# Integrating Flexible and Efficient Image Acquisition with Open software

EuBIAS 2015

Paris

# Overview

- NIS Elements and Open Software
- NIS Elements General Extensibility
- JOBS: the experiment builder
- Illumination Sequence

# NIS Elements and Open Software

NIS Elements is strong in acquisition and general imaging:

- In addition to Nikon devices we support:
  - over 40 camera models (EMCCD, sCMOS, CCD) and
  - over 60 3rd party devices (Spinning disks, lasers, filters, stages, incubators, …)
- General analysis and measurement (Ratio, Counting, Tracking, …)
- 3D Segmentation and Visualization

Open Software is strong in specific imaging:

- Niche hardware support (robotic well-plate loaders)
- Implementations of novel imaging techniques (light-sheet)
- Dedicated advanced image analysis algorithms (thunderStorm)
- Statistics and Data Presentation tools (R)
- Complex data-mining pipeline (CellProfiler)

# NIS Elements General Extensibility

## Macro language

- Load functions from 3rd party DLLs (e.g. to control simple devices)
- Control RS232 devices via reading/writing to serial port
- Run other processes
  - Save Image in NIS
  - Process in ImageJ (blocking)
  - Reload in NIS

## Command line options

- NIS Elements as Slave
- Run NIS Elements from another app
  - Execute given task in NIS (blocking)
  - Continue the pipeline

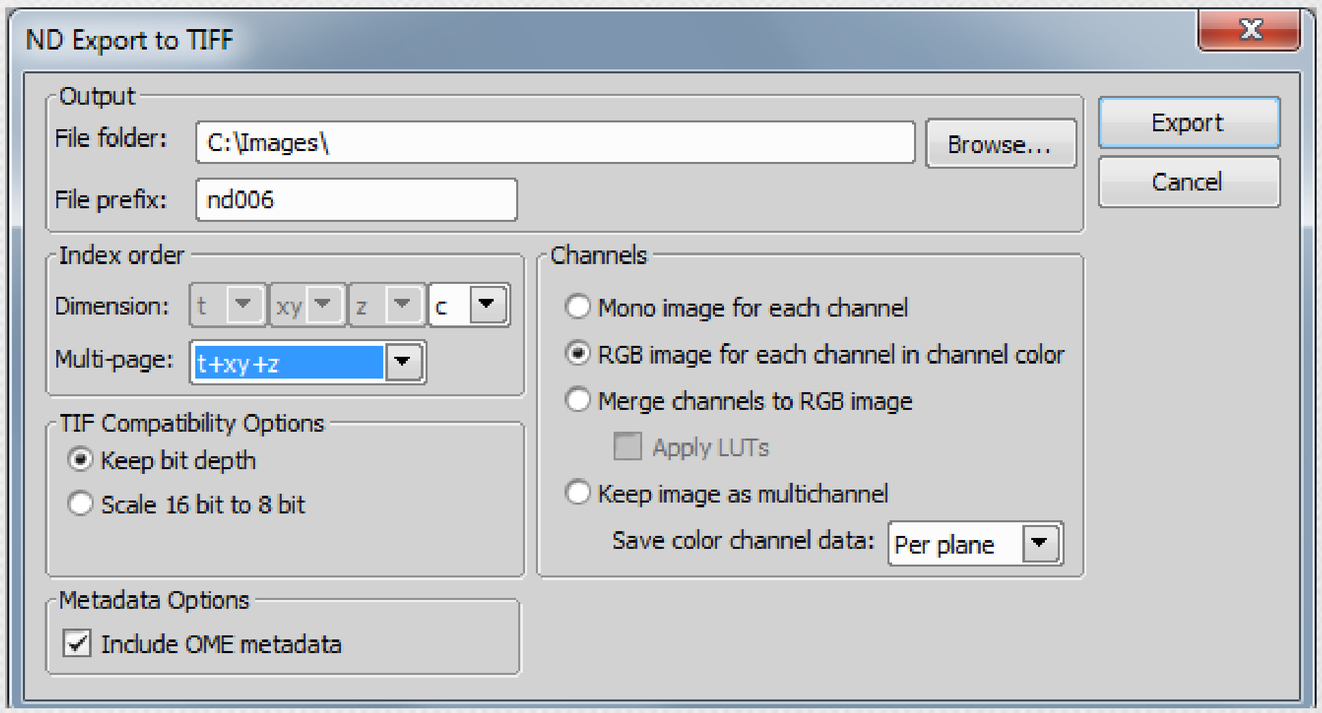
```
#import("MyModule.dll");
#import int MyFunction(LPSTR file);

char OutBuffer[1000];
char currentFilename[MAX_FILE];

int testImportedFunc()
{
   Get_Filename(FILE_IMAGE, currentFilename);
   MyFunction(currentFilename);
   return TRUE;
}

int testRS232()
{
   OpenPort(6, 115200, 8, "N", 1);
   WritePortEx(1, "1PA1.436", 13, 1, OutBuffer, 0);
}

int testImageJ()
{
   ImageSaveAs("C:\images\exchange.tif", 2, 0);
   ImageClose();
   Int_ExecProgramWait("C:\Fiji.app\ImageJ-win32.exe"
                       " -headless -macro C:\macros\nis.ijm");
   ImageOpen("C:\images\exchange.tif");
   return TRUE;
}
```

