

# Nikon Ti2 R-619

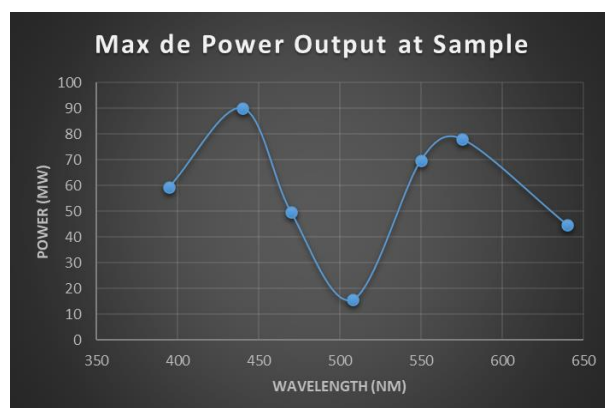
## Illumination Quality Assessment

### Maximum power at sample

This measure evaluates the maximum power of each laser. It accounts for the quality of the laser, their alignment and the transmittance of the overall microscope light path.

Measurements are made at the sample using the 20x-0.75 DIC objective. The laser power is set to 100%. The PFS Dichroic is out of the light path. For 395nm 470nm 550nm and 640nm the quad band DAPI/GFP/Cy3/Cy5 dichroic was used. For 440nm 508nm and 575nm the Triple band CFP/YFP/mCherry was used.

Wavelength (nm)	Max Power Output at sample (mW)
395	59.4
440	90.1
470	49.8
508	15.8
550	69.9
575	78.2
640	44.8



These results agree with expected output performance.

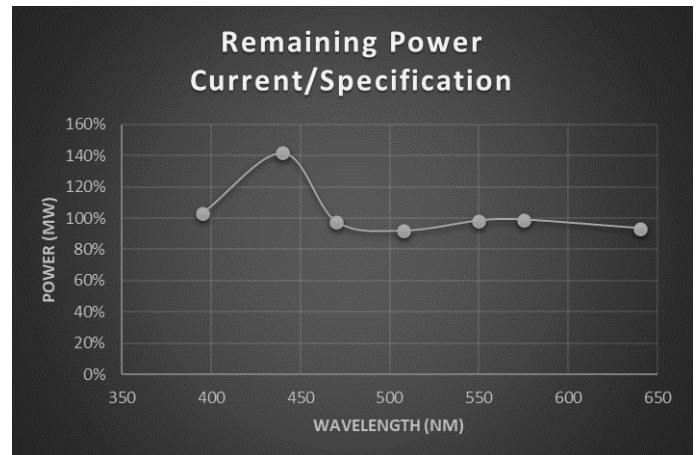
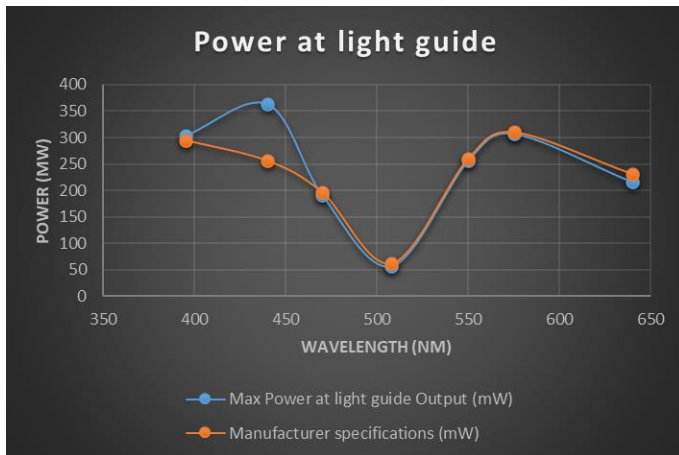
### Maximum power at liquid light guide

This measure evaluates the maximum power of each laser without taking in account the microscope light path. It accounts for the quality of the laser, their alignment, and the transmittance of the liquid light guide.

Measurements are made at the output of the liquid light guide. The laser power is set to 100%. It is best to use a new liquid light guide to make this measure.

