An Introduction to version 2.0
Agenda

• General overview of the MetaMorph NX

• 4D Viewer

• How to use some features in MetaMorph NX
The Ribbon Interface

- Takes the place of toolbars and dialog boxes
- Orange Buttons: Option can be toggled on or off
- Down Arrow: Pull out for more options
- Galleries: visually choose different options to configure
- Active Context: the currently selected Ribbon ‘Tab’
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The Image Grid

- Takes the place of image windows
- Display single or multiple images all at once
- Configure how the grid gets displayed
- Choose the current Z or T being viewed using sliders
- Displays which T, Z, Stage, being displayed
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The Filmstrip

- Gives visual access to different parts of the experiment
- Makes it easier to select data to view
- Can be configured to show any dimension
  - Datasets in Experiment
  - Stage Positions
  - Time Points
  - Z Planes…
The 4D Viewer

- Button on Home Toolbar
- Opens Time / Z Experiments into a 4D Dataset View
- Allows color overlays, transparency, isosurfaces
- Make movies or rotations
- Switch to Slice Mode to get view of arbitrary cutout of 3D slices
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Quick Access Toolbar

- Displayed at the top or bottom of Ribbon
- Always displayed, no matter which Ribbon is used
- Good for putting often-used tools
  - Dataset Grid Layouts
  - Toggle overlays
- Galleries can be selected from the Quick Access Toolbar
- To add to the Quick Access Toolbar – Right Click on Tool in Ribbon
- To remove from the Quick Access Toolbar – Right Click on Tool in Quick Access Toolbar
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Using MetaMorph NX

Features of the software to make it easier to use
Image Scaling

- Displayed under Image Grid
- One Scale Bar for each channel
- Histogram displayed inside Scale Bar
- Options for:
  - Fixed: Absolute min/max intensity
  - Normalized: % of pixels for min/max
  - Camera: Full scale of camera used to acquire image
- Calipers for setting min/max
- Can also use Channel Display
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Image Zoom

- Change Zoom via Mouse
  - Put mouse over image and scroll using mouse wheel
- Home ribbon also has options for zooming
- Zoom overlay appears when the entire image can’t be seen in the grid.
- Move the zoomed view window around image using clear part of the zoom overlay
- Zoom overlay can be toggled on and off.
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Opening Images in MetaMorph NX

Opening, creating Experiments from external data
Images in MetaMorph NX are always associated with an Experiment.

Within each experiment is a group of related images called a Dataset.

A Dataset is considered all the images from a single acquisition.

For example: If you run Multi Dimensional Acquisition it produces one Dataset. Run the same MDA again and it produces a second Dataset.

Datasets also include the related measurements and object masks made on those images.

An Experiment may hold multiple datasets.
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An Experiment may hold multiple datasets.

The Home Ribbon has a Dataset Gallery to select different datasets in the experiment.
Experiments

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  - A Dataset is considered all the images from a single acquisition.
  - For example: If you run Multi Dimensional Acquisition it produces one Dataset. Run the same MDA again and it produces a second Dataset.
  - Datasets also include the related measurements and object masks made on those images.
- An Experiment may hold multiple datasets.

The Filmstrip can also be used to select between Datasets in the current experiment.
Working with experiments

• Application Menu
  • Holds tools for working with the current experiment files
  • Also tools for MetaMorph NX Administration.

• New Experiment
  • Create a new experiment
    – Gives details where the experiment will be created
  • Set Experiment Defaults
    – Change the location on disk where experiments are saved
    – Change names given to experiments and datasets
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• **Application Menu**
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• **Open Experiment**
  • Loads a previously saved experiment into MetaMorph NX
  • Experiments are saved into a folder with their name on it
  • Always open the ‘Experiment.mexp’ file.
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Experiment File Structure

Experiment Path

Experiment Name

File name: Experiment.mexp
Experiment File Structure

Datasets

Experiment File

Select an Experiment to Open

Organize  New folder

Favorites
  Desktop
  Downloads
  Recent Places

Libraries
  Documents
  Music
  Pictures
  Videos

Homegroup

Computer
  Win7 (C:)
  SYSTEM (D:)