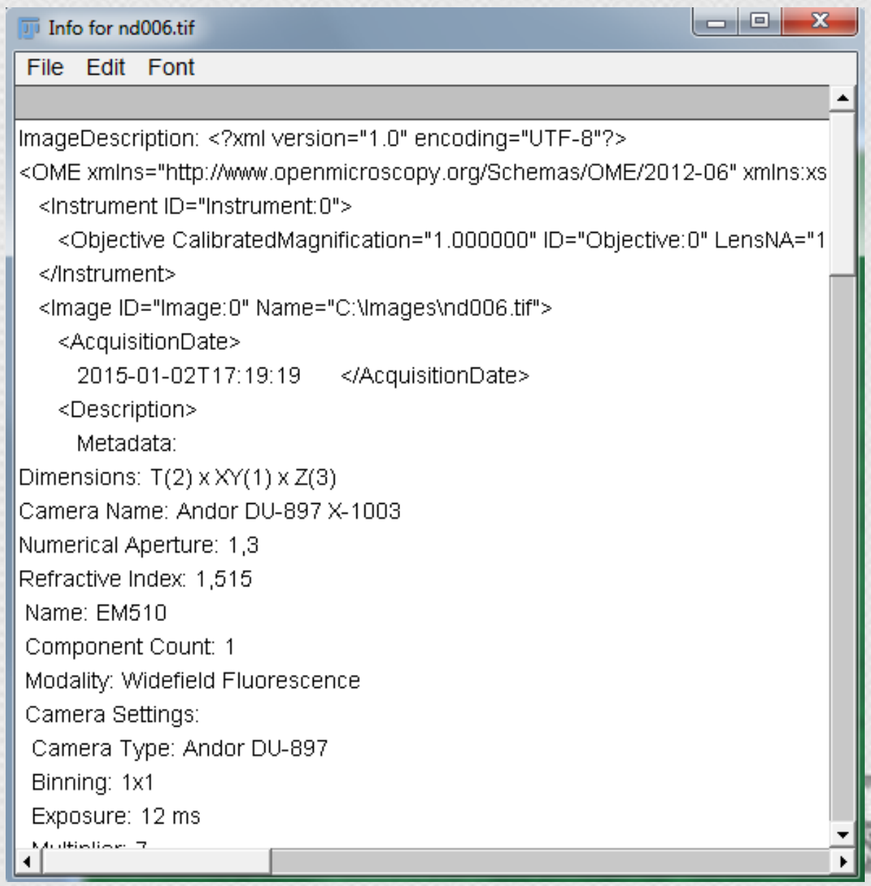
# NIS Elements General Extensibility

- ND2 File Format SDK

  Register and download from:
  http://www.nd2sdk.com/


- OME TIFF export



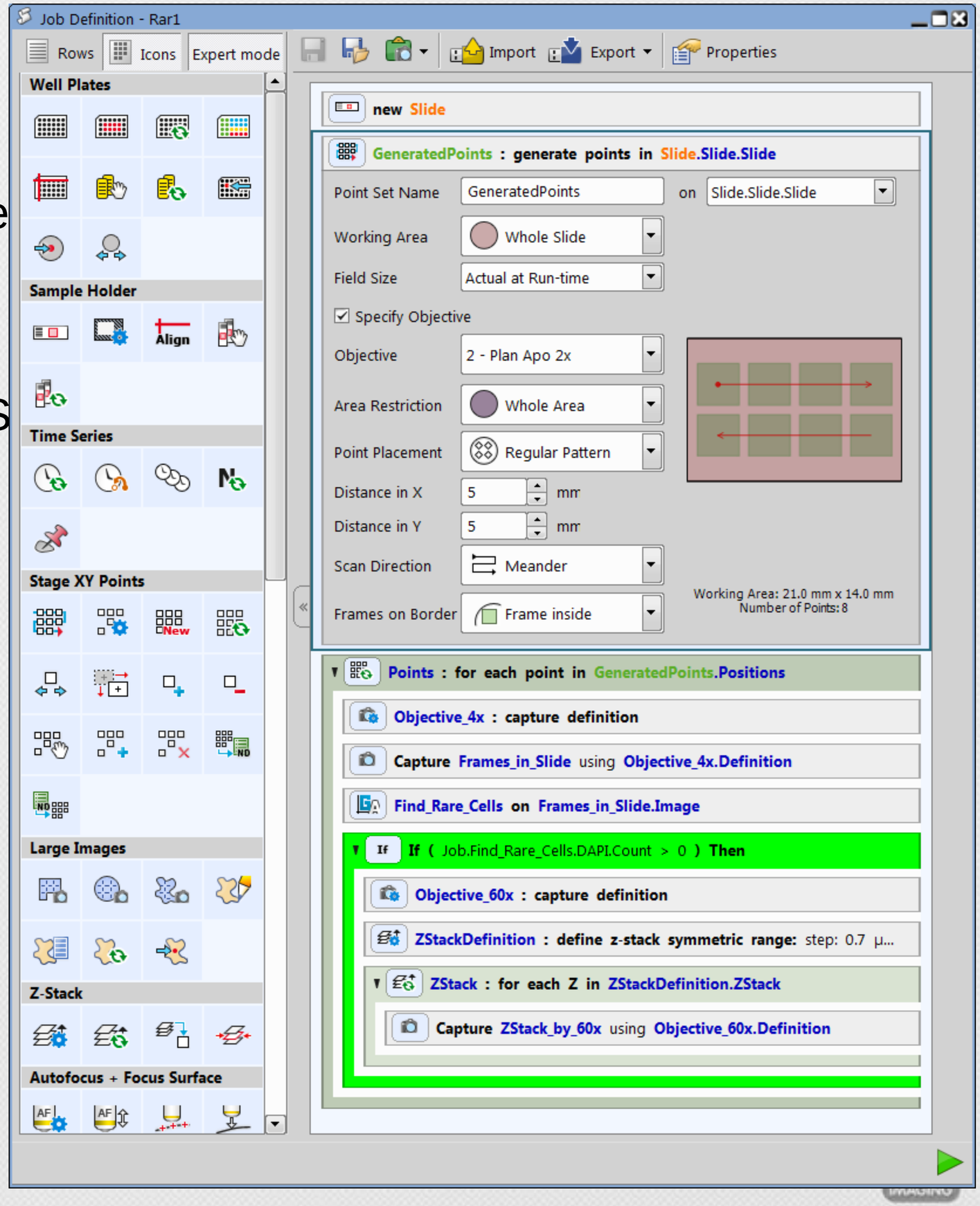



# JOBS

HCS and Live-cell imaging bring ne
challenges in terms of flexibility.

NIS Elements face them with JOBS

- Visual experiment building
- Non-orthogonal acquisition
- Conditional acquisition
- *Analysis during acquisition*
- *Macros during acquisition*
- *Storage as TIFFs (OME soon)*



NIS Elements
Imaging Software
Advanced Solutions for your imaging World

Job Definition - NewJob4*

Rows | Icons | Expert mode | Import | Export | Properties

**Well Plates**
- Define Plate
- Select Wells
- Loop over Wells
- Label Wells
- Align Plate
- Plate Loader
- Manual Plate List
- Loop over Plates
- Move to Well
- Move to Well Center
- Move to Previous/Next Well

