—Photogravure—

Multispectral Imaging of Pancreatic Mixed Acinar-neuroendocrine-ductal Carcinoma with Triple-immunoenzyme Staining

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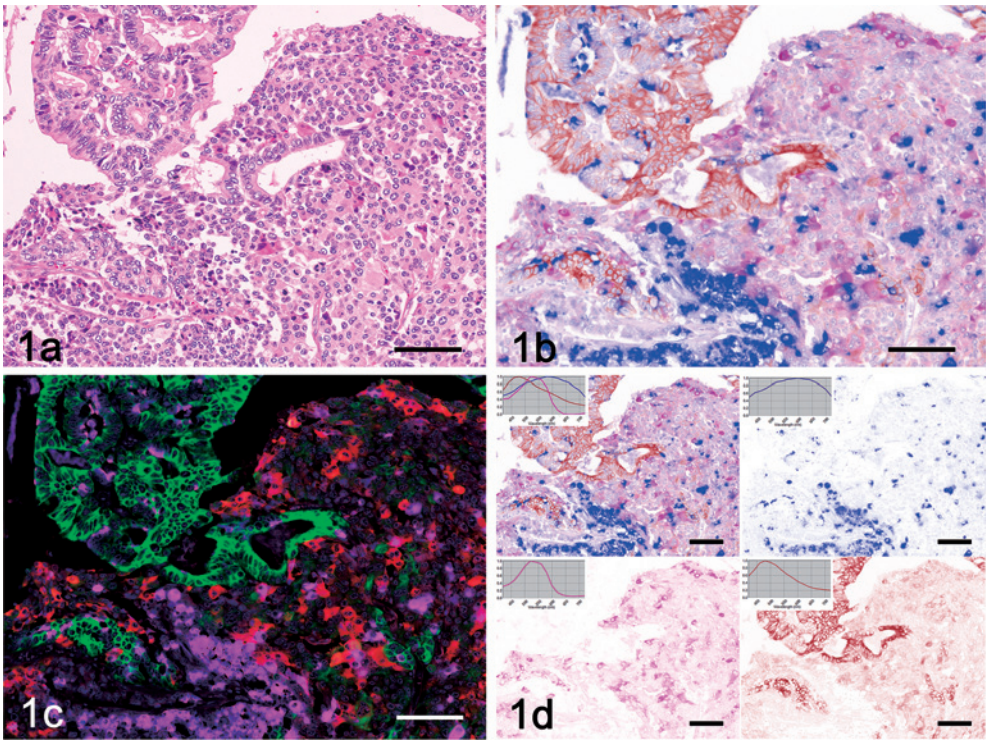

Fig. 1

Multiple immunoenzyme staining is a useful technique for visualizing the localization of multiple antigens using different chromogens on a tissue section. However, when the multiple antigens localize in the same cells, the mixed colors are indistinguishable to the human eye and color red-green-blue cameras. Multispectral imaging is a new technique that can solve this problem[1,2]. Pancreatic mixed acinar-neuroendocrine-ductal carcinoma is an extremely rare neoplasm consisting of 3 cell components with acinar, neuroendocrine, and ductal differentiation (**Fig. 1a**)[3]. **Figure 1b** shows a pseudocolored composite image acquired from a specimen stained with trypsin (acinar marker, blue), synaptophysin (neuroendocrine marker, magenta), and cytokeratin 7 (ductal marker, brown) using a multispectral imaging system (Nuance, PerkinElmer Inc., Waltham, MA, USA). The system acquired the

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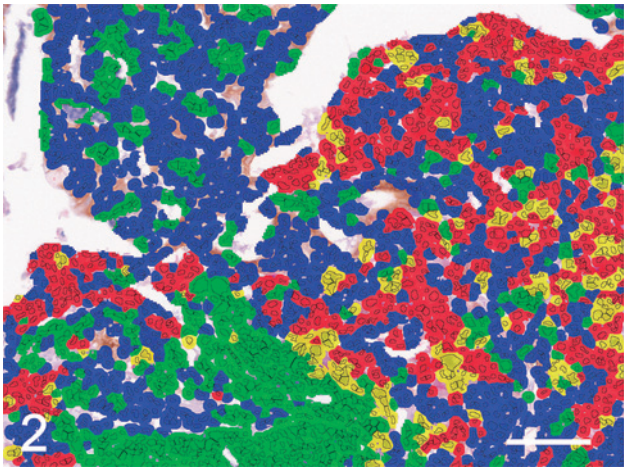

Fig. 2

individual spectra of 3 chromogens (5-bromo-4-chloro-3-indolyl phosphate [BCIP], new fuchsine, and 3,3′-diaminobenzidine [DAB]) through a set of tunable liquid crystal filters. On the basis of the spectral data sets, the colors can be converted into fluorescent images for optimal visual contrast (**Fig. 1c**). The acquired image can also be spectrally unmixed into 3 individual images displaying only 1 chromogen data (**Fig. 1d**). Morphological and cellular phenotyping analysis by inForm software (PerkinElmer) revealed that 30.93% of tumor cells were trypsin-positive (green and yellow), 31.89% were synaptophysin-positive (red and yellow), 7.54% were double-positive (yellow), and 44.73% were double-negative (blue). A total of 2,493 cells were present (**Fig. 2**).

Fig. 1 (a) Hematoxylin and eosin staining of pancreatic mixed acinar-neuroendocrine-ductal carcinoma. (b) Pseudocolored composite image (Nuance) acquired from triple-immunoenzyme staining with trypsin (mouse monoclonal antibody, MAB1482; Millipore, Temecula, CA, USA) in blue (BCIP), synaptophysin (rabbit polyclonal antibody; DAKO, Glostrup, Denmark) in magenta (new fuchsine), and cytokeratin 7 (mouse monoclonal antibody, clone OV-TL 12/30; DAKO) in brown (DAB). (c) The image in (b) was converted to a pseudofluorescent composite image with trypsin (magenta), synaptophysin (red), and cytokeratin 7 (green). (d) Composite image (upper left) and spectral unmixed images (upper right, trypsin; lower left, synaptophysin; lower right, cytokeratin 7). The spectral curves of the chromogens are shown in the top left corners of individual images. Scale bar=100 μm.

Fig. 2 Automated cellular segmentation using inForm software. Green indicates trypsin-positive cells, red synaptophysin-positive cells, yellow double-positive cells, and blue double-negative cells (total cell number: 2,493). Scale bar=100 μm.

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