

Instructions for the Olympus DSU spinning disk confocal microscope

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Start up

1. press the ON/OFF switch on UPS1 to power up the computer, monitor, and cameras, log on when Windows is fully loaded
 2. press the ON/OFF switch on UPS2 to power up the microscope, ASI controllers, CoolLED, and Lambda XL light source
 3. launch Metamorph (MM) via the desktop shortcut; MM controls all devices except
 - the intensity of the CoolLED which must be set using the ↑ and ↓ buttons on the control pod
 - the 3 neutral density filters in a carrier on top of the microscope, which can further reduce the intensity of the CoolLED
 - ND2 = 50 % transmittance
 - ND16 = 6 % transmittance
 - ND 2.0 = 1 % transmittance
 - the DIC slider
 4. Default startup settings
 - illum: 00 BF Eyes
 - mag: 10× BF, unless other objective lens has been selected
 - z-drive: ASI Z
 - Lambda XL: 100 % intensity, ND-144
 - CoolLED: 50 % Intensity
 - 3 neutral density filters in a carrier on top of the microscope are in
 - DIC slider is out
 5. if you need epifluorescence, go to the Taskbar, click Start Lambda lamp to enable emission, lamp is operational when the status reads LIT 100% on the LCD display of the Lambda XL
- Caution:* Do not turn off the lamp within the first min of operation, and keep away from the light guide and camera.
- Note:* The taskbar is launched on startup or do Journal > Load Taskbar > load
 c:\Olympus\TASKBARS\default startup Taskbar.JTB

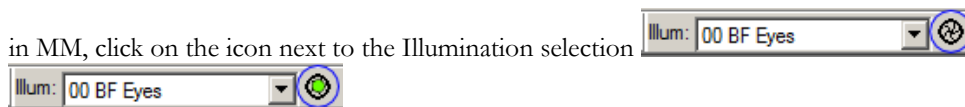


Load the prep

1. start with 10x BF; lower the objective by pressing ESC on the microscope, if necessary
2. rotate the condenser or flip it up to gain access to the stage
3. load the prep on the stage insert; if you want to record any positions and reuse them in subsequent sessions, a consistent way of loading is necessary
4. with the joy stick on the ASI controller, manipulate the area of interest over the objective; tap the top button to toggle between fast and slow mode (shown as f and s on the controller's LCD display)
5. use the ▼ FOCUS ▲ buttons on the microscope to bring the objective to about 3 mm beneath the area of interest
6. in MM use the Illum drop down menu to select a setting for Eyes to locate your sample
 - for bright field, use 00 or 01; adjust the CoolLED power to 4 % with the ↓ button on its control pod
 - for epifluorescence, select a setting appropriate for the fluorophore of interest, use 02 - 04



7. in MM, click on the icon next to the Illumination selection



to toggle the shutter open

8. focus with

- the microscope's focus knob: turning away from you lowers the objective away from the stage; use F/C to toggle fine and coarse mode; range is over a few mm
- Z knob on the ASI controller: turning clockwise lowers the objective; range is $\pm 175 \mu\text{m}$

Acquire an image

1. on the Taskbar, click to select either the ORCA-Flash or EM-CCD camera

2. click Camera Acquire Settings to launch the Acquire panel

- on the left pane, enter an exposure time appropriate for the desirable frame rate (see [camera exposure settings](#) in the Appendix)

- on the upper pane of the Display tab, check Auto scaling for optimal display of the current intensity range

Annoyance: Unchecking Auto Scaling will leave the Min and Max handles at the last used positions, one needs to drag those to the left and right ends to get the full range.

Tip: It may be easier to adjust the display level here than at the live window; adjustment made at the live window may not update the position of the handles here correctly.

- on the lower pane of the Display tab, check Display saturation markers: pixels with 0 intensity are displayed in cyan and saturated ones are red; however, beware of confusion when using a cyan or red LUT

Annoyance: Changing camera may confuse MM about the status of the check box, i.e., it does not display saturation markers even when the box is checked; deselect and reselect the check box can fix it.