**Sample Holder**
- Define Slide
- Define Stage Area
- Align Slide
- Manual Slide List
- Loop over Slides

**Time Series**
- Time Lapse
- Redefine Time Lapse
- Time Sequence
- Repeat N times
- Keep Object In View

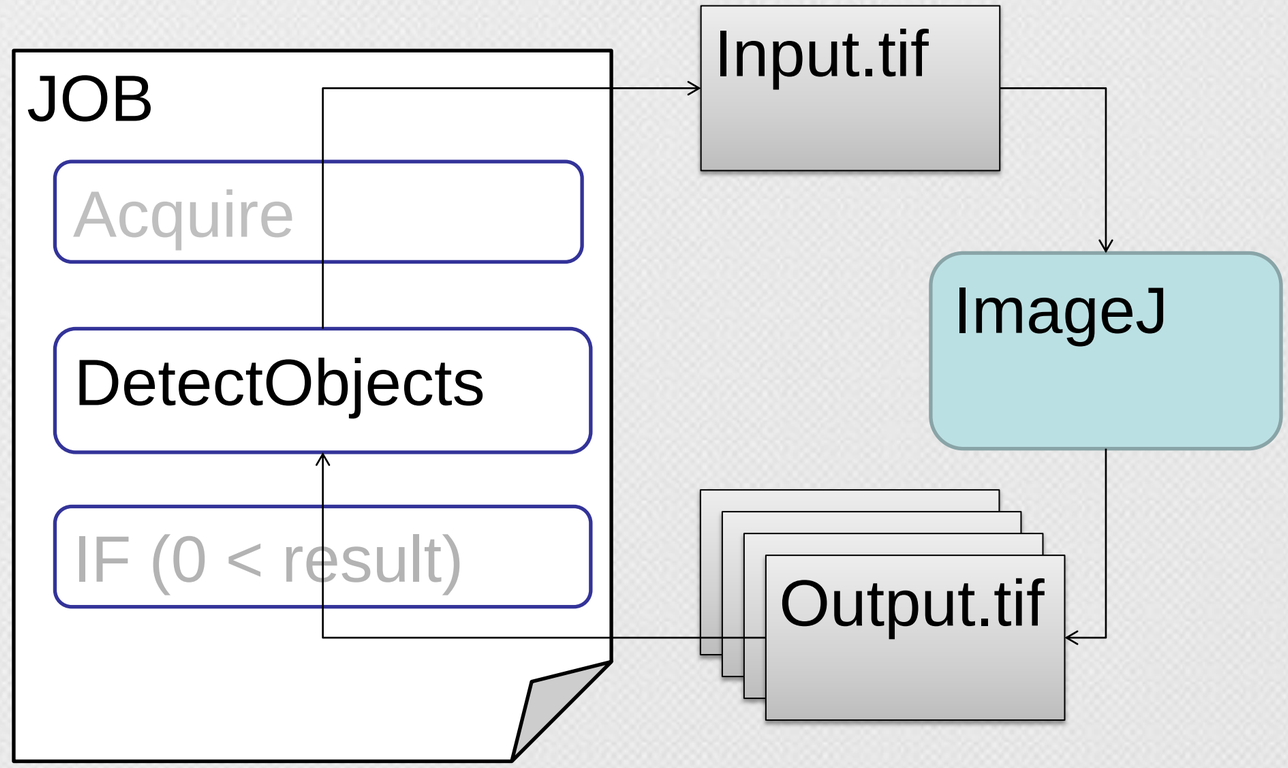
**Stage XY Points**
- Generate Points
- Predefined Points
- New Point Set
- Loop over Points
- Move to Previous/Next Point
- Offset Point Set
- Add Point to Point Set

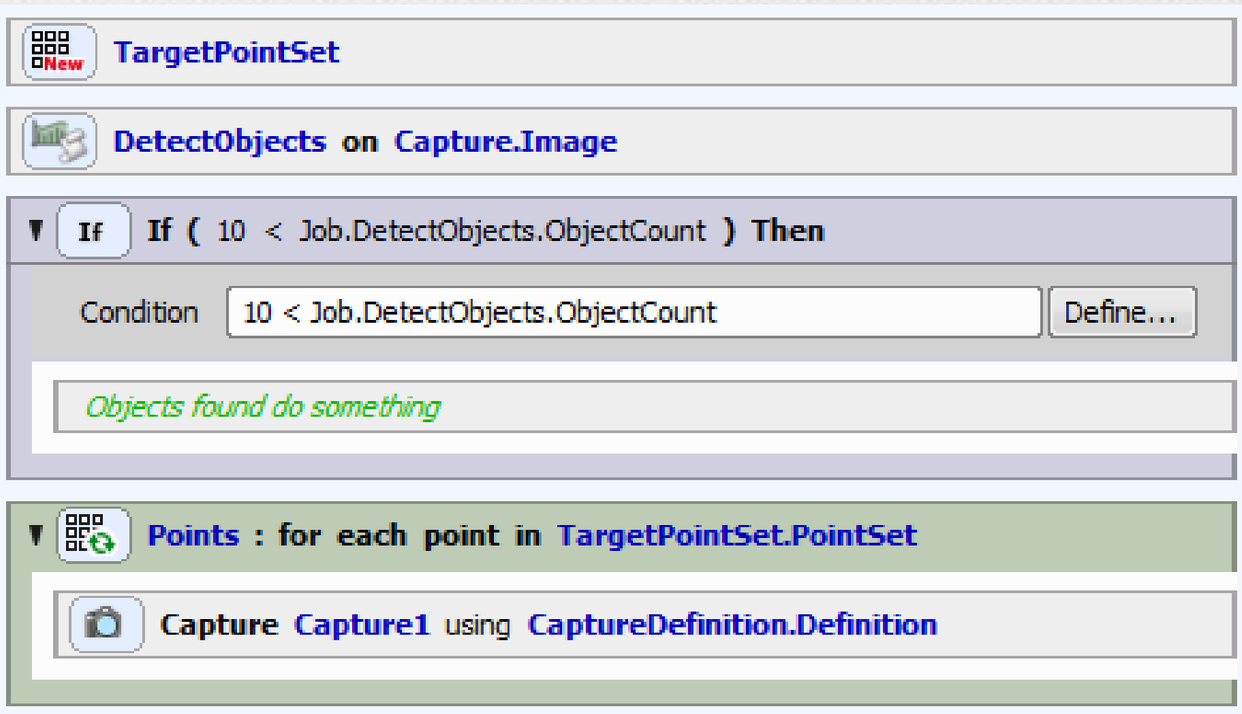
Drag tasks from the Left pane and Drop them here

