myFX 2014 v1.1.0

User's Guide

April 2014

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1 About myFX

What is myFX?

We have developed biologist-centric software that contains computational methods to automatically process and analyze images depicting embryonic gene expression in the fruit fly *Drosophila melanogaster*. It facilitates creating digital descriptions of spatial patterns in images and enables measurements of pattern similarity and visualization of expression across genes and developmental stages. It is closely linked with our FlyExpress (http://www.flyexpress.net/) resource, allowing users to search thousands of previously recorded gene expression patterns to find co-expressed genes by image comparison.

This User's Guide is located in the installation directory of myFX as well as on the myFX website. It contains useful information on how to use all of the features myFX has to offer, as well as examples. myFX does need an Internet connection for some features (such as searching FlyExpress for co-expressed genes and sending bug reports). The User's Guide, the Watch Directory, and the Processed Directory can be accessed via the top menu bar.

The myFX team is directed by Sudhir Kumar and consists of an application programmer (Ivan Montiel), developmental biologists (Charlotte Konikoff and Mary Packard), computer scientists (Qian Sun and Jipeing Ye), and technical associates (Bremen Braun and Adam Orr).

How Does myFX Work?

myFX works by hosting a server on either your local machine, or a central machine, and creating a view for accessing the content on the server. For most installations, the server and the view are integrated into one download and installation.

myFX utilizes image processing techniques for isolating embryos from images, creating "expression profiles" from those isolated embryos, and generating images that are representative of a developmental stage, anatomical view, and image source. myFX comes with multiple image processing techniques for use on a wide variety of images.

How Do I Install myFX?

For Windows PCs:

1. Go to http://www.flyexpress.net/myfx/download.php to download myFX.
2. Sign up and click Download.
3. Check your email for a link to the software download. You may want to check your Spam folder if it takes awhile to receive an email from noreply@flyexpress.net.
4. Click the link for the Windows Setup Installer Executable.
5. Download and Run the Windows Setup Installer Executable.
6. Follow the Setup Installer directions. If the Java JRE cannot be found on your system, it will prompt you to install it. The installer comes with the correct version of the JRE and will then proceed to install it for you. The Java 7 JRE is required for myFX to run, without it, myFX will not be able to finish launching.
7. Launch myFX.

For Macs:

1. Go to http://www.flyexpress.net/myfx/download.php to download myFX.
2. Sign up and click Download.
3. Check your email for a link for the software download. You may want to check your Spam folder if it takes awhile to receive an email from noreply@flyexpress.net.
4. Click the link for the Mac DMG File Download.
5. Download and Save the Mac DMG File.
6. Open the DMG file (Disk Image file) as seen in Figure 1, that you downloaded from the myFX website.
7. A folder should open with instructions on how to install myFX. If you have administrator privileges, you can simply drag and drop the myFX.app into the Applications folder. The DMG file has a shortcut for the Applications folder for your convenience. If you do not have administrator privileges, you can drag and drop the myFX.app to your Desktop.
8. The Mac application comes bundled with the Java 7 JRE required for myFX to launch, no additional steps are necessary to install Java.
9. Launch myFX by clicking on myFX.app.

Figure 1. The myFX Disk Image folder. This folder contains the myFX.app, a shortcut to your Applications folder, a copy of the myFX User's Guide, and the License for myFX. The Disk image folder is a folder that is mounted onto the Mac system when the DMG file is opened.