File name: Experiment.mexp
Working with experiments

• **Application Menu**
  • Holds tools for working with the current experiment files
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• **Open Images**
  • Opens images acquired outside of MetaMorph NX into a **New Experiment**
  • **Open Image**
    – Select a single image, or import a .ND file from MetaMorph
  • **Channel / Time / Z Series**
    – Import multiple images or stage is a series of the given dimension
  • **Stage Series**
    – Opens multiple images – each image is considered a new stage location.
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Two Stage Positions

One Channel
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Working with experiments

- **Application Menu**
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  - Also tools for MetaMorph NX Administration.

- **Add Images to Experiment**
  - Opens images acquired outside of MetaMorph NX adding them to the **Current Experiment** as a new Dataset
  - **Open Image**
    - Select a single image, or import a .ND file from MetaMorph
  - **Channel / Time / Z Series**
    - Import multiple images or stage is a series of the given dimension
  - **Stage Series**
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Measurements

Basic Measurement Tools in MM NX
The New Measure Ribbon
The New Measure Ribbon

- MetaMorph 2.0.1 has re-designed the measure ribbon
  - Reorganized tools like Regions, Thresholding, and Modules
  - Added space for the Application Modules
  - Added tools for exporting data
  - Added tools for turning thresholds and Module masks to Regions of Interest.
Measurement Tools

- MetaMorph 2.0.1 has re-designed the measure ribbon
  - The Left side of the Ribbon contains tools for making measurements.
Region Tools

- Regions
  - The first section is for Regions of Interest.
  - There is a gallery for choosing the type of Region to draw.
  - There is a drop down menu for configuring Region Measurements
  - There is a button to Measure Regions
Region Tools

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Region Tools

- The first section is for Regions of Interest.
- New Auto Find Tool helps place Regions by clicking on an object of Interest.
- There is a gallery for choosing the type of Region to draw.
- The Region then is drawn automatically around the object.
- There is a drop down menu for configuring Region Measurements.
- There is a button to Measure Regions.
Region Tools

- Use the Crosshairs to click on an object of Interest
- The Region then is drawn automatically around the object
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TIP

- Press the ALT key and click on the image to continue to place Regions of the same type.
Threshold Tools

- Threshold
  - The next section is for Thresholding.
  - There is a drop down menu for setting the Threshold
  - There is a button to Measure Thresholded Areas
  - There is a button to turn the Threshold overlay on or off
  - There is a drop down menu for configuring how Thresholds are turned to Regions
  - There is a button to turn Thresholds into Regions
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Low and High Range Calipers
Threshold Mask Cleanup
Threshold Tools

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## Modules

- **Module**
  - The next section is for Modules.
  - There is a gallery for selecting which Application or Custom Module to run.
  - You can click on the Module to configure it.
  - Custom Modules can be edited or deleted.
  - Use Create Module to make a new Custom Module.
  - Press Measure to run the selected Module.
Modules

- The next section is for Modules.
- There is a gallery for selecting which Application to run.
- You can click on the Module to configure it.
- Custom Modules can be edited or deleted.
- Use Create Module to make a new Custom Module.
- Press Measure to run the selected Module.
Modules

• Module

• The next section is for Modules.

• There is a gallery for selecting which Application or Custom Module to run.

• You can click on the Module to configure it.

• Custom Modules can be edited or deleted.

• Use Create Module to make a new Custom Module.

• Press Measure to run the selected Module.
Many of the Application Modules also have the Click To Find feature

- Modules are easier to setup – no measuring intensities or sizes needed

- Modules also have Fast Algorithm options
  - Perform similar to Standard algorithm, but run 2x faster (or more)
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• Measured Images

• Measure Range defines what Images to measure using any of the 3 Measure buttons.

• If no options are turned on, only the Visible images are measured

• Turn on All Time, All Z, and All Stage to measure your entire dataset.
• **Measurements**

  • The Right side of the Ribbon contains tools for after you make measurements.

