

inForm software allows image analysis of proprietary PerkinElmer images taken with Nuance, Maestro, TRIO, or Vectra, along with standard image formats such as .tif, .jpg and .png images.

Below, we've highlighted the new features of inForm 2.1.

- ▶ Spectral Library Development
- ▶ Stain Selection
- ▶ Synthetic Spectra
- ▶ Stain Management
- ▶ Adding Markers
- ▶ Pathology Views
- ▶ Cell Phenotyping
- ▶ Cell Segmentation Improvements



## ► Spectral Library Development

Within the Build Libraries tab, inForm can automatically create spectra from your singly stained samples.

Load any image taken with Mantra or Vectra 2.0.7, select the chromogen or fluorophore, and click extract!

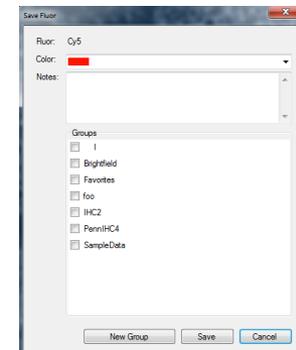
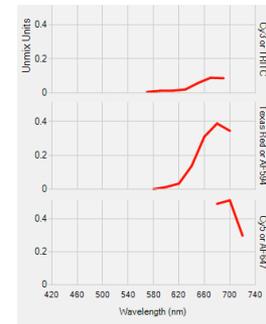
View your extracted spectrum in the spectral chart; if you like it, save it to the store.

You'll be able to choose a display color, add any notes, and add the spectrum to any groups you may have before saving it.

Load Image:

Sample Format:

Fluor:



Save Fluor

Fluor: Cy5

Color:

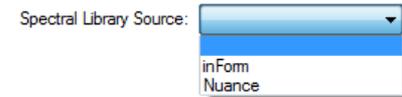
Notes:

Groups

- 1
- Brightfield
- Favorites
- foo
- IHC2
- PerkinHC4
- SampleData

## ► Stain Selection

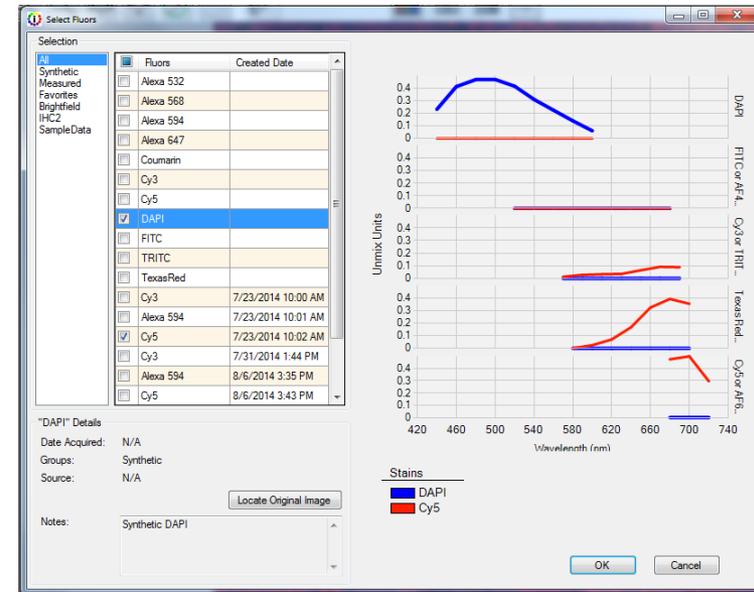
Use your newly created stains in an inForm project or algorithm. Start by loading an image taken with Mantra or Vectra 2.0.7, then choose inForm for the library source.



Select your stains. inForm will only show stains that are compatible with your imagery.

You can narrow your stain list down by group, and see the details of any stain you choose.

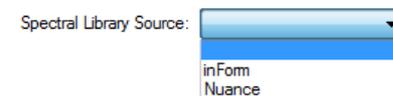
If needed, select autofluorescence directly from the image. We recommend using an unstained tissue sample.



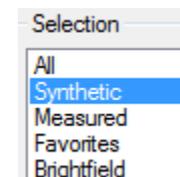
## ► Synthetic Spectra

For fluorescent images acquired with Mantra or Vectra 2.0.7, inForm provides synthetically made spectra. These spectra are mathematically generated from the expected response of the fluor with the Perkin Elmer lamp and filter sets.

