

### **Introduction of Spinning Disk Confocal Microscope**

In traditional confocal microscope, the galvanometer-based scanning mechanism can only scan the specimen pixel by pixel, thus the scanning speed of the system is relatively low. To increase the scanning speed, microlens-enhanced dual-spinning Nipkow disk technology can be applied, shown in Figure 1 below:

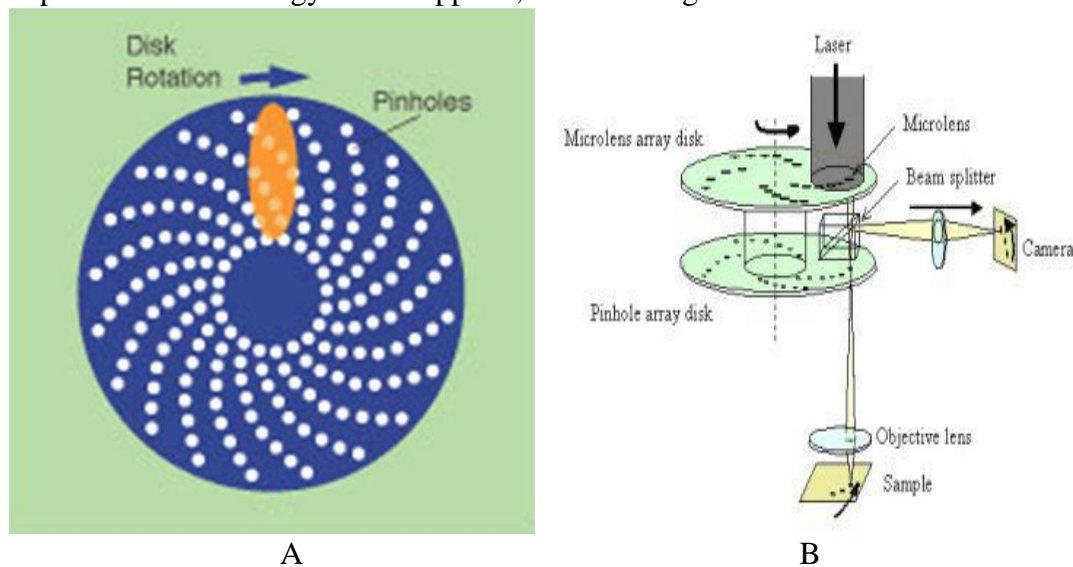
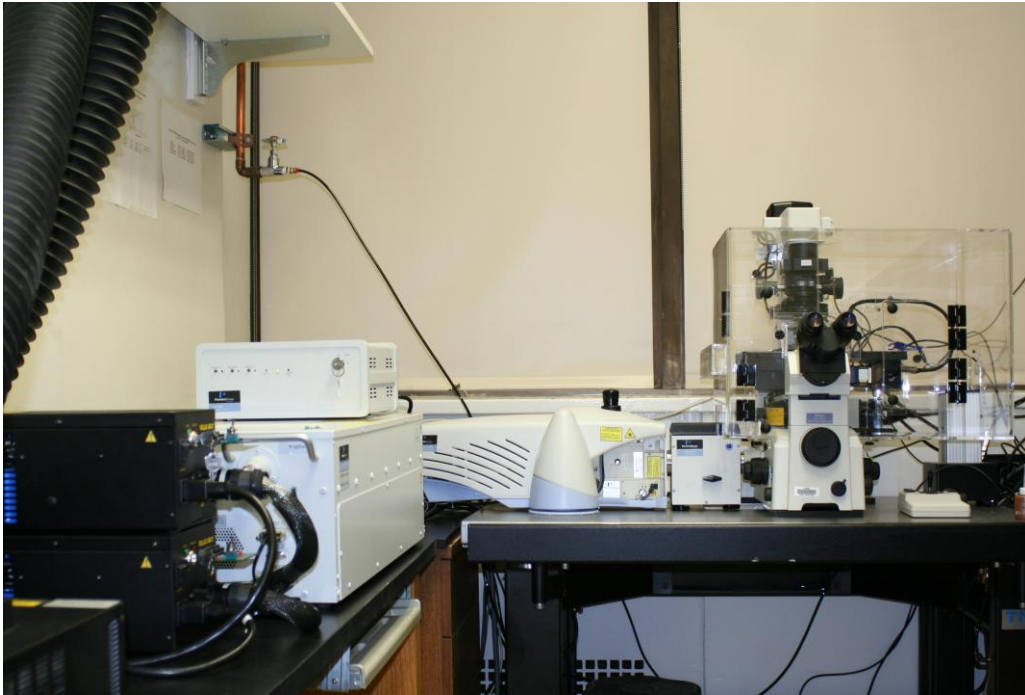