2 The User Interface

Parts of the User Interface

The main user interface consists of the Menu, a Toolkit, Helper Bar, and Grid. Each part of the interface contains controls used to help organize or analyze embryo images.
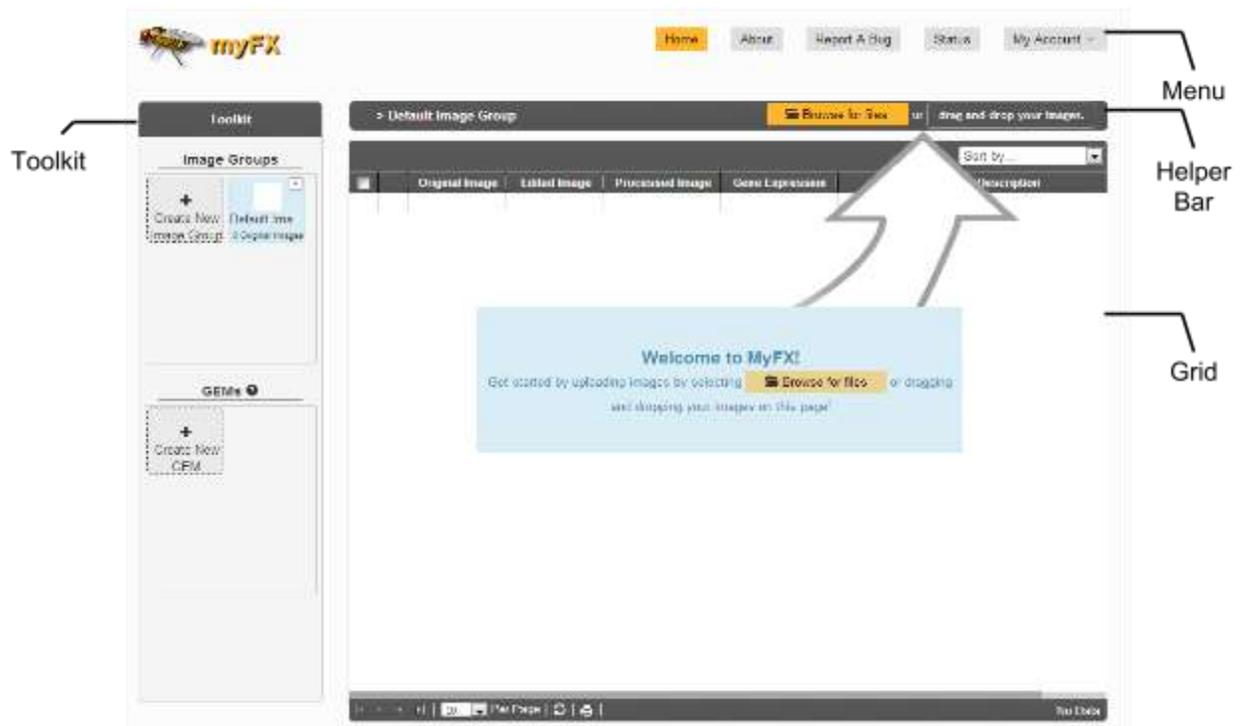

Figure 2. The myFX Interface. The interface is split into four major sections: the Toolkit, the Menu, the Helper Bar, and the Grid (or table).

The Menu Bar

The Menu Bar contains buttons that navigate to different views:

- The "Home" button navigates to the home page, which is the standard interface.
- The "About" button navigates to a view containing information concerning myFX, including links, acknowledgements, and citation information.
- The "Report A Bug" button navigates to a form for reporting software related bugs and issues.
- The "Status" button navigates to a grid view that displays the current queue of images that the back end server is processing.
- The "My Account" button is a dropdown menuthat allows you to view your profile, edit your profile, change your password, or log out.

The Toolkit

The Toolkit contains Image Groups and GEMs.

Image Groups

Image Groups are user-created logical organizations of images. Image Groups can be created by clicking on the "Create New Image Group" button, which will then prompt for a name for the Image Group. To rename an Image Group, double-click its icon and rename it.

To move a single image from one group to another, drag and drop the image. To move multiple images from one group to another, select them by checking the checkboxes, and then drag and drop the images to the desired Image Group. Note that "Check All" only selects (and moves) images on the current view of the Grid.

Image Groups are ordered chronologically in the order in which they were created. Each Image Group has a preview image that represents the latest image that has been manipulated at the most advanced level of work. The order of precedence of this image preview is: Original Image, Edited Image, Processed Image, GEMs. Therefore, if the Image Group has a GEM created, the preview will be that of the most recent GEM manipulation, even if an Edited Image was created and manipulated after the GEM creation.

GEMs

Genome-wide Expression Maps (GEMs) are summaries of spatio-temporal expression patterns for a specific stage (or stage range) and anatomical view within an Image Group. They summarize spatial patterns and relative expression levels from a set of user-specified images in myFX. Custom GEMs in myFX are made using the same methods FlyExpress uses to create Image GEMS.

