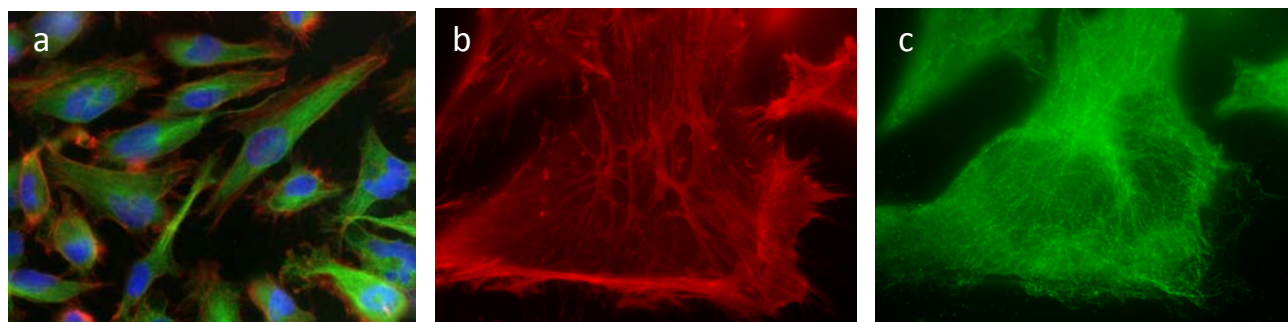


Immunostaining Cells on the M04

Sample Protocol

The ability of our system to automate rapid flow switching in a cell culture environment lends itself as a useful alternative to traditional labor-intensive procedures for immunostaining cells. The protocol below offers an example of how the ONIX system can be used to immunostain α -tubulin, while simultaneously staining cells' nuclei and actin filaments, easily and efficiently.

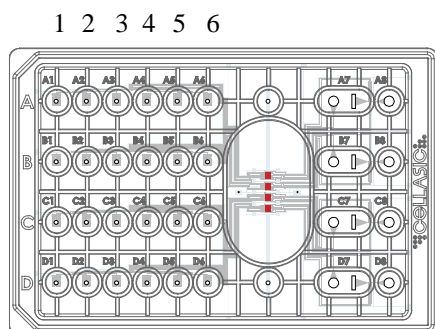


HeLa cells immunostained in the M04 microfluidic plate. (a) 20x fluorescence image, (b) 100x image of actin staining, and (c) 100x image of tubulin staining.

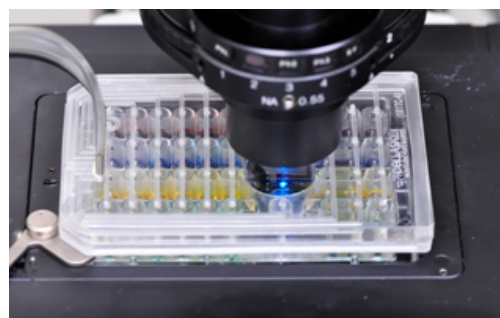
Setup

1. Load and culture the cells of interest in a M04 plate
2. Download and save the “M04-Immunostaining-Protocol.txt” file found on the website
3. On the day of the experiment, prepare 0.5 ml of each of the six solutions given in the table below

	Solution	Details	Purpose	Wells
1	PBS	Phosphate buffered saline (without $\text{Ca}^{2+}/\text{Mg}^{2+}$)	Washing	A1-D1
2	Paraformaldehyde	4% Formaldehyde	Fixing	A2-D2
3	PBT	0.1% TritonX-100 in PBS	Permeabilizing	A3-D3
4	10% NGS	10% non-immune normal goat serum (Invitrogen)	Blocking	A4-D4
5	1° Ab Solution	1 $\mu\text{g}/\text{mL}$ anti- α -tubulin (bovine) mouse IgG, 10 $\mu\text{g}/\text{mL}$ Hoechst 33342, in 10% NGS (Invitrogen)	Binding α -tubulin Staining nuclei	A5-D5
6	2° Ab Solution	1% BSA, 5 $\mu\text{g}/\text{mL}$ Alexa Fluor® 488 goat anti-mouse IgG (H+L), 8 mg/mL Alexa Fluor 546 phalloidin, in PBS (Invitrogen)	Tagging α -tubulin Staining actin	A6-D6