Figure 1 Microlens-enhanced dual-spinning Nipkow disk technology

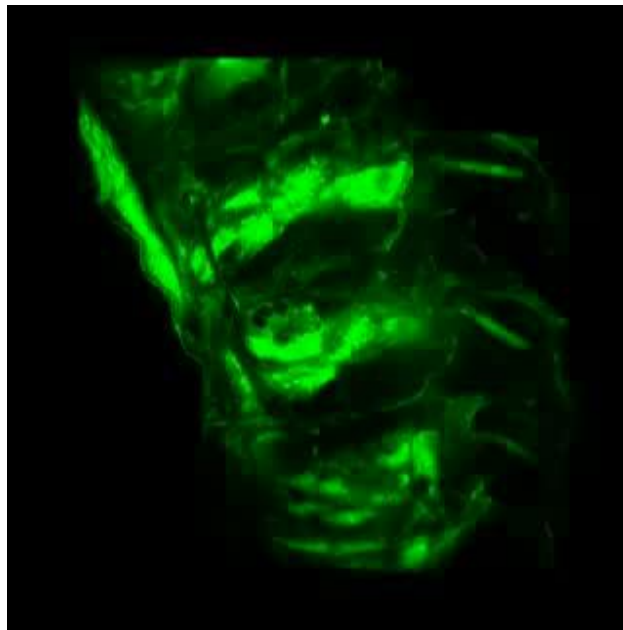
A: Nipkow disk; B: Microlens-enhanced dual-spinning Nipkow disk

The Nipkow disk (shown in figure 1-A) refers to a type of scanning disk with multiple, symmetrically placed spirals of pinhole apertures through which illumination light is passed and split into multiple 'minibeams'. When spun, the light scans the specimen in a raster pattern. As the disk rotates, each beam emerging from a pinhole scans across a portion of the field. Light emitted from the specimen can be detected to generate a real image of the specimen that can be projected onto a detector – normally sensitive, cooled, digital CCD camera or the human eye. To ensure good confocality with this multi-beam technology, the pinholes on a Nipkow disk must be placed up to 10 diameters apart. This limits the light throughput of traditional Nipkow disks to only 1% of the light shining onto the specimen. To overcome this problem, second collector disk containing microlenses is placed in front of the Nipkow disk, as shown in figure 1-B. The microlenses—one for each pinhole—ensure that most of the light illuminating the disk is focused onto the pinholes. Transmission efficiency is increased from 1% to nearly 70% , allowing the specimen to be illuminated with a sufficient quantity of light. In this type of scanning mechanism, since multiple pinholes are illuminated at any one time, the specimen is excited by multiple mini-beams simultaneously. Thus compared to the traditional confocal system, the scanning speed is increased up to 1000 times.

The available system using the above spinning disk technology in our Bio-imaging facility is the UltraView ERS confocal system from PerkinElmer, shown in Figure 2 below:



A



B

Figure 2 UltrView ERS system and demo image  
A: UltraVIEW ERS confocal system; B: Sample image by spinning-disk confocal

## Starting-up procedure



Figure 3 Power buttons for UltrView ERS system

A HAMAMATSU Camera Control Unit: ON

B Xcite 120 Fluorescence Illumination Unit: ON (wait for 5 minutes for best performance)

C NanScan Z Control Unit: ON

D Nikon Microscope Power Unit: ON

E Incubator Temperature Control Unit: ON

F UltraView ProScan II Unit: ON

**Caution: A-F controls are all wired to a power strip on the shelf.**

G UltraView ProSync Unit: ON (the power button is on the back side of the unit)

H Laser Control Unit: ON

I Argon Laser Power Unit: power button ON, rotate the key to the right

J Krypton Laser Power Unit: power button ON, rotate the key to the right

## Shutdown procedure

I & J: rotate the key from right to the top, wait until the cooling systems stop, then turn off the power button.