- under the Special tab, check that frame average is set to 1
- stay in the Special tab for access to the gain setting and digitizer selection

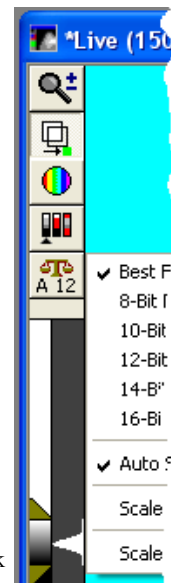
- ORCA-Flash is 12-bit, gain setting from 0-255
- EM-CCD is 16-bit with 2 modes
 - std mode: gain (1-5)
 - EM mode: gain ($0.5 \times -5 \times$), EM gain (4-1200)

- on the lower left pane, click Show Live to bring up the live window which has menu bar on the left, click Stop Live or F2 to close the live window

- zoom: left click zooms in, right click zooms out
- overlay
- LUT
- threshold
- bit depth: higher bit depth provides more intensity levels i.e., better definition but results in a larger file size and acquires at a slower frame rate
 - 8-bit (0-255)
 - 10-bit (0-1023)
 - 12-bit (0-4095)
 - 16-bit (0-65535)
- live histogram with min and max handles for display

Tip: The default has auto bit depth (Best Fit Range) and Auto Scale enabled. Once you are in the ballpark of a good image, fine tuning is best done without these auto settings.

Warning: Overexposure can damage the EM-CCD camera. It is configured to beep and shutdown in such condition. One should press F2 or click Stop Live immediately when that happens. Reduce illumination intensity, lower the gain, or decrease exposure time can resolve this. You may need to click on Reset and Reset protection in Acquire panel to reactivate the camera.



3. adjust the illumination intensity: use the least amount of light and shortest exposure time to keep the prep alive

- for epifluorescence, the Lambda XL has a ND filter adjustable from 1 to 144 (max intensity), and lamp intensity from 25 % to 100 %; for more stable output, use $\geq 30 \%$

Tip: each Illumination setting has a preset lamp intensity and ND value, it is always a good idea to check before going live by doing either one of the following

- Device > Device Control, then Component control to select either Lambda XL Lamp Intensity or Lambda XL Neutral Density Shutter A
- Device > Configure illumination
- check line 2 & 4 on the display panel of the Lambda XL

- for transmitted light, the CoolLED starts up at 50 % but usually only needs less, e.g., 4 % with no ND filters or 50 % with ND 2.0 at 10×

Warning: ND filters are especially useful when using the EM-CCD camera to avoid damaging the EM circuit from overexposure at lower magnifications

Things to consider:

- ideally, one would increase the exposure time or illumination intensity to get an image with the max bit depth and keeping the brightest desirable features just below saturation
 - for samples of low signal intensity, in order to achieve a desirable frame rate, one may have to sacrifice image quality e.g., use higher gain, reduce bit depth, reduce image dimension (binning, ROI) etc.
4. focus with either the microscope's focus knob or z knob on the ASI controller
 5. click Acquire to capture an image
 6. do File > Save...

Simple acquisitions

- z-stack (make sure to focus with the selected z-device)
 - by specifying top and bottom
 1. do Devices > Focus... to open the dialog box
 2. select the Configure tab
 3. focus to the top of the stack, hit Set Top
 4. optionally, focus to your point of reference in the stack, hit set Home
 5. focus to the bottom of the stack, hit Set Bottom
 6. do Acquire > Acquire Z Series to open the dialog box
 7. enter the desirable z step size in the textbox of microns(s): [*size*]
 8. select Start at: Top
 9. select Move to: Bottom
 10. select After: Final Position
 11. select mode of Image Storage
 12. select Shutter: [Current Shutter] to use the currently active Illumination setting
 13. click OK to acquire
 - by specifying the number of planes and z step size
 1. do Acquire > Acquire Z Series to open the dialog box
 2. enter the number of planes
 3. enter the desirable z step size in the textbox of microns(s): [*size*]
 4. focus to top of the stack
 5. select Start at: Current Position
 6. select Move to: Planes*Spacing
 7. select After: Final Position
 8. select mode of Image Storage
 9. select Shutter: [Current Shutter] to use the currently active Illumination setting
 10. click OK to acquire
- Stream Acquisition
 - stream time
 1. do Acquire > Stream Acquisition
 2. select Acquisition Mode: Stream to RAM for max speed
 3. enter the number of frames
 4. select Initial Illum: [Current Shutter] to use the currently active Illumination setting
 5. click Acquire
 - stream z
 1. set up the same as stream time above, number of frames is now z positions i.e., # of planes
 2. check the box "Use with high-speed Focus Motor" (select ASI Z for max speed)
 3. click on the now activated Focus tab to specify the z-stack parameters (see z-stack above)
 4. select Initial Illum: [Current Shutter] to use the currently active Illumination setting
 5. click Acquire
 - stream λ
 1. set up the same as stream time above, number of frames is now wavelength sets
 2. check the box "Use with high-speed Wavelength changer"
 3. click on the now activated Wavelength tab to specify the number of wavelengths and Illumination setting for each