GEMs are rendered as heat maps, with the darkest regions indicating domains where the largest number of images show gene expression:
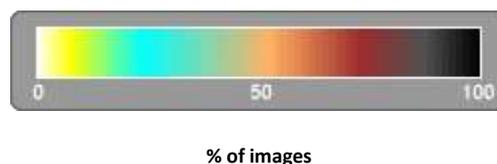
% of images

Figure 3. The GEM heat map scale. The "darker" the color, the higher percentage of images there are

with expression at that point.

The Helper Bar

The Helper Bar contains the name of the current image group as well as the "Browse For Files" button. The "Browse For Files" button opens up a File Chooser dialog that allows you to select individual or multiple files for upload into myFX. Images uploaded using "Browse For Files" will be added to the currently selected Image Group.

The Grid

The Grid contains all the data associated with uploaded images. The data stored by myFX includes Gene Name, Stage Begin, Stage End, Anatomical View, Gene Expression Type, Number of Embryos, and Pubmed ID. This data is shown in the Grid under the Description column. Genes have link outs to FlyBase, which, when clicked, open up the FlyBase report for that gene. Pubmed IDs also have link outs, which when clicked, perform a search of that Pubmed ID in FlyExpress.

Original Images are stored by myFX.   The user's file name for the image is stored by myFX, however the images are given unique names in the myFX file structure. This allows for multiple files with the same name to be uploaded and not interfere with each other.   Original Images are stored in the application directory. For Windows machines, the standard location is:

```
%localappdata%\myFX\MyFXImages\original
```

It is recommended that you do not move, add, or rename files in the \MyFXImages\ folder.

Each Original Image can have an Edited Image. The Edited Image column only appears if an Original Image being displayed on the Grid has an Edited Image. Edited Images are created by clicking on the Original Image, and clicking the Paint button or by rotating/flipping the image. If an Edited Image has not been created by the user, myFX assumes that the Original Image is the Edited Image.

Each Edited Image can have a Standardized Image, which is created using the standardization methods provided. The standardization process attempts to isolate a single embryo from an Edited Image, orient the embryo so that the Anterior-Posterior (A-P) axis is horizontally displayed in the image with the anterior positioned to the left, and resize the embryo to a standard image size (320 x 128 pixels).

Each Standardized Image has three (3) black-and-white Extraction Patterns. These are created by clicking "Extract Expression Patterns".   The Expression Patterns represent gene expression present in an embryo, with black pixels indicating expression presence and white pixels indicating absence. The center

expression profile should reflect gene expression in the original image, while left and right profiles represent under- and over-expression, respectively.

There is a checkbox feature to allow batch editing, standardization, and expression pattern extraction of selected embryos. By checking at least one image via the checkbox column on the left side of the Grid, a menu will appear on the same line as the sort drop down. This menu allows you to edit the details of selected images, standardize the selected images, extract expression patterns from the selected images, delete the selected images, or download the selected images as a zip file. Alternatively, there is a dropdown menu in the Image Group's thumbnail in the Toolkit. The options available in this dropdown are: Standardize All, Extract All, Delete Image Group, and Download Image Group. These actions are applied to all images in the entire Image Group.

The check all button on the top left of the Grid selects all of the currently visible embryos that are on the page being displayed.

3 Using myFX

myFX can complete a variety of tasks, including:

- Standardizing images of *Drosophila* embryos
- Organizing data into Image Groups
- Searching for FlyExpress genes with overlapping expression
- Creating custom GEMs from original data

Creating An Account

Select "Create a New Account" and fill in the required fields. You must Log In to process images and search FlyExpress for genes. Note that myFX does not share your information with anyone. When creating an account, myFX will log you in automatically into the newly created account.

Image Loading and Processing

1. Embryo images can be uploaded to myFX by either browsing for files, dragging and dropping, or using the "Watch Directory" (see the Watch Directory section for more information). Acceptable file formats include *.bmp, *.jpg, *.png, and *.tiff. The minimum image resolution recommended is 72dpi. Higher resolutions are accepted and will result in higher quality processed images, but may delay image loading and processing times.
2. Images will automatically process using the default Standardization Algorithm (Canny edge detection) after loading. To use a different algorithm, go to the Processed Image column and click the arrowhead to open the dropdown menu and choose "Re-standardize." Processing multiple images using the same algorithm is also possible in myFX – check the boxes adjacent to the embryos of interest and choose "Standardize Selected" from the top menu.