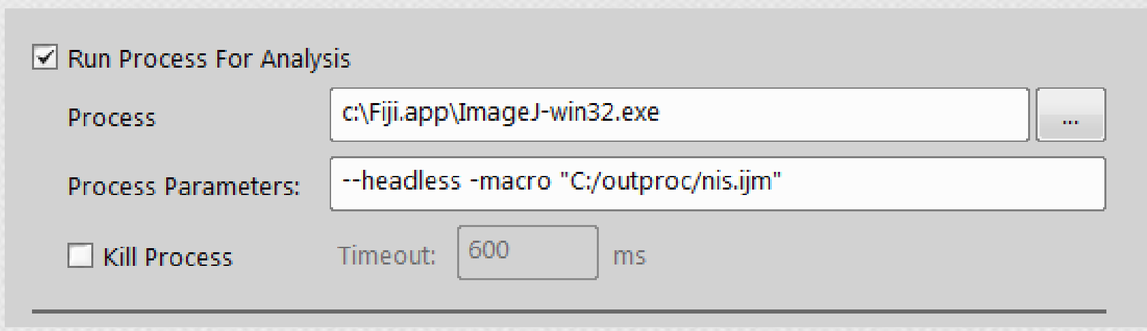
# The OutProc Analysis Task

A process is run on each frame.

It reads input image and writes its output mask images and text results.