Wavelength (nm)	Max Power at light guide Output (mW)	Manufacturer Output (mW)	Percentage Current/Intial
395	304	295	103%
440	363	256	142%
470	191.3	196	98%
508	57	62	92%
550	256	260	98%
575	307	310	99%
640	216	231	94%

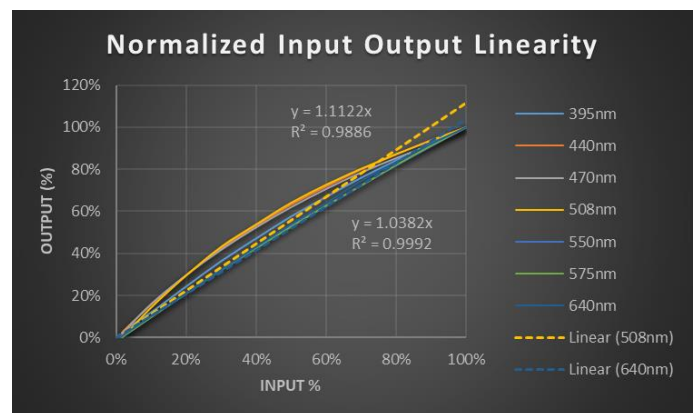
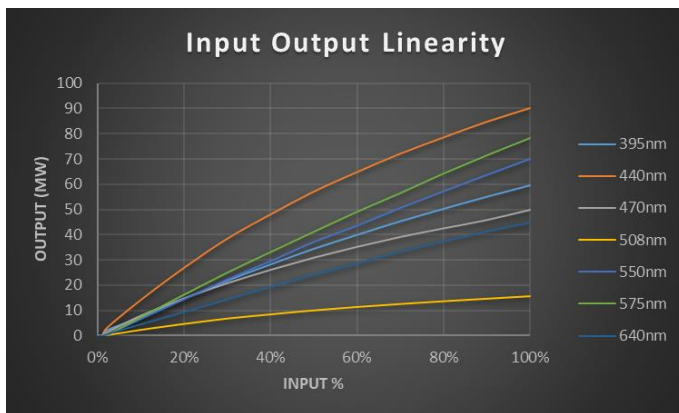
These results are within the manufacturer specifications. The light source remains above 90% of the original power output. This is likely due to the liquid light guide usage.



### Input Output linearity

This measure evaluates the linear relationship between input command and output power for each laser line.

Measurements are made at the sample using the 20x-0.75 DIC objective. The laser power varies between 0 and 100% by 10% increment. The PFS Dichroic is out of the light path. For 395nm 470nm 550nm and 640nm the quad band DAPI/GFP/Cy3/Cy5 dichroic was used. For 440nm 508nm and 575nm the Triple band CFP/YFP/mCherry was used.

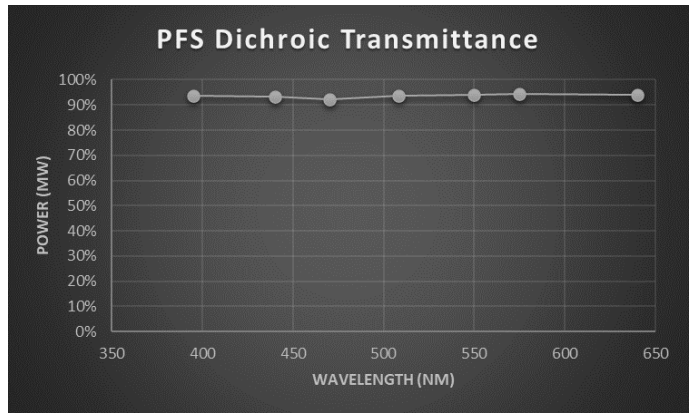
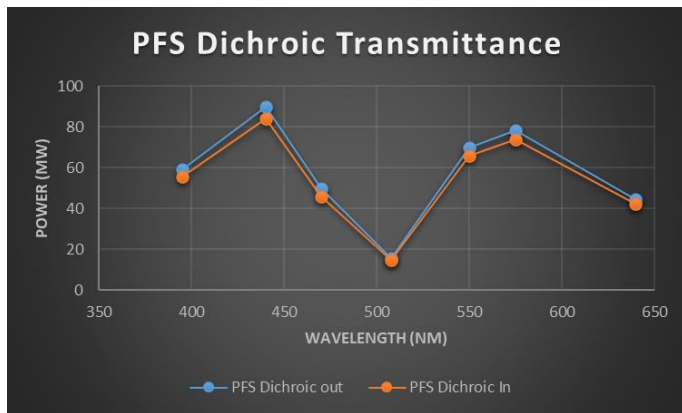


The relationship between input command and output power is fairly linear considering the technical aspect of the Spectra X. 640nm laser line is the most linear whereas 508nm is the least linear.