4. click Acquire

- time series
 - timelapse
 1. do Acquire > Acquire Timelapse
 2. enter desirable Time Interval and Duration
 3. select mode of Image Storage
 4. select Illum: [Current Shutter] to use the currently active Illumination setting
 5. click OK to acquire
 - z stack time series
 1. set up the same as timelapse above
 2. check the box "Acquire Z-Series on each interval"
 3. click on the now expanded Z-Series Acquisition Settings to specify the z-stack parameters (see z-stack above)
 4. select Illum: [Current Shutter] to use the currently active Illumination setting
 5. click OK to acquire

Applications and procedures

- Multi Dimensional Acquisition (MDA): for combinations of timelapse, multi-location, multi-channel, z stacks, and streaming
 1. launch from Taskbar or Toolbar
 2. in Main tab, check any desirable mode
 3. define the file save location, base file name, and description
 4. set up each section
 5. review the setting in the Summary tab
 6. click Acquire to proceed
 7. (optional) save the setting

Annoyance: When you launch MDA, settings last used will override any current settings including Illumination, lamp power, ND filter setting, camera selection etc. The settings are not synced until some parameters are changed usually from the MDA panel.

- Scan Slide (SS): for tiling and stitching
 1. launch from Apps > Scan Slide
 2. in main tab, check any desirable mode
 3. define the file save location, base file name, and description
 4. set up the camera

Inconsistent design: Settings used in SS is independent of any current settings including Illumination, lamp power, ND filter setting, camera selection etc. When the EM-CCD camera is selected, one of the 2 gain settings is absent and can only be set in the Acquire panel.
 5. proceed with calibration unless you are going to load an existing one for the same objective and camera combination
 6. review the settings in the Summary tab
 7. click Scan to proceed
 8. (optional) save the setting
- Tiling with z-stack, currently only deals with single-channel
 1. select the desirable Illumination setting
 2. set up for collecting a z-stack
 3. launch Tiling with z-stack from the Taskbar and follow the on-screen instructions for
 - setup the path and file name
 - setup % overlap
 - set the coordinates for the boundaries of the object of interest
 - optionally, open a log file to record the XY coordinates of each tile
 - acquire z-stack and then close each one when done
 4. stitching can be done in ImageJ using the Grid/Collection stitching plugin