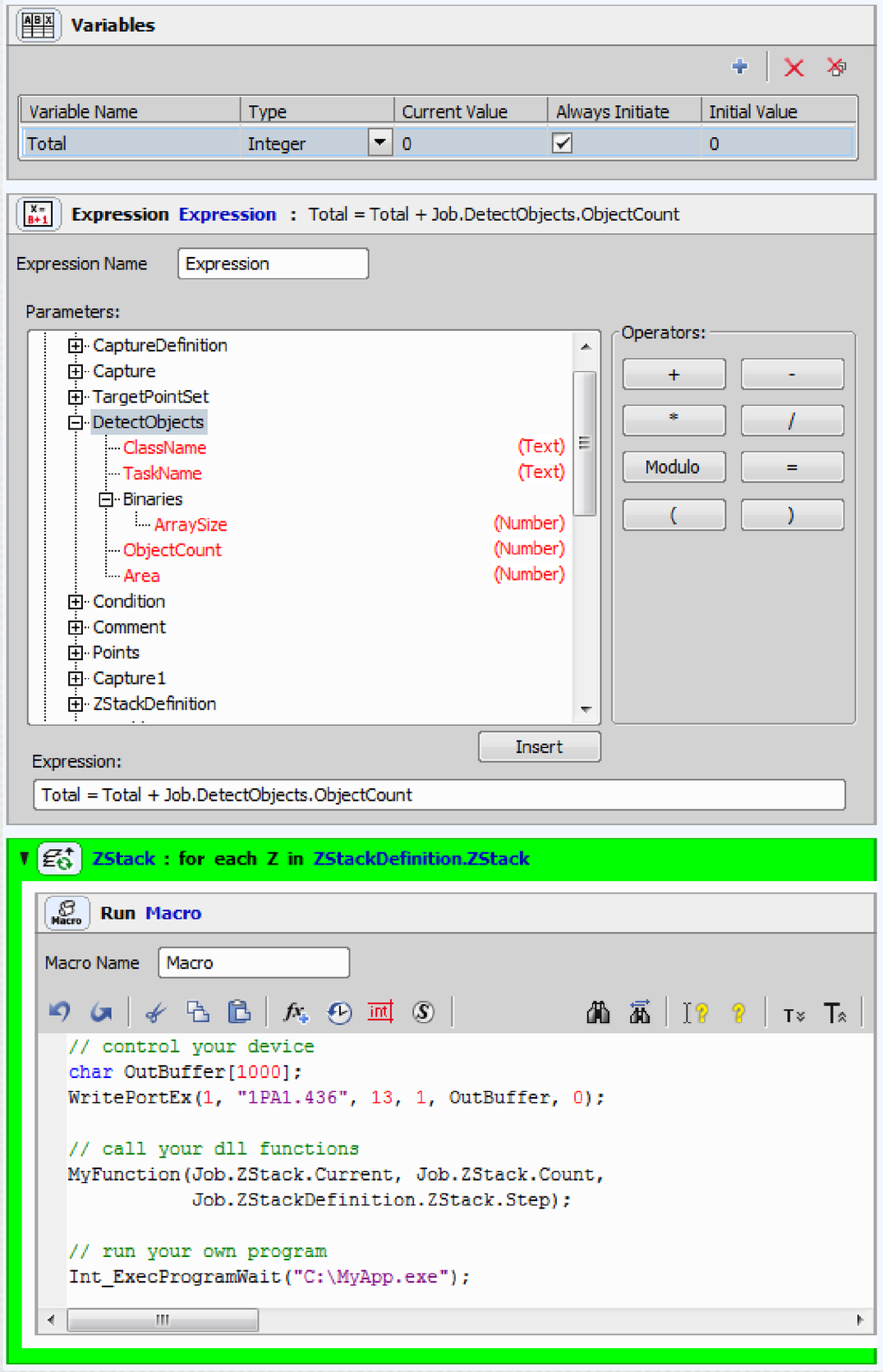



# Macros in JOBS

When Variables and Expression tasks are not enough:

- There is “Run Macro” task
- It is executed based on its position and the block it is in.
- The hardware is in defined state when it is executed.
- It can access other task parameters
- It can modify some (delays, steps, …)