   Note that selected "Check All" in the checkbox column of the Grid allows one to change all images on the current Grid page. To Re-standardize all images in a large Image Group at once,

the dropdown option from the Image Group's thumbnail in the Toolkit provides the option to "Standardize All."

3. Manual image editing may be necessary if an embryo does not process properly (for example: If an image with multiple embryos does not process correctly, white out everything but the embryo of interest so that it is the largest object in the picture). Double-click the original image and select Paint from the left menu. The Paint toolbar will appear below the image, which can now be manually edited. Saving creates an edited image which can then be processed. Note that myFX will never overwrite an original uploaded image. Not that editing an original image will not update the processed image or the extraction images.

4. Processed images should be oriented in an anterior-to-the-left and dorsal-on-top manner. To flip embryos on the A-P or D-V axis, double-click the image and select the appropriate option. Save the image when it is oriented correctly.

5. "Extract Expressions" to capture the gene expression in a processed image. Adjust the color channels and other parameters if necessary. The center expression profile should reflect gene expression in the original image, while left and right profiles represent under- and over-expression, respectively. When finished, click "Extract Expression Patterns." The expression profiles can be further modified by double-clicking and using Paint, if desired.

It is important to note that not all standardization techniques are meant to work on all types of images. The following are a few examples of how certain techniques work better for certain types of images:

- The "Canny Edge" standardization technique works well on BDPGimages.
- Fly-FISH type images can sometimes standardize better with the "Variance" standardization technique.
- The "Subtract Background" and "Variance" standardizations work well on confocal stacked images.

Watch Directory

The Watch Directory is located in the myFX program folder. Within the Watch Directory, each user can upload their original images to myFX by adding the images into the folder labeled with their email address they used to log into myFX when creating an account.

Loading images via the Watch Directory is a way of adding large datasets to myFX. A large data set is any number of embryo images over 100 in size. The Watch Directory is a folder in the myFX application that is located within the myFX folder structure.

For Windows installations, it is normally located in:

```
%localappdata%/myFX/watchDirectory
```

Note that `%localappdata%` is a shortcut in Windows. It can be typed in any Windows Explorer in the address bar. For example, on Windows 7, this directory is an alias to C:\Users***[username]***\AppData\Local.

For Mac installations, it is in

[Installation_Directory]`/myFX.app/Contents/Resources/watchDirectory`

For normal installations, the installation directory is "/Applications":

`/Applications/myFX.app/Contents/Resources/watchDirectory`

The easiest way to get to this directory is to open a Finder window, and hit command+shift+G (⌘⇧ G), and then type in the path above.

This folder can also be accessed from from either the Windows or Mac version by opening up the toolbar of myFX and selecting Folders->Open Watch Directory.

In order to use the Watch Directory, simply copy and paste your images to be uploaded to myFX "*[installation_directory]*/myFX.app/Contents/Resources/watchDirectory/*[email]*", where the email is the email of the user you want to have the images owned by. They can either be added while myFX is running or not running. If myFX is running, the images will immediately start being processed as they are detected by myFX. Otherwise, the images will be processed the next time myFX is started.

Images in the Watch Directory will be deleted after they are processed. If you do not want your original images deleted, please copy and paste your files into the directory.

The following is a detailed explanation of how myFX handles the Watch Directory:

- If myFX is open, it will be watching the directory for changes if your operating system supports this feature. When a file is created, it is queued to be added to the user's account, specified by the folder's username. If the folder name does not match a user's name, no action is performed. If a folder name does match a user's name, the files are queued for processing in myFX and added to a new Image Group labeled "Manual Upload *currentdate*". Once images are processed and added, they are deleted from the Watch Directory.
- If myFX is not open, then the files are not processed until myFX is open. Upon launch, myFX will poll the directory for files, and add them to a queue for processing. If the folder name does not match a user's name, no action is performed. If a folder name does match a user's name, the files are queued for processing in myFX and added to a new Image Group labeled "Manual Upload *currentdate*". Once images are processed and added, they are deleted from the Watch Directory.
- Folders for users should be automatically created when the user originally creates their myFX account. There should be no need to add new folders to the Watch Directory.