## PFS Dichroic Transmittance

This measure evaluates the transmittance of the PFS dichroic.

Measurements are made at the sample using the 20x-0.75 DIC objective. The laser power is set to 100%. The PFS dichroic varies in and out of the light path. For 395nm 470nm 550nm and 640nm the quad band DAPI/GFP/Cy3/Cy5 dichroic was used. For 440nm 508nm and 575nm the Triple band CFP/YFP/mCherry was used.

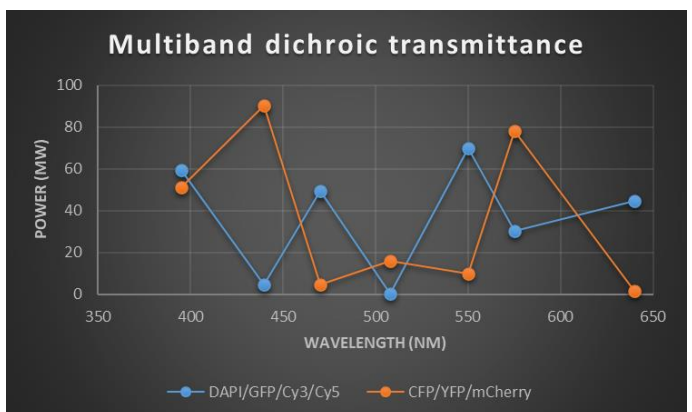
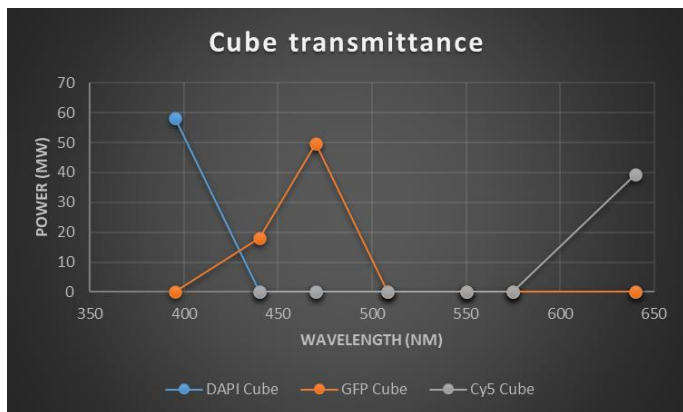


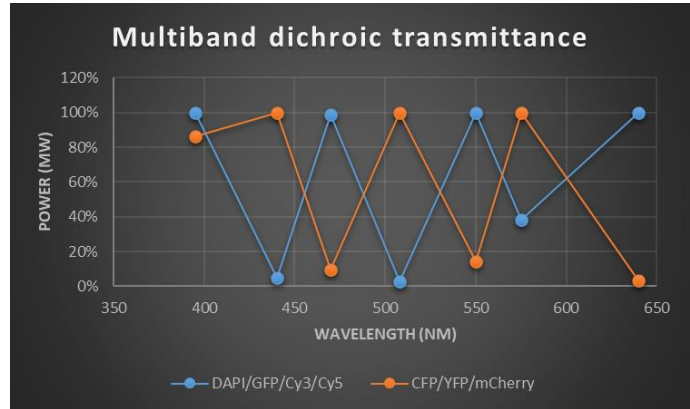
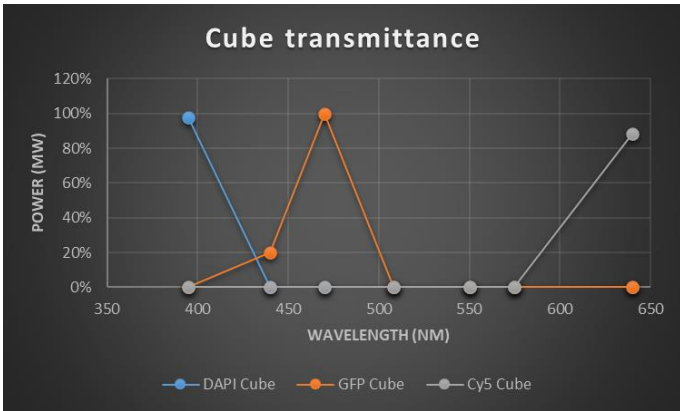
The PFS dichroic transmits between 92% and 94% of the light.