While singly stained samples are ideal, they aren't always available. Synthetic spectra will allow you to quickly see what's present in a multiplexed sample. With an image loaded, choose inForm as your library source.



Narrow your selection by selecting the "Synthetic" group. Only synthetic spectra will appear.



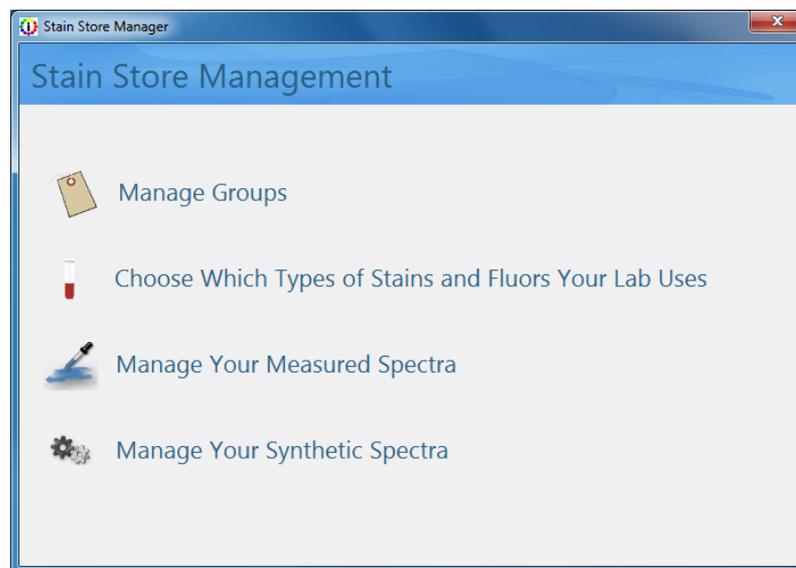
When you're done selecting spectra, choose an autofluorescence sample directly from the image.



## ► Stain Management

inForm lets you control which stains and fluors appear for everyday use. With the new Stain Store, you can

- Manage Groups to keep your study's spectra together and easily identifiable.
- Choose the stains and fluors that your lab works with commonly
- Organize the stains and fluors that you've measured from singly stained samples
- Organize the PerkinElmer fluorescence synthetic spectra



## ► Adding Markers

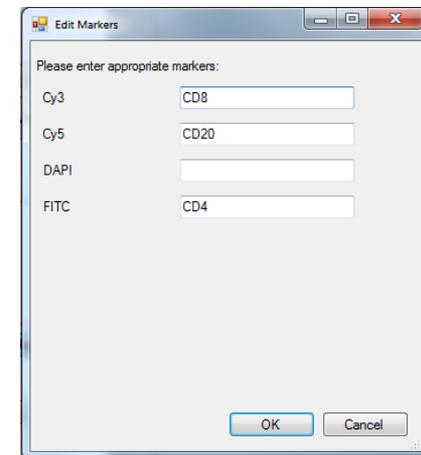
Need to remember which spectra are bound to which proteins? You can add markers to your spectra, which are saved on a per-project basis.

In the Prepare Images editor, click Edit Markers after you've selected your library.

Add the relevant proteins for this set of images. It's ok to leave some blank. When you click ok, the library names will be updated to reflect the new information.

Note: inForm treats changed markers as if it were a new library. We suggest setting your markers first before continuing on to other processing steps.

Edit Markers...

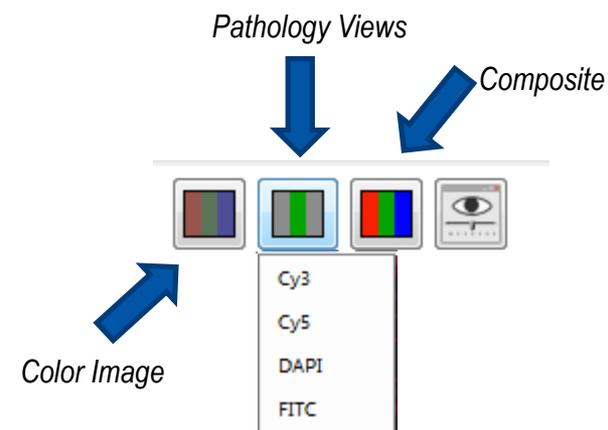


## ► Pathology Views

You can display your multiplexed fluorescent imagery as if it was a set of Hematoxylin + DAB serial sections with Pathology Views. By default, all newly created fluorescence inForm projects contain Pathology Views.