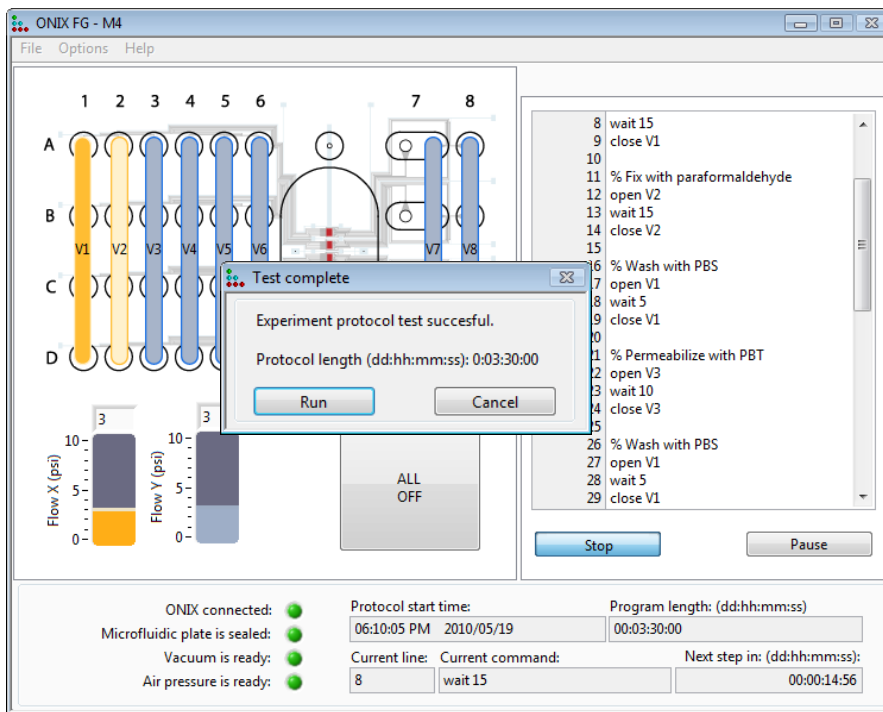
Fill the 6 inlet wells with staining reagents



Seal to ONIX manifold for flow scheduling

Protocol

1. Gently aspirate all wells in the M04 plate
2. Fill each well with 100 µl of solution, as indicated in the table above (leave 7 & 8 wells empty)
3. Vacuum seal the M04 plate to the ONIX manifold
4. Open the ONIX FG software and select M4 under the Mammalian tab
5. Go to File > Open Protocol, and select the "M04-Immunostaining-Protocol.txt" file
6. Run the protocol (it should take 3 hours 30 minutes)
7. After the program has finished, unseal the plate from the manifold
8. View cells with a standard fluorescence microscope using DAPI, FITC and CY3 filters to image nuclei, microtubules and actin filaments, respectively
9. For long term storage cover all wells of the plate with film or foil to prevent evaporation



```

% Immunostaining Protocol

setflow X 3
setflow Y 3

% Wash with PBS
open V1
wait 15
close V1

% Fix with paraformaldehyde
open V2
wait 15
close V2

% Wash with PBS
open V1
wait 5
close V1

% Permeabilize with PBT
open V3
wait 10
close V3

% Wash with PBS
open V1
wait 5
close V1

% Block with NGS
open V4
wait 60
close V4

% Incubate with primary antibody
open V5
wait 45
close V5

% Wash with PBS
open V1
wait 15
close V1

% Incubate with secondary antibody
open V6
wait 30
close V6

% Wash with PBS
open V1
wait 10
close V1

end
  
```

The ONIX FG software allows automating the wash and incubation steps for immunostaining. The laminar flow nature ensures rapid exposure to the cells as well as thorough washing. Values in the flow program (right) can be modified under the "Protocol" tab of the software once opened.