
CK20	Negative
TTF-1	Negative
ER	Negative
PR	Negative
Mammaglobin	Negative
S100	Negative
WT1 N-terminus	Negative
CA125	Focally Positive
CEA (P)	Positive
SYN	Negative
CA 19.9	Rare cells positive
CDX2	Negative
GCDFP-15	Negative
AE-1/AE-3	Positive
MOC 31	Rare cells positive
p63	Positive
SP-A	Negative



BRAF and KRAS DNA sequencing requires **at least 20% tumor in tissue**- then a go for Sanger or Next generation DNA sequencing

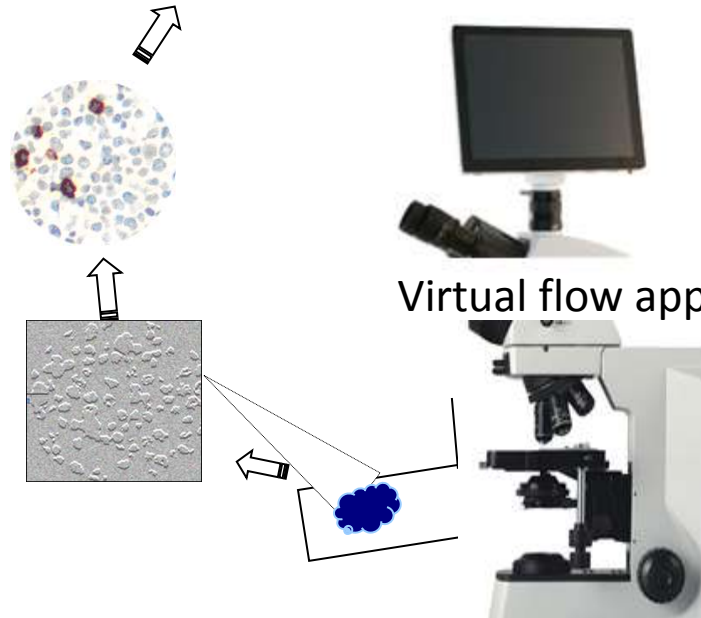
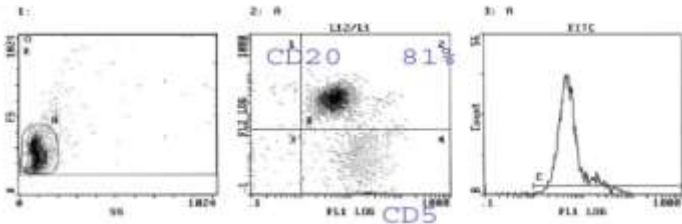




**HEALTH ALLIANCE LABORATORY SERVICES**

COULTER® EPIC300 Luminescence Flow Cytometry Report  
 D:\NOV90\NODEBA\A057124.LMD\_XL.W34131.Run base protocol

OP ID: RAH



# “Virtual Flow Cytometry” of Immunostained Lymphocytes on Microscopic Tissue Slides: iHCFLOW™ Tissue Cytometry

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US Patent No: 7,899,624

Virtual flow cytometry on immunostained tissue-tissue cytometer

4 ALSO PUBLISHED AS: 20070020697

RESEARCH APP BIOMARKERS

NOT ANTIGEN QUANT

For CLIA LAB  
DIAGNOSTICS  
Or TISSUE METRICS

Works with any  
microscope with any CCD



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## QUANTITATIVE MORPHOLOGICAL AND MOLECULAR PATHOLOGY OF THE HUMAN THYMUS CORRELATE WITH INFANT CAUSE OF DEATH

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## Virtual Flow cytometry in Pathology

There is a **better way to just eyeballing** and estimating metrics in everyday pathology images using digital pathology and virtual flow cytometry.

Virtual Flow does true counting with ratios based on single cells, the positive over total cells ( positive and negative cells) to generate a dual population dot plot parametric displays which is next generation above area analysis.

The virtual flow extend to hematometrics or to oncology metrics using a novel workshop paradigm applicable to wide variety of any color digital images to create custom final segmentation results to get data from pictures and to push traditional pathology to a next generation computational pathology .

# Computational Pathology

- The advances may help in augmenting traditional pathology into computational pathology in line with precision medicine and genomics.



## PATHOLOGY SOLUTIONS

### Consultant: Hemani Cualing MD

ABP Board Certified in Hematopathology  
 ABP Board Certified in Anatomic and Clinical Pathology

Fellowship Trained in Hematopathology and Surgical Pathology with over 30 years of pathology experience

Univ of South Florida Dept of Pathology/Cell Biology

Cutaneous Lymphoma Cooperative Group, Cincinnati, OH and Tampa, FL

IHCFLOW is a company specializing in diagnostic hematopathology as well as interpretation of IHC (ImmuNoHistoChemistry) and FLOW Cytometry results to achieve the most accurate and timely diagnosis.

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Please include the billing information and address of consultee. A set consultation fee is charged to the consultee pathologists or office (per consult).

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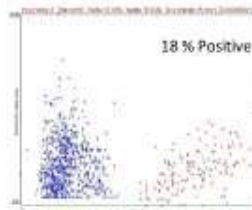
Ki-67 IHC stain



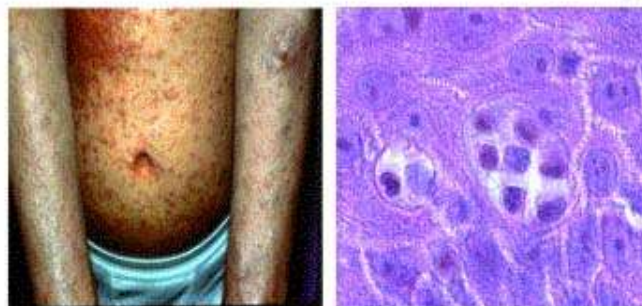
VirtualFlow



Positive Cells



18% Positive



IHCFLOW, Inc. is also a company that seeks to advance diagnosis through **Hematometrics**: digital tools to quantify pathology microscopic images such as **VirtualFlow™**.

Hematometrics are computerized tools to help pathologists. These tools are technology at work for pathologists. They are like microscopes, aiding the pathologist to see the underlying metrics of tissue special stains.

The intelligent guess or estimate that is used currently in diagnostic microscopy interpretation of tissue diagnosis could be made more accurate and precise by hematometrics such as **VirtualFlow™**. Diagnosis is made by pathologists. Results for display only. We have modules to analyze bone marrow cellularity and bone marrow fibrosis, immunofluorescence targets, carcinoma burden quantification, lymphoid stains subset analysis, and even counting tumor cells like Reed-Sternberg cells.. all from paraffin stained slides.

SALES: [WWW.IHCFLOW.COM/virtualFlow™](http://WWW.IHCFLOW.COM/virtualFlow™)



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Hematometric

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 10000

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HEMATOPATHOLOGY

DIAGNOSTICS