Pathology Views are available via our new toolbar shortcuts. Select any component to view it as a H+DAB image. If there is an autofluorescence component, it is displayed to look like an H&E image.

To convert existing projects to use Pathology Views, you must change the settings in the View Editor. Select any component view, and display it as Brightfield.

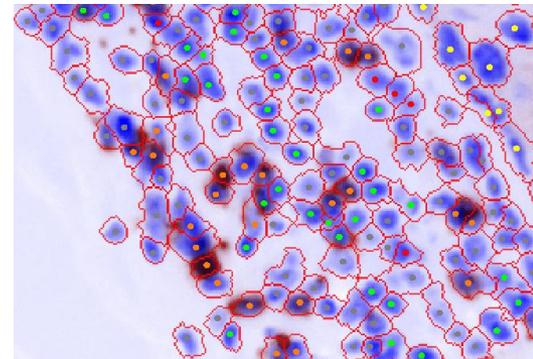
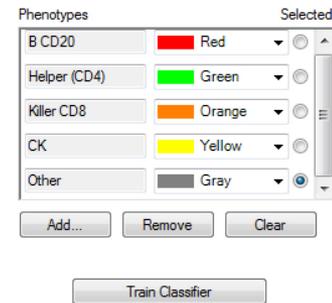


## ► Cell Phenotyping

With Cell Phenotyping, you can train inForm to identify cell types based on their staining and morphology. Cell Phenotyping is optimized for cancer immunology with PerkinElmer's OPAL staining.

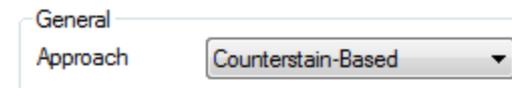
Identify cell phenotypes and set examples. Then train the classifier. Once a classifier is trained, it can be used on any similar images.

inForm will assign phenotypes to all cells in an image, and adds a phenotype column to its cell data tables.



## ► Cell Segmentation Improvements

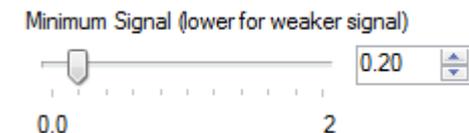
inForm has a new counterstain-based nuclear segmentation algorithm. To try it out, select the Counterstain-Based algorithm type in the cell segmentation settings.



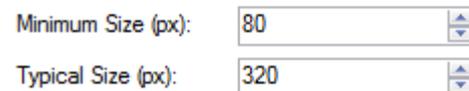
Choose your counterstain and process the image. We've found it's easier to adjust when you know where the algorithm is starting from.



If the algorithm is picking up too many or too few pixels in the nuclear mask, adjust the Minimum Nuclear Signal.



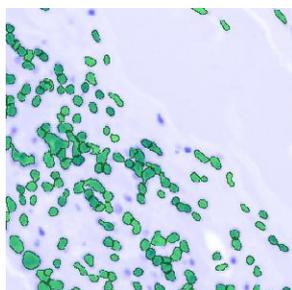
Set your minimum and typical nuclear sizes to help the algorithm determine initial splitting parameters. Use the Data View Tool if you need help determining those sizes.



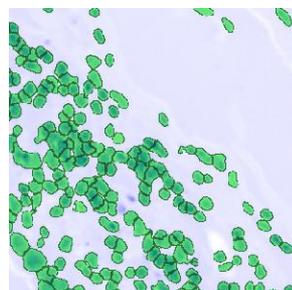
## ► Cell Segmentation Improvements, Continued...

If you need to split more or less, adjust the splitting.

Once you have good nuclei, you can finalize their shape by having them grow or shrink.

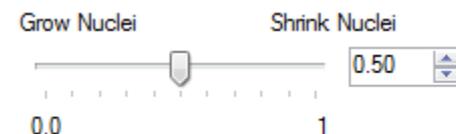
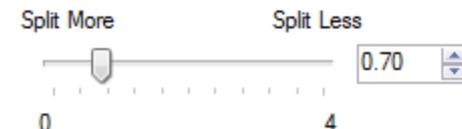


Shrunken nuclei



Grown nuclei

If you have good membrane staining, this can aid in your splitting. We suggest undersplitting in the initial algorithm, then turning on membrane splitting for best performance.



Use Membrane Signal To Aid Segmentation