## Filters Transmittance

This measure evaluates the transmittance of each filter in the filter wheel.

Measurements are made at the sample using the 20x-0.75 DIC objective. The laser power is set to 100%. The PFS dichroic is out of the light path. 3 filter cubes (DAPI, GFP, Cy5) and 2 multiband pass filters (DAPI/GFP/Cy3/Cy5 and CFP/YFP/mCherry) were used.





The cubes behave normally.

DAPI cube reflects 100% of 395nm only.

GFP cube reflects 100% of 470nm and 20% of the 440nm.

Cy5 cube reflects 90% of 640nm.

The multiband dichroics behaves as follow:

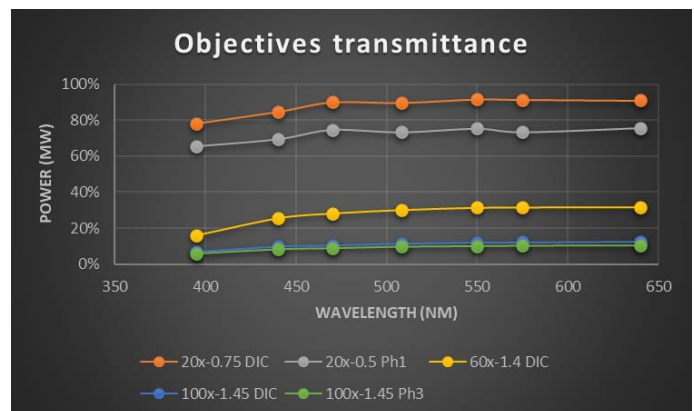
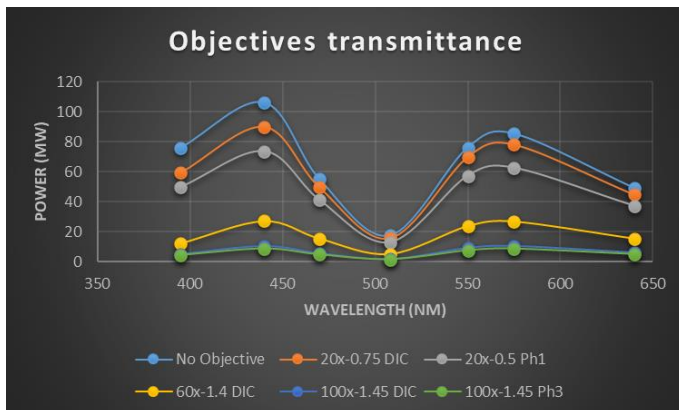
DAPI/GFP/Cy3/Cy5 reflects 100% of 395nm, 100% of 470nm, 100% of 550nm, 18% of 575nm and 100% 640nm but blocks 440nm, 508nm and 82% of 575nm.

CFP/YFP/mCherry reflects 90% of 395nm, 100% of 440nm, 100% of 508nm, 18% of 550nm, 100% of 575nm but blocks 470nm and 640nm.

## Objectives Transmittance

This measure evaluates the transmittance of each objective.

Measurements are made at the sample using all available objectives and compared to no objective. The laser power is set to 100%. The PFS dichroic is out of the light path. For 395nm 470nm 550nm and 640nm the quad band DAPI/GFP/Cy3/Cy5 dichroic was used. For 440nm 508nm and 575nm the Triple band CFP/YFP/mCherry was used.



