Using ImageJ to Create a Videogram

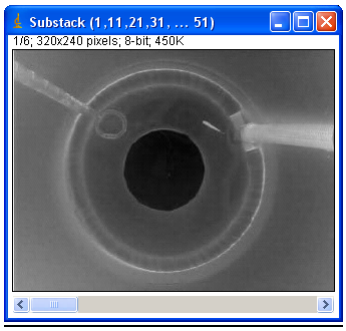
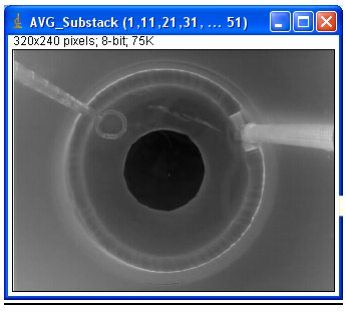
This document outlines a step by step procedure to produce a videogram from a short sample movie

Requirements/Preparation:

1. **ImageJ**
   The procedure uses the MacBiophotonics ImageJ release which bundles a number of necessary plugins (AVI Reader, Substack Maker, Handle Extra File Types)
   http://www.macbiophotonics.ca/imagej/
2. **Sample video**
   The procedure relies on the movie being opened directly in ImageJ. This only works if the movie is uncompressed. Therefore, do any **one** of the following:
   - Use an uncompressed AVI movie and load it into ImageJ using the AVI Reader plugin http://rsbweb.nih.gov/ij/plugins/avi-reader.html
   - Use a compressed movie and convert it to an uncompressed movie using another video processing program, and use the AVI Reader plugin.
   - Use a compressed movie and convert it to a series of uncompressed (TIFF, TARGA, BMP, etc.) images using another video processing program, and then use the File: Import: Image Sequence… command in ImageJ to create a stack of grayscale images from the series of image files.

The sample movie used in this example is available in both compressed and uncompressed versions at:
http://people.stfx.ca/rwyeth/vidsimages.html or contact Russell Wyeth rwyeth@stfx.ca

ImageJ commands to produce a videogram from the uncompressed video "sample2.avi"

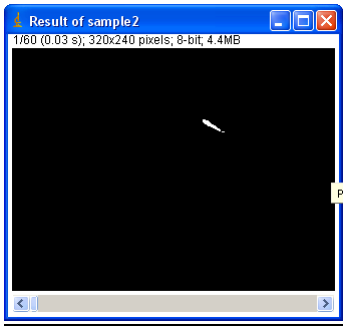
Menu Command in ImageJ *(v1.42l, MacBiophotonics release)*	Image Window Result
1. File: Open browse and select "**sample2.avi**" **Open** *Only uncompressed AVI files can be opened by ImageJ.* First Frame: **1** Last Frame: **60** ☐ Use Virtual Stack ☑ Convert to Grayscale ☐ Flip Vertical **OK**	
2. Edit: Invert Process all 60 images? There is no undo if you select "Yes" **Yes**	
3. Plugins: Stacks – Reducing: Substack Maker Enter either range (e.g. 2-14) or a list (e.g., 7,9,25,27): **1,11,21,31,41,51** **OK** *This stack will be used to create the mean image.*	
4. Image: Stacks: Z Project… Start slice: **1**; Stop slice: **6** Projection Type: **Average Intensity** **OK** *This creates a poor mean image, with a considerable 'shadow' of the fish's motion, yet still suffices to demonstrate the method. A longer video providing more widely spaced frames (selected in step 3) would produce a mean image with little trace of the fish.*	

5. Process: Image Calculator…
   Image 1: **Sample2.avi**
   Operation: **Subtract**
   Image 2: **AVG_Substack(1,11,21,31…51)**
   ☑ Create New Window
   ☐ 32 bit (float) Result
   OK

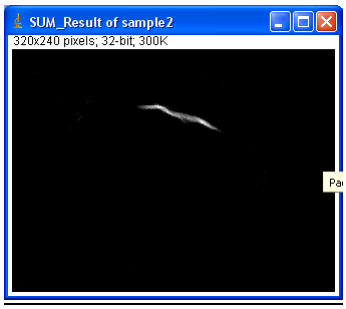
   Process all 60 images? ? There is no undo if you select “Yes”
   Yes

6. Image: Adjust: Threshold…
   [threshold minimum slider, top]: **20**
   [threshold maximum slider, middle]: **255**
   [threshold display, bottom]: **Red**
   Apply

   Convert all images in stack to binary?
   ☐ Calculate Threshold for Each Image
   ☑ Black Background
   OK



7. Image: Stacks: Z Project…
   Start slice: **1**;
   Stop slice: **60**
   Projection Type: **Sum Slices**
   OK



8. Image:Type:8 bit