- If a filename change or a folder name change MUST occur, keep in mind that myFX currently cannot listen to folder name changes. If a folder name change must occur, please restart myFX in order for myFX to "see" the new name of the folder. When a folder is created in Windows, it is automatically registered with myFX as "New Folder". Changing the name after its creation cannot be registered with myFX due to Operating System constraints.
- The Watch Directory does not support ZIP files. If you have a zip file, extract it to the directory. Zip files will be ignored by myFX.

Images processed from the Watch Directory appear in a new Image Group in myFX. The Image Group is named as "Manual Upload *[currentdate]*."

The Processed Files Directory

The Processed Files Directory is a directory within the myFX application that stores files that have been processed by the myFX Watch Directory. When a file from the Watch Directory is completed and entered into myFX, the file is moved to the Processed Files Directory. Files in the Processed Files Directory can be moved, deleted, and edited without affecting myFX.

The Processed Files Directory can be accessed via the application menu bar on either the Mac or Windows version of the application under Folders-> Processed Files Directory. The folder can also be accessed from the operating system's file system by navigating in Windows machines to:

```
%localappdata%/myFX/processed
```

Note that `%localappdata%` is a shortcut in Windows. It can be typed in any Windows Explorer in the address bar. For example, on Windows 7, this directory is an alias to C:\Users*[username]*\AppData\Local.

Or on Mac machines, open a Finder window, hit command+shift+G (⌘⇧ G), and then type in the following path:

[Installation_Directory]`/myFX.app/Contents/Resources/processed`

For normal installations, the installation directory is "/Applications":

```
/Applications/myFX.app/Contents/Resources/processed
```

Entering Embryo Data

Double-click the Description Box to input information. The Edit icon (⬚)in a data row can also be selected. To enter the precise stage of an embryo, type the stage in the Stage Begin and Stage End fields. To enter the same data for multiple embryos, select them using the check boxes and choose "Edit Selected" from the top menu. Note that stage and anatomical view must be entered to create GEMs and to search for overlapping patterns in FlyExpress.

When batch editing embryo image data, if annotations for the selected images differ, the fields that are different will be locked. Any field locked will not be overwritten when saved. You can unlock a field by clicking on the lock. This allows you to edit the field for all the images selected.

Searching for Overlapping Expression Patterns

After extracting expression patterns, users will have the ability to search FlyExpress for genes with similar and overlapping expression patterns. Images must also be assigned a stage (or stage range) and anatomical orientation for searching. To search for FlyExpress images with overlapping patterns, go to the "Gene Expression" column and click one of the numbered expression variation patterns. Next, click on the drop-down menu arrow and "Search FlyExpress for Genes." A FlyExpress GEM image that is representative of the stage, view, and image source provided will be displayed with a group of expression points. If desired, these expression points can be moved around the GEM by clicking and dragging.

Creating Custom GEMs

myFX can create custom GEMs from original data. Custom GEMs are made from images of the same stage (or stage range) and anatomical view within an image group. First, select the image group you would like to make a GEM for. Next, select "Create New GEM" in the Toolkit. A box will appear where the stage (or stage range) and anatomical view can be specified. Finally, click "Create GEM."

Custom GEMs in myFX are created using the same methods FlyExpress uses to make Image GEMs. Relative expression levels in a GEM are rendered as a heat map:
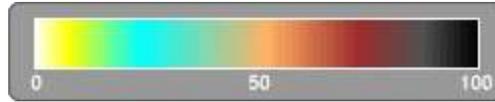
% of images

Searching Your Data

You can currently search your own Expression Images by clicking on a GEM created from a data set and selecting "Search for Embryos." This will bring up a sketching panel where you can paint on areas of interest. By clicking search, myFX takes your painted areas and checks all of your images with Expression Images that are associated with that GEM. If a point of expression lies in the same location as a point of interest, the image gets +1 point. In the results table, the Points column denotes how many points that image has in common with the points of interest.

A common concern is why an image can have more points in common with the points of interest than the number of points of interest.

Acknowledgments

Citation Information

Montiel I, Braun B, Konikoff C, Packard M, Sun Q, Ye J, and Kumar S (2013) myFX: Turn-key software for laboratory desktops to analyze spatial patterns of gene expression in *Drosophila* embryos. (In preparation)