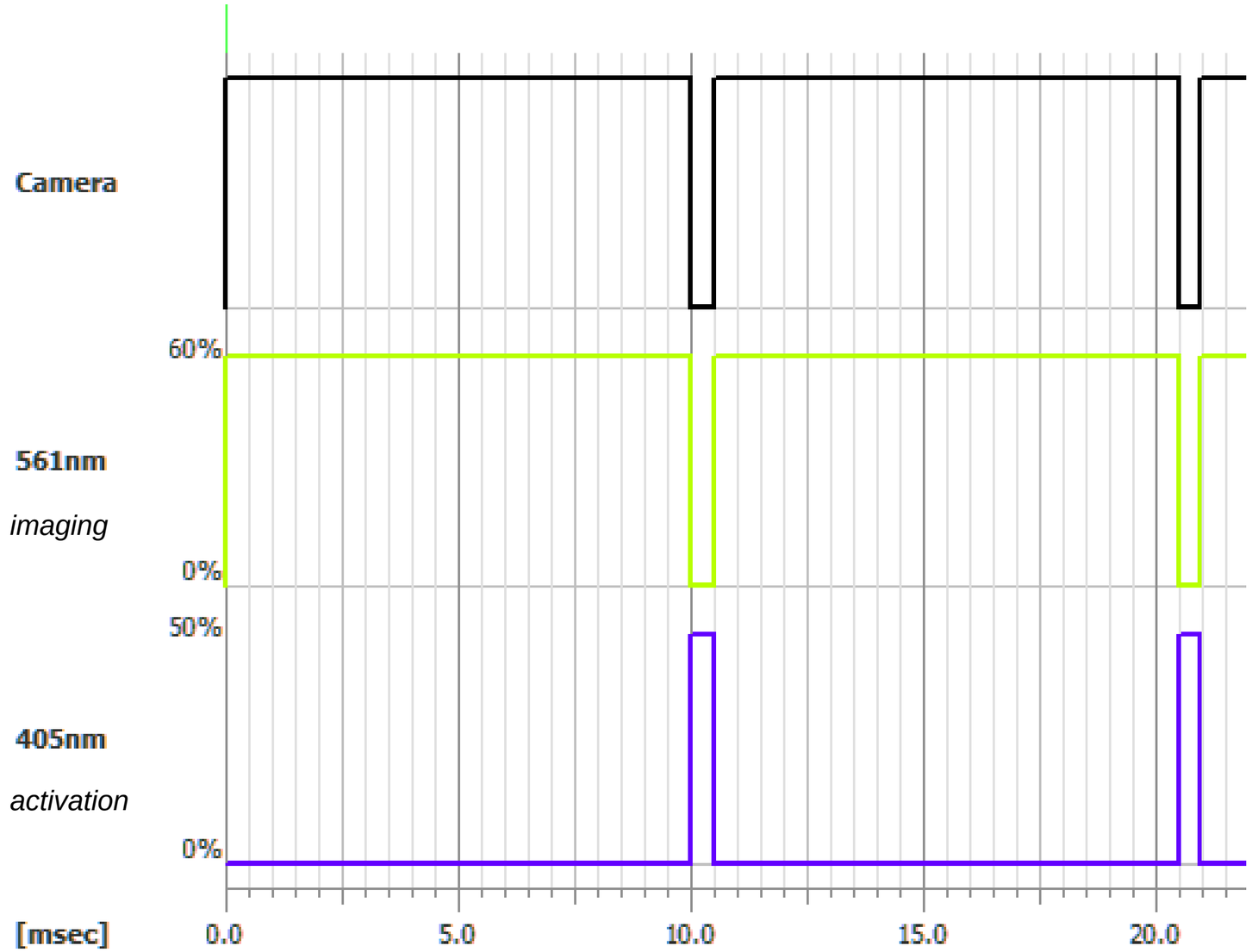
# Illumination Sequence

- Experiment driven by TTL-only signal
- Master of timing is camera FIRE signal
- Waveform generator is NIDAQ card
- Integrated devices:
  AOTF, LED, piezoZ, DMD

+

- ANY device with analog interface
- ANY device with TTL interface

*Example: Photo-activation during transition period*



# NIS Elements calculate – camera timing for

- Sensor mode: normal/overlapped
- Binning /ROI
- Vertical Shift speed
- Rolling/Global shutter

*Figure 220.*

**Flash 4.0**
sensor = overlapped, rolling shutter
transition = 10 msec, @ 2048 x 2048
exposure = 20 msec

| | Cycle Definition | | |
|---|---|---|---|
| | Transition 10 msec | Global Exposure 10 msec | Transition 10 msec |
| Camera | | | |
| TTL signal (Global Exposure) | Low | High | Low |
| Illumination | | | |
| Cycle Time | 20 msec | | |

*Figure 221.*

**iXon 897 Ultra**
sensor = normal
readout = 24.6 msec
expozice = 50 msec @ 512x512
Vshift = 3.3 microsec
Illumination mode: Follow Exposure

| | Cycle Definition | | | |
|---|---|---|---|---|
| | Exposure 50 msec | Transition 1.7 msec | Dead 21.2 msec | Transition 1.7 msec |
| Camera | | | | |
| TTL signal (Exposure) | High | Low | Low | Low |
| Illumination | | | | |
| Cycle Time | 74.6 msec | | | |