A-H: turn off the power button.

## Spinning Disk Microscope Operation

### Control switches

Functions for the control switches are described below:



Figure 4 Control switches for UltrView ERS system

A: Work Mode Switch: switching the system between different work modes  
Position 5: for laser scanning mode  
Position 1: for eyepiece observation

B: Fine focus adjustment

C: Coarse focus adjustment

D: Locking ring: **Do not adjust these two rings.**

### Objectives

Currently we have the following three objectives available:

- (1) 20X objective with NA= 0.75
- (2) 60X oil objective with NA= 1.49
- (3) 100X oil objective with NA= 1.4

### Filter wheel

The filter wheel has the following four positions:

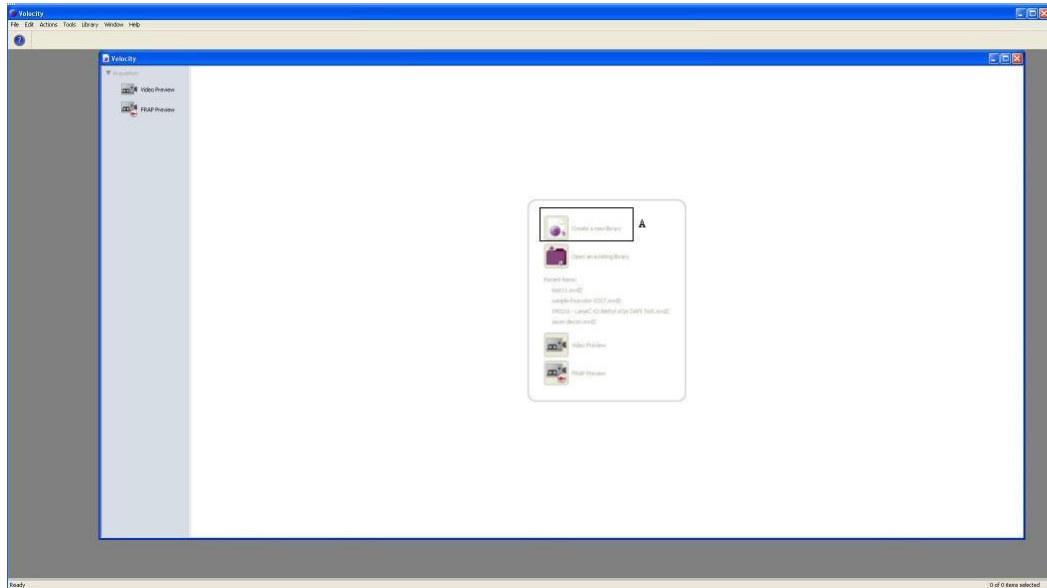
- (1) Position 1: for laser scanning
- (2) Position 2: for green channel illumination
- (3) Position 3: for red channel illumination
- (4) Position 4: for UV channel illumination