  • Such as selecting or displaying data, and converting Masks to Regions.
Measurement Tools

- Measurement Set
  - A Measurement Set contains all the result, the numbers and the masks, from any one measurement.
  - There is a gallery to choose which Measurement Set to work with.
  - There is a button to turn the masks associated with the active Measurement Set into Regions
  - There is a button to configure what Objects to keep in for Display.
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  • There is a button to configure what Objects to keep in for Display.
Measurement Tools

- **Measurement Set Configuration**
  - Choose which Measurement Columns will be displayed.
  - Filter un-desired Objects out.
Measurement Tools

Measurement Set Configuration

- Choose which Measurement Columns will be displayed.
- Filter un-desired Objects out.
Measurement Tools

- Measurement Display
  - The next section controls different ways of displaying the Data
  - Show Table will display a table with all the Object data on it.
  - Show Summary will show a table with statistical summary information
  - Add Line Graph and Add Scatter Plot will plot the data
Measurement Tools

There are different ways of displaying the Data.

- **Show Table** will display a table with all the Object data on it.
- **Show Summary** will show a table with statistical summary information.
- **Add Line Graph** and **Add Scatter Plot** will plot the data.
When a Line Graph or Scatter Plot is shown, the Graph Ribbon becomes available.

- The Graph Ribbon allows you to resize and move the displayed area of the graph.
- The Graph can have different Layouts, controlling what labels and text get displayed.
- Graphs can have different styles, controlling colors, line thicknesses and data points.
- Graph Settings can be used to further modify the style.
The Graph Ribbon

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When a Line Graph or Scatter Plot is shown, the Graph Ribbon becomes available. The Graph Ribbon allows you to resize and move the displayed area of the graph. The graph can have different layouts, controlling what labels and text are displayed. Graphs can have different styles, controlling colors, line thicknesses, and data points. Graph settings can be used to further modify the style.
Measurement Tools

- **Working with data logs**
  
  - Measurements made in MetaMorph NX are always maintained in a ‘database’ – you do not need to open a log file to record measurement values.
  
  - Measurements are stored with the Dataset and maintained between MetaMorph NX sessions (i.e. if you close NX and re-open it the measurements are still there).
  
  - You can export a **displayed data table** to Excel or to a file
    - Displayed Data Table: You export the table as displayed – including the column and object filtering performed in the ‘Configure Results’ panel.
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MetaMorph NX can directly export to Excel 2003, 2007, or 2010 if it is installed on the system.
Or MetaMorph NX can write data to an XLS file or CSV file if Excel is not present on the computer.
Using the Analysis Builder

Custom Modules
Why Custom Modules? (aka the analysis builder)

• Application Modules are great but…
  • Many times customers need to ‘add to’ what App Modules provide
  • So many things to measure – not all application modules

• Not Journals!
  • Easier to use than journals
  • Most segmentation tools available to journals are present
  • UI makes it easier to find the tool, test options, without getting lost in menus or minutia

• Custom Modules are Modules
  • Can be run exactly like application modules
  • Measurements are logged the same as application modules
Custom Modules

- Use the Analysis Builder to make custom modules

- Open the Analysis Builder using the Create Module on the Modules palette
A New Dataset View:

The Analysis Builder opens up in a New Dataset View.

The display, image grid, data tables, and filmstrip are independent of the original dataset view used to create it.
The Ribbon:

All the Segmentation tools are available from the ribbon, grouped into 3 palettes based on what they do.

Find Objects: Automated tools to create Object Masks

Modify Objects: Tools for changing Object Masks (filters, logical operations…)

Modify Images: Tools for modifying grayscale images (Morphology, Image Arithmetic…)

Organized so all the tools are easy to find – no hunting through menus…
The Module:

Steps in the module are displayed as discrete cards. Each card has all the parameters need to configure that step.

Easier to read than journals, the cards are friendlier and less intimidating than the programming look of journals.
The Filmstrip:

Each step in the module has its own thumbnail result in the filmstrip.

Easier to identify what each step does, and if it is working.