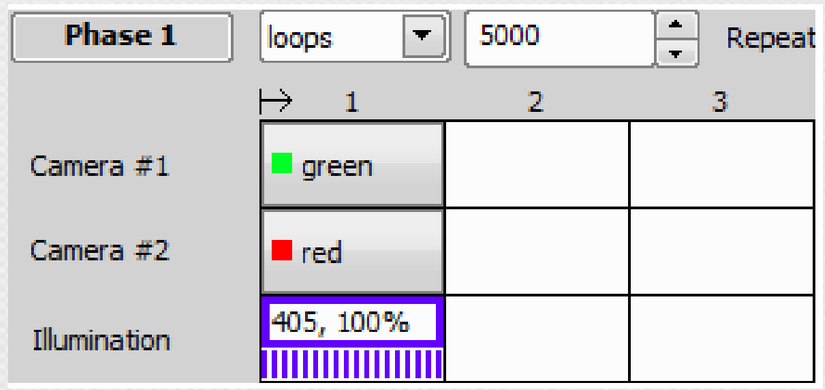
*Figure 222.*

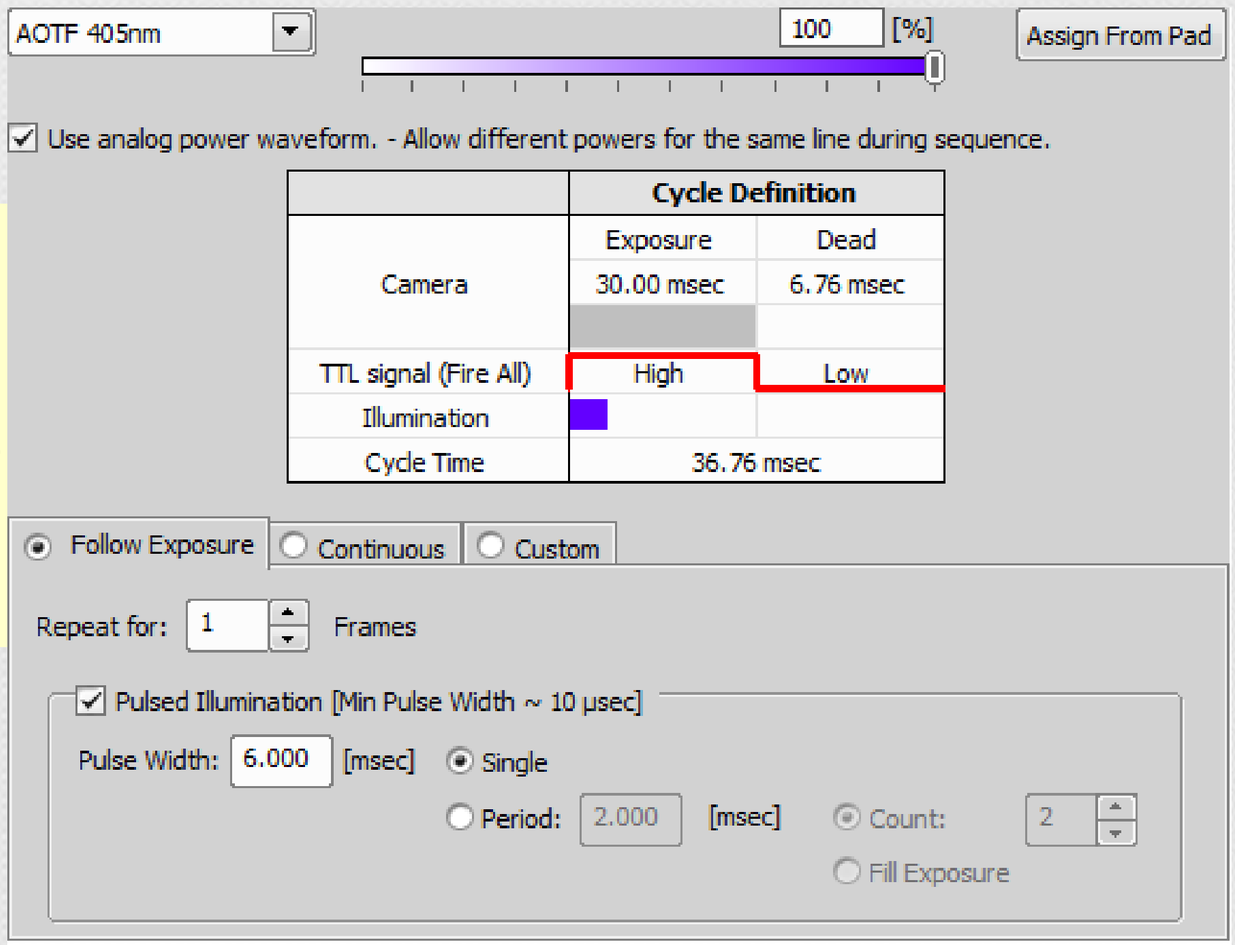
**iXon 897 Ultra**
sensor = overlapped
transition = 1.7 msec
exposure = 1 frame, 19 msec @ 512x512
Vshift = 3.3 microsec
Illumination mode: Follow Exposure

| | Cycle Definition | |
|---|---|---|
| | Exposure 19.3 msec | Transition 1.7 msec |
| Camera | | |
| TTL signal (Exposure) | High | Low |
| Illumination | | |
| Cycle Time | 21.0 msec | |

# A user defines – how to

- Organize camera frames into channels
- Illuminate sample (AOTF, LED):
  follow exposure, pulsed illumination,
  during dead time, …
- Change patterns (DMD)
- PiezoZ stacks, set PiezoZ offset,




- For a device with analog/TTL interface
  - set value at the given time-points during camera cycle: exposure-start, exposure-start + offset, exposure-end, during dead-time

# Thank you…

Questions?