Check out

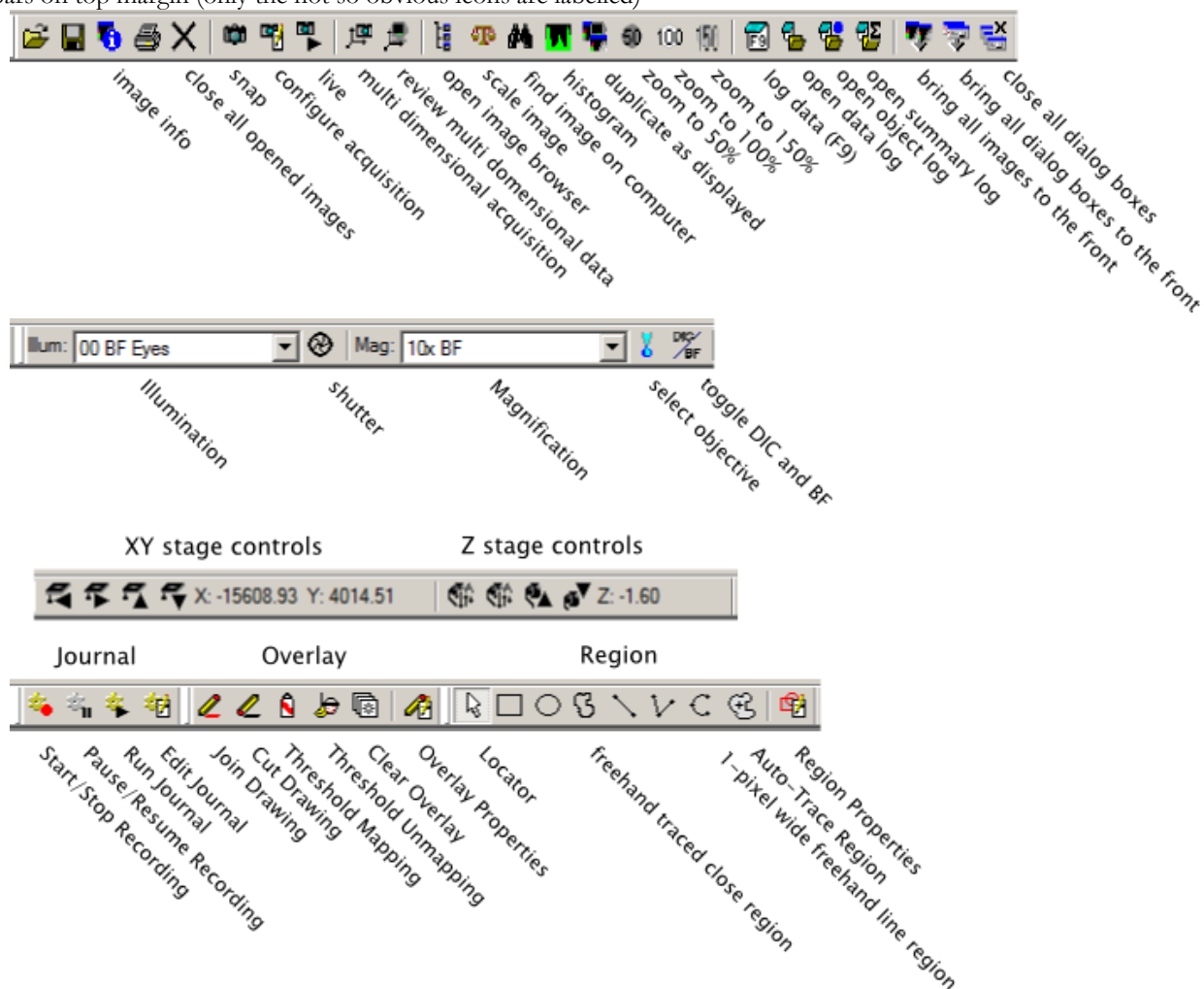
1. in Metamorph, click "Turn-Off Lambda Lamp" on the Taskbar to turn off the light source; it is also OK to leave the lamp on for the next user
2. hit Esc on the microscope to lower the objective turret, remove your prep, remove oil/immersion fluid on objectives as needed, switch to the 10× objective
3. save all your files and quit Metamorph

4. copy your data to external storage media or network file servers as needed
5. submit your usage using the google form
6. if no one signs up for the next 2 sessions, please proceed to shutdown everything; otherwise, just log out of Windows
 - shutdown Windows
 - press the ON/OFF switch on UPS1 for 2 S to power it off
 - when the Lambda XL display shows the lamp temperature ≤ 40 °C, press the ON/OFF switch on UPS2 for 2 S to power it off
 - put the cover back on the microscope
7. clean up the work area

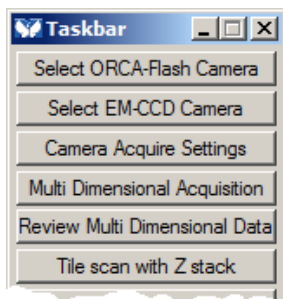
Appendix

Metamorph basics

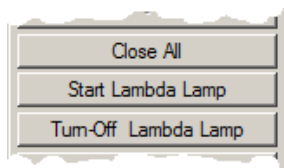
1. frequently used operations are grouped in
 - tool bars on top margin (only the not so obvious icons are labelled)



