Technology Solutions for RESEARCH

'Virtual' flow cytometry: objective counting of cells licensed to JMC Surgery ENT Dr. Luginbuhl for easy counting of stromal cells

A new method could provide an important quantitative tool for research scientists and clinical researchers who need a tool to count cells in an automated rapid and objective manner.

Recently, a medical student interning with Dr. Adam contacted IHCFLOW and presented to us a problem common in research facilities: counting immunostained cells in about a thousand of microscopic slides. Not daunted by the task of manually counting, the student nevertheless ask the question, is there a better way to go through the slides in a systematic, objective, real time operation short of manually drawing grids on the glass slides and counting cells inside the grids. They were interested in counting Heme Oxidase and VEGF positive cells, see below for some results.

On Feb 21, 2012, he sent us this email:

"My name is Giuseppe and I am a medical student researching at Jefferson Medical College. I am looking for software solutions to analyzing the cell number on virtual slides stained with IHC . Can your product analyze cells stained with H&E, and with DAB staining? I need a simple positive cell count. Do you have a trial software that I could use to see if it would work on my slides. I have 1000 (approx) slides that need to be analyzed for a cell count. There is some variance in terms of background and over-staining.

He sent us 4 images to play with initially and subsequently we trained the virtual Flow with more images and a sample data validation with images shown below after several iteration of custom software training and revalidations. After validation, the department acquired the license to use the technology for its research on July 2012.

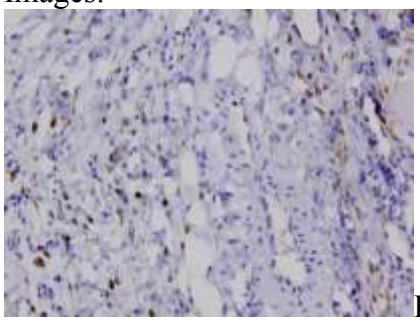
Data Validating manual and virtualFlow counts

TESTS ANTIBODIES TO STROMAL CELLS : HEME OXIDASE (HO) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

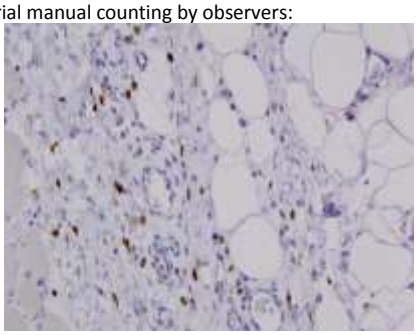
> HO Test 1: My count = 96, Vflow count = 88
> HO Test 2: My count = 53, Vflow count = 58 minus 5
> HO test 3: My count = 83, Vflow count = 84 minus 1
> HO Test 4: my count = 139, Vflow count = 138 minus 1
> HO test 5: my count = 14, Vflow count = 11 minus 3
> VEGF test 1: My count = 168, Vflow count = 169 minus 1
> VEGF test 3: My count = 0, Vflow count = 0 minus 0

With low COV and SD, 95% CI 0 to 3 difference. See images below for trial manual counting by observers:

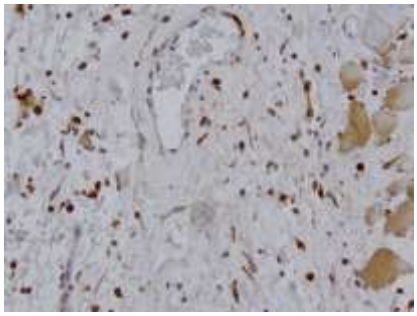
Images:



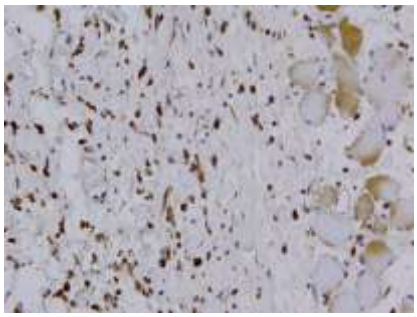
HO 1



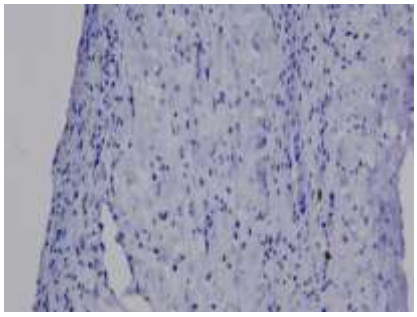
HO 2



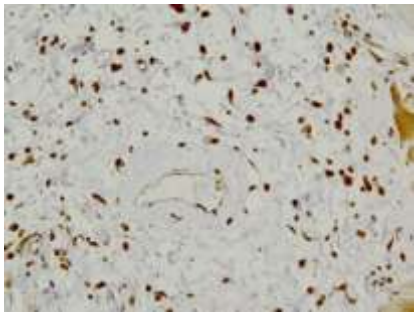
HO 3



HO 4

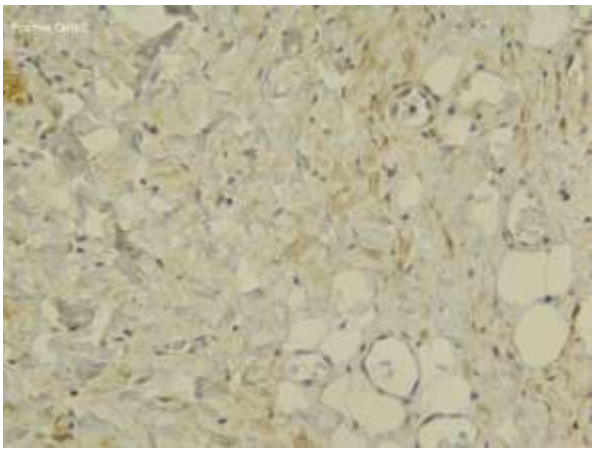


HO 5

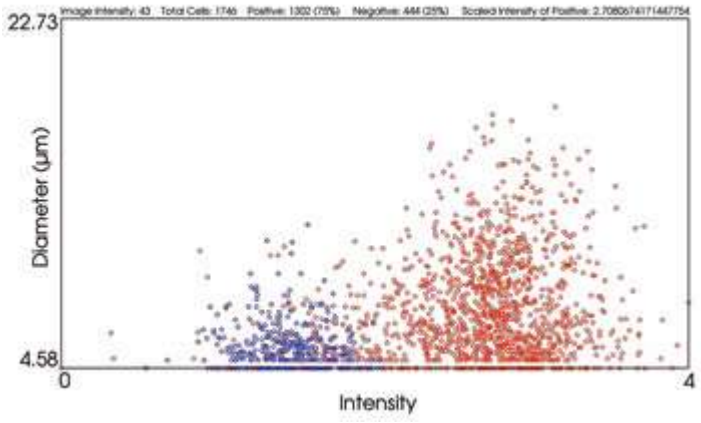


VEGF 1

VEGF3 counts



Another example initially published by PHOTONICS Gary Boas, for detecting lymphocytes in lymphomas:
As reported in the Jan. 15, 2007 issue of *Cytometry Part B*, the method converts immunohistochemistry data to a two-parameter dot-plot display, much like those found in flow cytometry. Thus it provides a sort of "virtual" flow cytometry, offering the objectivity of the latter method but with relatively inexpensive tools that are readily available to most researchers.



The software developed by the researchers generates a two-parameter dot-plot display, similar to those found in flow cytometry, offering the objectivity of the latter method but with tools generally available to hematopathologists and other clinicians.

Immunohistochemistry techniques typically do not provide population-based statistics, with positive and negative cell counts. "The current paradigm is to calculate the antigen density in particular unit areas, or 'hot spots,'" said Hernani D. Cualing, "This method departs from that paradigm by looking at tissue cell by cell. It provides cell-based analysis."

To be able to extract flow cytometry-like results from immunohistochemically stained cells, the researchers had to address a number of issues Other groups have reported methods of separating positive stain from nonstained pixels, thus obtaining objective information from stained tissue samples. Some have described hybrid microscope flow cytometers, for example; others have applied immunofluorescence techniques on slide substrates and used laser scanning instrumentation to acquire information about cell populations. Such approaches typically require complex —and expensive — technology not commonly found in pathologists' and oncologists' offices and labs.

Researchers have developed a method for converting immunohistochemistry data into objective, flow cytometry like when counting cells by using immunohistochemically stained slides — such as the one shown here, with red AEC staining of cancer lymphocytes with Hematoxylin counterstain.