Easier to navigate back to a step which may be causing a problem – just click the offending thumbnail.
Custom Modules

- The Custom Module Ribbon
  - Modify Image: Tools for processing grayscale images
  - Find Objects: Tools for creating masks and detecting objects from grayscale images
  - Modify Objects: Tools for changing or selecting masks.
• Modify Image
  • Tools for modifying real image data, grouped into functional sections
    • Arithmetic: Adding, Subtracting, etc… two images or an image and a constant value
    • Morphology: Filters based on shape, size and relative intensities
    • Special: Color separation techniques, basic filters…
• **Modify Image**
  
  • Tools for modifying real image data, grouped into functional sections

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- **Modifying images**

- **Close Open**
  - Source: Channel 3
  - Size (pixels): 13
  - Filter Shape: Circle
  - Grayscale Reconstruction: 
  - Sequential Filters: 
  - Result: Close Open
  - Apply
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- Arithmetical Operations
  - Add
  - Subtract
  - Multiply
  - Divide
  - Minimum
  - Maximum

- Morphological Operations
  - Erode
  - Dilate

- Special Operations
  - Average Filter
  - Color Separate RGB
  - Color Separate HSL
  - Color Separate CIE-Lab
  - Gaussian Filter
  - LoG Filter
  - Distance
  - Mask to Image
  - Select Image from Dataset

- Custom Module
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Modify Objects

- Modify Objects
  - Tools for resizing, selecting, or reshaping objects on a mask
    - Example object selecting tools:
      - Logical Operations
      - Keep/Remove Marked Objects
      - Filter Mask
    - Example object resizing tools:
      - Grow Objects
      - Grow Objects Without Touching
      - Shrink Objects
    - Example reshaping tools:
      - Invert Object
      - Watershed
      - Fill Holes
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• **Find Objects**
  • Tools for finding objects in grayscale images and making masks for them.
  
  • Simple to use tools like:
    – Auto Segmentation: No Configuration Needed
    – Auto Threshold: Like auto thresholding in MM – for Bright or Dark objects
    – Simple Threshold: Set Min / Max intensity of objects
  
  • Tools like the Application Modules use:
    – Auto Find Blobs for finding things like nuclei
    – Find Blobs for finding things like cytoplasm
  
  • And a few more tools:
    – Find Fibers for tube or process detection
    – Find Round Objects for truly round things (like puncta)
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Find Objects

- **Auto Segmentation**
  - Find all kinds of Objects with NO values to configure.
  - Great for objects of all sizes and shapes
  - Does not split touching objects
  - May need to filter small objects out
Find Objects

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- **Simple Threshold**
  - Identify objects based on their absolute intensity
  - Set a Low and High Threshold Value
  - Inclusive On = Target intensities are between the Low and High values
  - Inclusive Off = Target intensities are those values NOT between the Low and High values
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Find Objects

• Find Round Objects
  • Great at finding round things
  • Splits touching objects
  • Best for small objects
  • Not best option for nuclei – nuclei can be more cigar shaped …
Find Objects

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Find Objects

- **Auto Find Blobs**
  - Great at finding objects with little or no configuration

  - With ‘Automatic’ option on – Zero configuration setup, just apply and go
    - Splits objects
    - may find smaller objects than you want
    - Can add Filter Mask to get rid of the small stuff

- Splits touching objects

- **Best tool for Nuclei**, or other relatively uniform objects
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Find Objects

- **Find Blobs**
  - Finds arbitrary shaped objects, like Cytoplasm
  - Finds borders of objects based on Local Intensity differences
  - Min / Max Width define the details of the object borders, not necessarily how big the objects are
    - Small Minimum Width shows all the blebs and formations on the edge of a cytoplasm
    - Large Minimum Width shows mostly the same area, but the smaller details will be clipped.
  - Will not split touching objects
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Approximate Minimum Width: 5
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  - Will not split touching objects

Approximate Minimum Width: 20

Source
- Punta

Approximate Maximum Width (μm): 30

Intensity Above Local Background: 500

Result
- Find Blobs

Apply

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Find Objects

- **Find Fibers**
  - Find long tubes or fibers, their branches, and objects too large to be fibers.