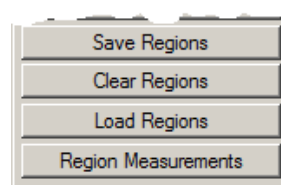
- Taskbar



camera selection and acquire settings, MDA and Tile scan



lambda lamp control



region tools

2. basic configuration

- Magnification (Mag): access via the tool bar drop down menu, selects the objective lens and the corresponding DIC prism in the condenser for transmitted light mode
 - bright field (BF)
 - DIC, also requires manually inserting the DIC slider above the front turret
- Illumination (Illum): access via the tool bar drop down menu, determines how the signal from the sample is generated and processed; exciter, beam splitter, and emitter in case of epifluorescence, power of the Lambda XL and ND setting, shutter operation, and DSU selection
 - front light path: uses the front filter turret with the binocular (Eyes, **settings start with 0**) or the front left side port ORCA-Flash camera (Flash, **settings start with 1**)
 - rear light path: uses the rear/DSU filter turret and the rear port EM-CCD camera with or without DSU (**settings start with 2**)
- z-drive: go to Device > Device Control > Configure tab, click on Select Z Devices... to choose Olympus Z or ASI Z
Annoyance: Any memorized locations will only contain the currently active z value which makes it hard to re-establish these locations across sessions; unless one chooses Olympus Z and set ASI Z to zero.
- notifications and crashes
 - in some dialog boxes e.g., MDA, MM will display a red square when it detects incompatible settings, a yellow square for non-fatal but questionable ones, and green for good
 - when MM/Windows fails or crashes, check the RMT indicator light on the microscope IX2 controller, it must be off before restarting MM; if RMT is stuck on, power cycle the controller (arrowhead): power off, wait 1 min, power on

3. reading MM files in ImageJ/Fiji

- ImageJ ([download from ImageJ website](#)) will read .tif files but will not know the spatial calibrations or other metadata
- do Plugins > LOCI > Bio-Formats Importer to load MM .tif, and .nd files to correctly apply the included metadata ([download loci_tools from OME website](#)); however, sometimes MM can record the wrong calibration values, so the following table of spatial calibration can be useful

Spatial calibration of both camera at full frame

Mag	EM camera, 512 × 512		Flash camera, 1960 × 1440	
	Description ¹	μm/pixel	Description	μm/pixel
4x	4x BF EM	4.020	4x BF Flash	0.903
10x BF	10x BF EM	1.622	10x BF Flash	0.362
10x DIC	10x DIC EM	1.616	10x DIC Flash	0.361
20x Air BF	20x BF EM	0.812	20x BF Flash	0.181
20x Air DIC	20x DIC EM	0.810	20x DIC Flash	0.181
20x Oil BF	20x Oil BF EM	0.810	20x Oil BF Flash	0.181
20x Oil DIC	20x Oil DIC EM	0.807	20x Oil DIC Flash	0.181
20x Water BF	20x Water BF EM	0.811	20x Water BF Flash	0.181
20x Water DIC	20x Water DIC EM	0.808	20x Water DIC Flash	0.182
40x Water BF	40x Water BF EM	0.402	40x Water BF Flash	0.090

¹as used in Measure > Calibrate Distances



Mag	EM camera, 512 × 512		Flash camera, 1960 × 1440	
	Description ¹	μm/pixel	Description	μm/pixel
40x Water DIC	40x Water DIC EM	0.400	40x Water DIC Flash	0.090
60x Oil BF	60x Oil BF EM	0.270	60x Oil BF Flash	0.060
60x Oil DIC	60x Oil DIC EM	0.270	60x Oil DIC Flash	0.060
60x Water BF	60x Water BF EM	0.270	60x Water BF Flash	0.060
60x Water DIC	60x Water DIC EM	0.269	60x Water DIC Flash	0.060
¹ as used in Measure > Calibrate Distances				

- no support for .scan of Scan Slide yet

Microscope operation

The description here is only valid when Metamorph is loaded. Some of the buttons, especially the keypad, have different functions assigned when the microscope is operated alone

1. buttons on the microscopes

- side:
 - FOCUS - turn towards the operator raises the objective
 - F/C - toggles fine and coarse focus control
 - ESC - drops the objectives to the lowest position