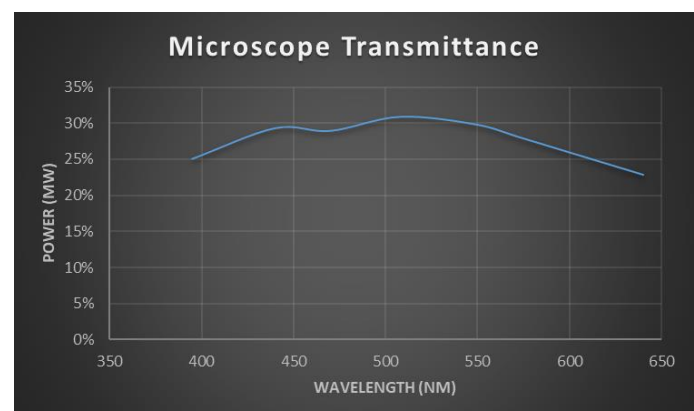
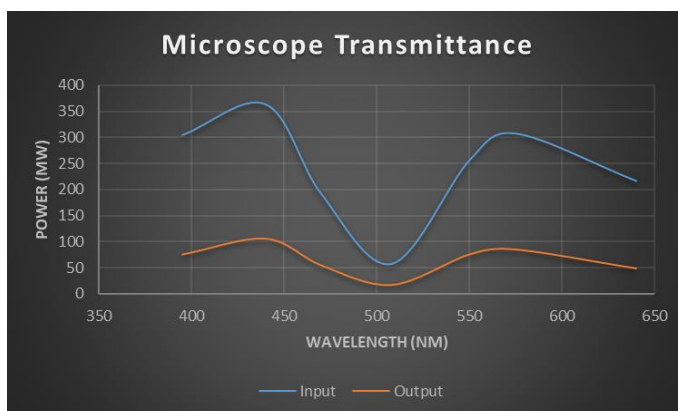
Objective	Average transmittance
20x-0.75 DIC	88%
20x-0.5 Ph1	72%
60x-1.4 DIC	28%
100x-1.45 DIC	11%
100x-1.45 Ph3	9%

This transmittance should be normalized for the diameter of the back aperture.

## Microscope Transmittance

This measure evaluates the overall transmittance of microscope lightpath from the back port to the sample.

Measurements are made at the sample with no objective and compared to measurements made at the liquid light guide. The laser power is set to 100%. The PFS dichroic is out of the light path. For 395nm 470nm 550nm and 640nm the quad band DAPI/GFP/Cy3/Cy5 dichroic was used. For 440nm 508nm and 575nm the Triple band CFP/YFP/mCherry was used.



On average the microscope transmits 28% of the light it receives.

## Liquid light guide Transmittance

This measure evaluates the transmittance of liquid light guide. Over time and usage liquid light guide transmittance decreases.

Measurements are made at the liquid light guide using the current guide or a new one. The laser power is set to 100%.

This measurement was not taken.

## Illumination stability (1 min)

This measure evaluates the stability of the illumination over time (1min).

Measurements are made at the sample. The laser power is set to 100%. The PFS dichroic is out of the light path. For 395nm 470nm 550nm and 640nm the quad band DAPI/GFP/Cy3/Cy5 dichroic was used. For 440nm 508nm and 575nm the Triple band CFP/YFP/mCherry was used. Data is recorded continuously for 1 minute.

	Min	Max	Average	StdDev	Coefficient Variation
395nm	59.3	60.9	60.0	0.4	0.63%
440nm	90.0	91.2	90.5	0.3	0.29%
470nm	48.9	50.1	49.4	0.3	0.56%
508nm	15.6	15.9	15.7	0.0	0.30%
550nm	68.2	70.8	69.5	0.7	0.94%
575nm	77.9	80.4	79.2	0.7	0.88%
640nm	44.6	45.4	44.9	0.2	0.35%

The coefficient of variation indicates what is the percentage of variation from the mean. Here we see that the illumination is stable at more than 99%.

### **Illumination stability (1h)**

This measure evaluates the stability of the illumination over time (1h).

Measurements are made at the sample. The laser power is set to 100%. The PFS dichroic is out of the light path. For 395nm 470nm 550nm and 640nm the quad band DAPI/GFP/Cy3/Cy5 dichroic was used. For 440nm 508nm and 575nm the Triple band CFP/YFP/mCherry was used. Data is recorded every 5 minutes for 30 seconds. Laser is ON at all time.

	Coeff Variation
0 min	0.94%
5 min	0.90%
10 min	0.12%
15 min	0.12%
20 min	0.12%

The coefficient of variation decreases after the first 10 minutes. We recommend to leave the laser ON and use the shutter for quantitative intensity based measurements.