## Velocity Software Operations

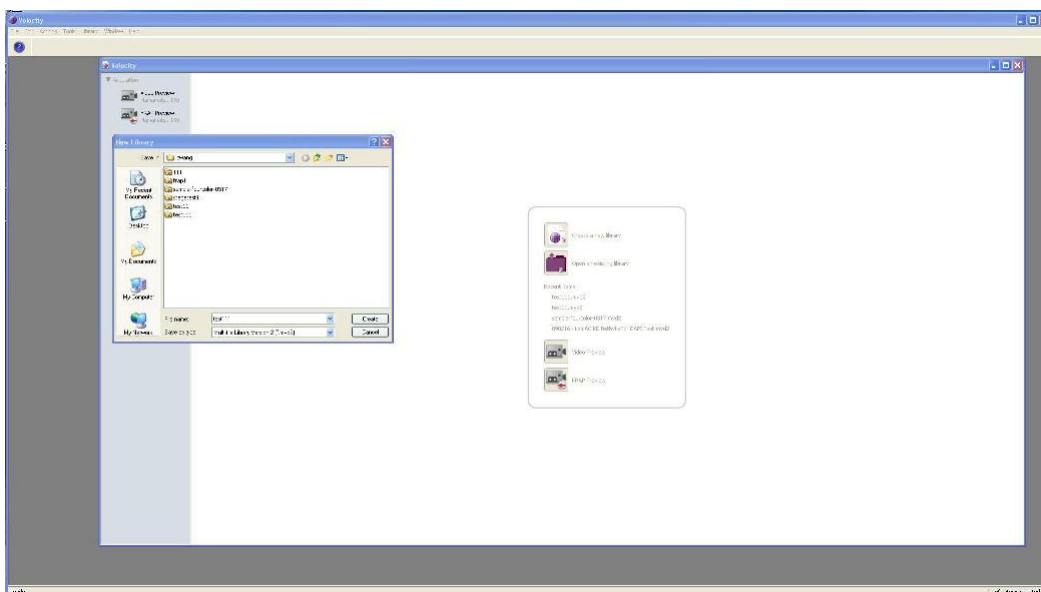
- (1) Start the **Velocity** software by double-click the following icon on the desktop:



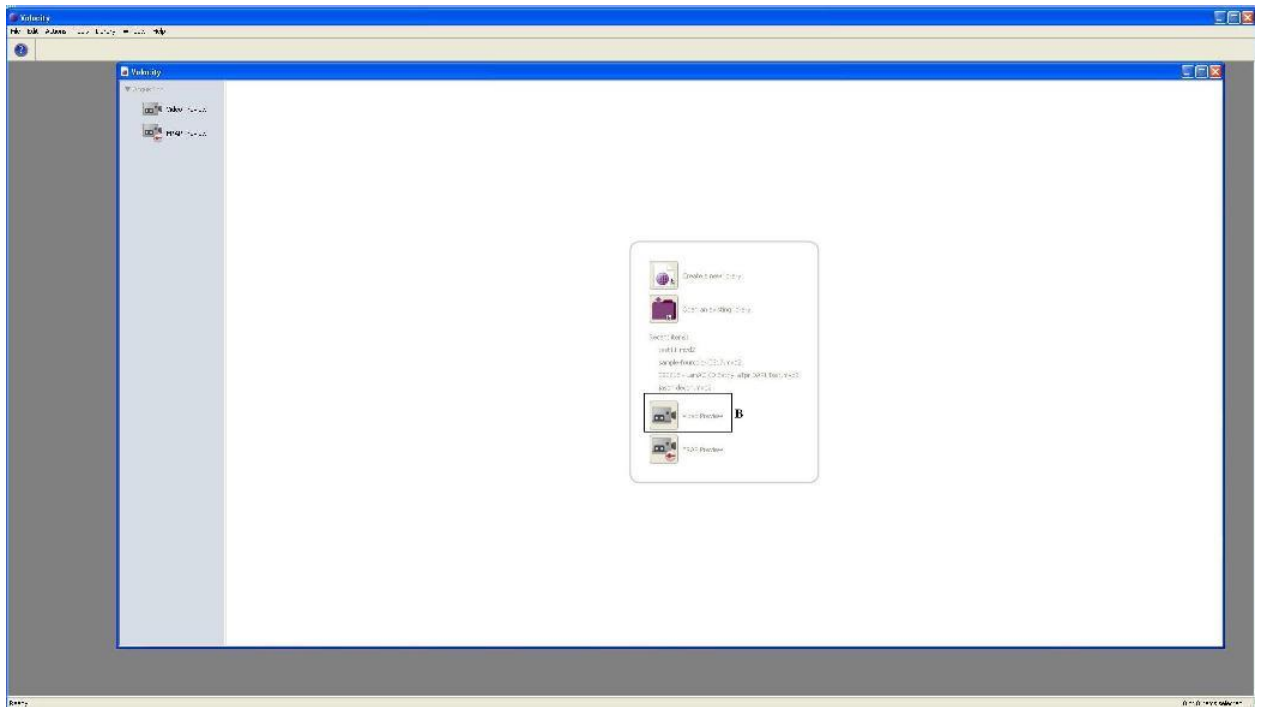
- (2) After **double-click** the **Velocity** icon, the following window appears:



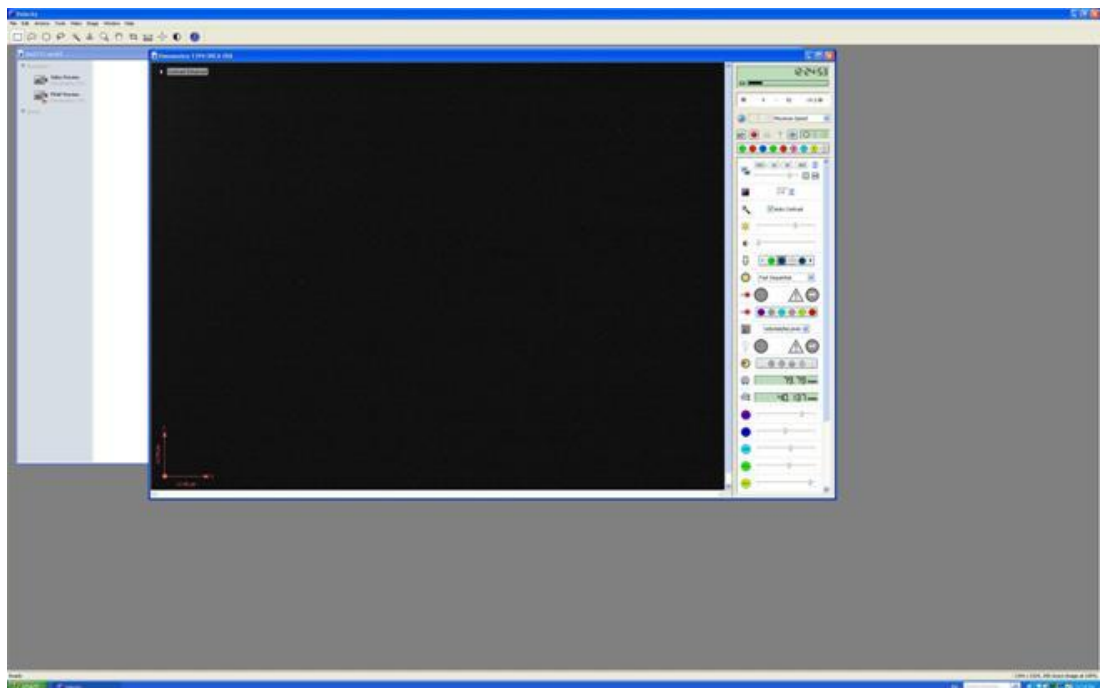
- (3) **Click** the icon in the Region A to create a new library for a new experiment, **assign** the new library (which will include all your experimental datasets) located in your own preferred folder:



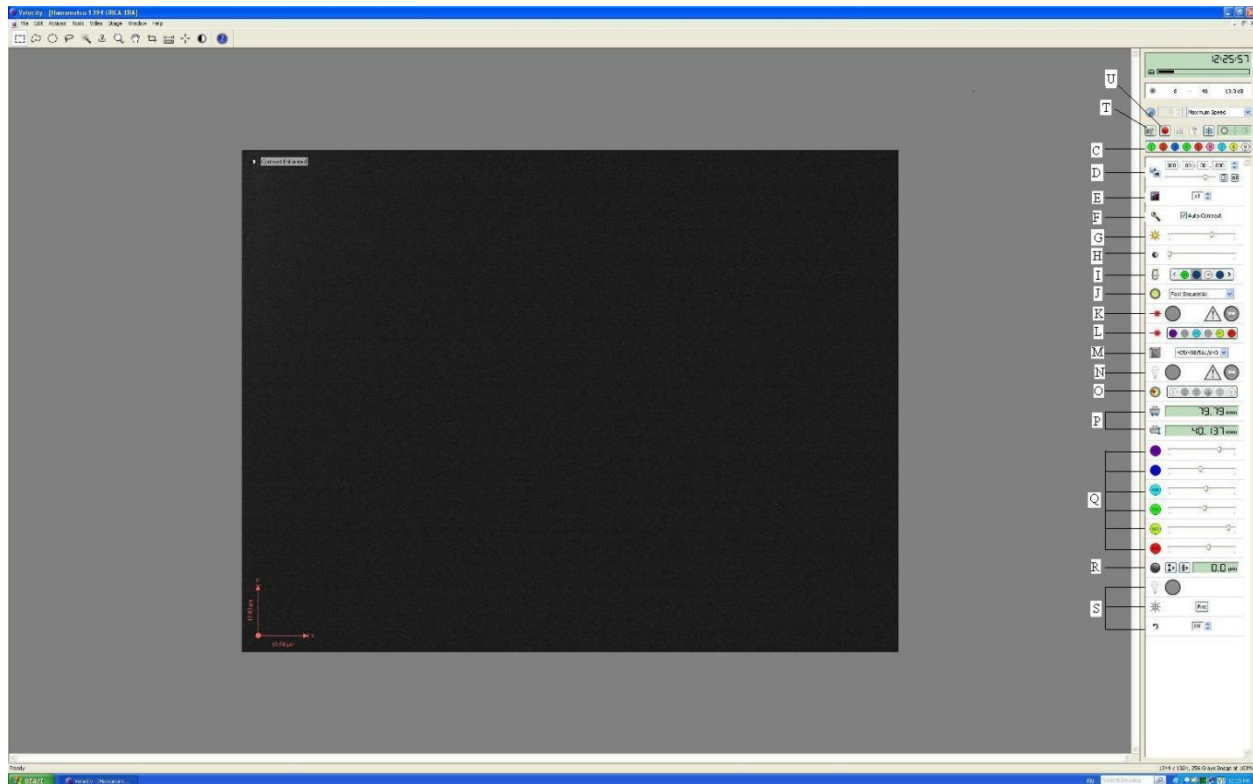
(4) Next **click** the icon in Region B to open the **Video Preview** window:



(5) **Video Preview** window is activated shown below:



(6) **Control icons** on the video preview window:



**C:** Channel selections: right-click each channel icon you will see the corresponding probe

**D:** Exposure time: longer exposure time will increase the signal intensity

**E:** Bin number: merge neighbouring pixels

**F:** Auto-contrast: auto-adjust the image contrast

**G:** Gain: signal amplification

**H:** Offset: background control

**I:** Objective: objective lens selection

**J:** Work mode: the system has two work mode (fast sequential & emission discrimination)

**K:** Laser shutter control: control the shutter for the laser beam illumination

**L:** Laser selection: control the different laser source

**M:** Dichroic mirror selection: dichroic mirror selections for best performance

**N:** Bright-field light control shutter: control the shutter for the bright-field light source

**O:** Emission filter: emission filter control on the signal receiving side

**P:** X-Y coordinates: the absolute X-Y positions of the stage center

**Q:** Laser intensity control: control the excitation power for specific laser source

**R:** Focus control: control the focal point along Z direction

**S:** Photo-kinetic control: **not used in regular acquisition procedure**

**T:** Single frame acquisition: acquire a single frame image

**U:** Multiple- image acquisition: acquire image sequences or multi-channel image

**Caution: the correct settings for the specific channel is shown in the following table**

Channel settings for the UltraView spinning disc confocal system

Channel No.	1	2	3	4	5	6	7	8	9
Dye description	GFP	RFP	DAPI	FITC	Rhodamine /TxRd	Cy5	CFP	YFP	Bright field
Excitation Wavelength(nm)	488	558	359	495	595	650	435	514	N/A
Emission Wavelength(nm)	509	583	461	519	613	670	475	527	N/A
Exposure Time	Adjustable								
Objective	20x/60x/100x based on application								
Discrimination Mode	F.S.*	F.S.	E.D.**	E.D.	E.D.	E.D.	F.S.	F.S.	N/A
Laser(nm)	488	561	405	488	561	640	440	514	N/A
Dichroic	405/488/561/640								
Emission Wheel position # & Wavelength(nm)	N/A	N/A	3 <b>485/705</b>	2 <b>527</b>	3 <b>455/615</b>	4 <b>485/705</b>	N/A	N/A	N/A

F.S.: Fast Sequential Mode  
E.D.: Emission Discrimination Mode