- Four masks produced
  - Fibers: Objects with Widths between the Min Fiber Width and Max Fiber Width
  - Non-Fibrous Objects: Objects with Widths > Max Fiber Width
  - Segments: Skeleton of Fibers – good for measuring length of Fibers
  - Branch Points: Single points where segments meet each other.
  - Segments + Branch Points = total Fiber system
Finding Granules In the Nucleus

An Example Custom Module
Granules in Nucleus

• **Goals:**
  • Count the number of Granules in a GFP stain that are localized to the Nucleus

• **Measurements to make:**
  • Nuclear Area and Intensity
  • Granule Count
  • Total Granule Area and Intensity
  • Average Granule Area

• **Inputs:**
  • DRAQ5 Nuclear Stain
  • GFP Stain localize to granules (in and out of nucleus)
Granules in Nucleus

- Example Images
Granules In Nucleus

• **Setup:**
  
  • Assign real names to be used for the input images

  • These names will be used throughout the Custom Module when referring to the ‘Input’ images

  • These names will be used when running the Custom Module from the Measurement Ribbon to assign the ‘Input’ images
Granules In Nucleus

- Finding the Nuclei
  - Auto Find Blobs using Click to Find
    - Turn Automatic Off
  - Click on a few Nuclei
  - Hit Apply to find all nuclei
Granules In Nucleus

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Granules In Nucleus

- **Finding Granules**
  - Separate Granules from background using Modify Images > Morphology > HDome
  - HDome uses a Relative Intensity Threshold
  - Don’t forget to provide meaningful names to the Result Images
  - After HDome, use Find Objects > Auto Segmentation to create a Mask of All Puncta
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Granules In Nucleus

• How Good Did Segmentation Do

  • Use Side By Side view to see!

  • Slide the Bar left and right to see which spots (on the Left) were turned into objects (on the Right)
Granules In Nucleus

- How Good Did Segmentation Do

- Use Side By Side view to see!

- Slide the Bar left and right to see which spots (on the Left) were turned into objects (on the Right)
Granules In Nucleus

• Making Measurements: Measure Tab
  • Custom Modules are designed to measure ‘Features within Objects’
    – For Example ‘Granules within Nuclei’
  • The first step is to measure the outer-borders: the Objects of Interest
    – In this case, the Nuclei
  • In the Object to Measure section:
    – Mask of Objects = Nuclear Mask
      – Define the widest area to measure in
    – Image to Measure = Nuclei
      – Grayscale image to get Intensities from
    – You can measure more than One image using the + icon.
• **Making Nuclear Measurements**

  • Press the … button to configure what values gets measured

  • Turn off the Right column of values. They are not needed for the ‘Object’ measurement.

  • Select which rows to measure from the Left column of values

    – Area, Integrated Intensity, Average Intensity

    – Can re-name the values to be more meaningful
Granules In Nucleus

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Select which rows to measure from the Left column of values

- Area, Integrated Intensity, Average Intensity
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Granules In Nucleus

- **Making Granule Measurements**

  - The Granules are ‘Features’ found inside the Nucleus.
  
  - Click on the ‘Add Feature Group’ button to add an new section for measuring the Granules.
  
  - Mask of Features = All Granules
  
  - Image to Measure = Puncta
  
  - Press … to select which measurements to make on Granules
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Granules In Nucleus

Making Granule Measurements

Press … to select which measurements to make on Granules

- The Left Column is the Average of all Features per Object
- The Right Column is the Sum of all Features per Object
  - Area on Left Column = Average Granule Area
  - Area on Right Column = Total Granule Area
- The last measurement on the Right Column is the Count of Features
- Measure:
  - Average / Total Granule Area
  - Average / Total Granule Intensity
  - Total Granule Count
• Saving the Custom Module

• At the bottom of the Module, provide a Measurement Name

• Select from a list of icons to display for the measurement

• Save the measurement to add it to the list of Modules

• Then close the Custom Module dataset view
• Running a Custom Module
  • Select the Module you made from the same list as the Application Modules
  • Click on it in the Ribbon
  • Assign Images
  • Run Measurement
• **Running a Custom Module**
  
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Running a Custom Module

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This part of the presentation will be handled in software.

Process images based on shapes and sizes

**Morphology Filters**