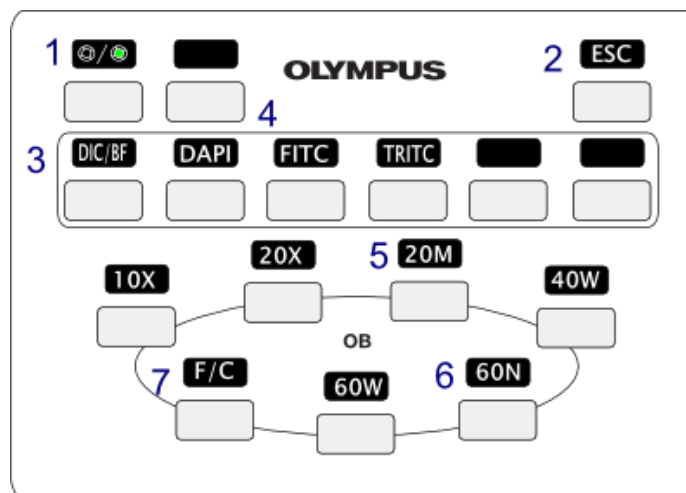


- front left:
 - top button - toggles Eye and front left camera port (should use the Illum settings instead)
 - ▼ FOCUS ▲ - rapid down or up movement of objectives
- front right: top button - coarse focus
- front lower: controls in the grey out area are not implemented



- keypad: configured for using the binoculars
 - shutter control
 - Focus ESC
 - DIC/BF toggle
 - DAPI/FTTC/TRITC: epifluorescent channels for Eyes
 - 20M: water or oil immersion, depends on the loaded set
 - 60N: 60× oil immersion or other exotic lenses, depends on the loaded set


7. Focus Fine/Coarse



2. switch objectives

- dry objectives: use the Mag pull down menu
- immersion objectives:

1. focus on object of interest

2. click on the  button on the top toolbar
3. select the Mag combination in the dialog box and click OK
4. follow the on-screen instruction to offset the stage to add immersion fluid
5. click Yes to return the stage to object of interest
6. when done, press ESC to lower the objective
7. remove the slide
8. remove the immersion fluid from the objective lens with Ross lens paper
9. remove any fluid from the slide and clean it first before selecting a dry objective

Details: When activated, the routine remembers the current xyz position and Mag selection, drops the z down, asks for the new Mag selection and switches to it when you click OK or Close, pops up a dialog box to prompt you to offset the stage to add immersion fluid. On clicking Yes, the stage returns to the remembered xyz position; otherwise, on clicking No, the routine restores the remembered Mag and the stage returns to the remembered xyz position.

3. switch objective sets

- currently we have 3 sets defined
 - default: 10×, 20×, 20× Water, 40× Water, 60× Water, and 60× Oil
 - set+20× Oil: 20× Water replaced by 20× Oil
 - set+4×: 60× Oil replaced by 4×
- to load a specific set
 1. request Facility staff to switch in the objectives and select the set in MMAdmin
 2. Facility staff needs to modify the Startup journal to load the appropriate Keypad (.okp) and Mag (.MGS) files
 3. user will need to manually load the appropriate calibration file (.CAL) in Measure > Calibrate Distances once

Immersion objective lenses with correction collar (the knurled ring with white numbers)



40×/1.15 NA water immersion objective, set at 0.17
(for 0.13 - 0.25 mm thick coverglass)



60×/1.2 NA water immersion objective, set at 0.17
(for 0.13 - 0.21 mm thick coverglass)

Note: Please consult facility staff if it is the first time you want to adjust the correction collar.

Camera exposure settings

Note: When the exposure in the software is set shorter than the minimum exposure time supported by the camera, Live view will be shown at the minimum exposure time as constrained by the hardware; however, the acquired image will be exposed at the shorter exposure time gated by the software.

ORCA-Flash camera: frame rates at some commonly used exposure times

exposure time (ms)	frame rate (FPS)
0.021-20	45-35
30	30
40	24
100	10
200	5
300	3
500	2

EM-CCD camera: frame rates at minimum exposure time of the 5 digitizers

digitizer	exposure time (ms)	frame rate (FPS)
690 kHz (std)	488.45	2
2.75 MHz (std)	122.12	8
690 kHz (EM Gain)	488.45	2
2.75 MHz (EM Gain)	122.12	8
11 MHz (EM Gain)	30.53	30