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Abstract

In flow cytometry, the FALS (Forward Angle Light Scatter) detector collects scattered light from a particle that intersects with a laser and delivers information roughly proportional to the size of the particle. Few innovations have occurred in the measurement of FALS since its introduction to flow cytometry 45 years ago until the recent introduction of a new FALS design in the Gallios Flow Cytometer from Beckman Coulter. In the past, flow Cytometers struggled to both differentiate near-size particles, and discriminate small particles from system noise. The new Gallios FALS design provides distinction of particles with small variations in size as well as ultra-clear discrimination of small particles from background noise. Leveraging concepts from the Mie Theory, which describes the pattern of light diffraction intensity relative to particle size and angle of light collected, this innovative FALS design in the Gallios flow cytometer is designed to optimize the detector for the users' choice of particle-size sensitivity. The optical system design and sketch of system geometry are described, as well as calculations of measured angles of light collection. Important design parameters including photo-sensitive detector diameter and principal focal length relationships are explained. The intent is to produce the highest quality "electronic image" of the particle thereby resulting in optimized signal to noise. This versatile forward scatter detector is controlled using 3 software controlled settings chosen for optimal versatility of a wide range of particle size characterization. The design can provide the clear resolution necessary to differentiate near-size particles of 0.4um from noise with 0.004% signal/noise population overlap and 0.5um from 0.4um particles with 0.002% signal/signal population overlap with one setting in the very same histogram. The new FALS detector design in the Gallios flow cytometer has expanded the capabilities of the popular parameter and opened the door to the "small-world". With a flexible and customizable detector, the Gallios expands the range of particle size resolution available in flow cytometry analyzers.

INTRODUCTION

In a Flow Cytometer, a stationary laser interrogates a passing cell/particle suspended in a stable flowing column of liquid. As the cell passes through the laser, multiple types of photon transformations simultaneously occur providing valuable information relative to cell fluorescence, cell complexity, and cell size. The desired parameters of cell fluorescence, cell complexity and cell size photons will each be collected in a different but specific domain using certain devices. The goal of these devices is to produce highest target photon detection rate possible as allowed by current technology. For discussion sake most flow cytometer designs;

- have placed cell-fluorescence collection orthogonal to the laser beam at -90° in the horizontal plane (laser beam path = 0°).
- have placed cell-complexity collection (Side-Scatter) orthogonal to the laser beam at +90° in the horizontal plane (laser beam path = 0°).
- have placed cell-size collection (FALS) directly in the path of, and normal to, the laser beam in the horizontal plane.

We will focus our discussion on the Cell-size photon collection otherwise known as Forward Angle Light Scatter detection. The following sketch attempts to convey the geometrical arrangement of the detectors. FALS has been a necessary entity for system triggering as well as a parameter full of information just waiting to be uncovered since the beginning of Flow Cytometry.

Through much effort, the Gallios FALS detector opens a door to the "small-world". Since limits on max cell diameter are the flowcell sensing zone aperture, we will focus on the difficulties of the "small-particle-world". The goal is to solve for the following issues, reach a conclusion, and as an end result to show the necessity for the Versatile Gallios FALS detection system.

Issue-1: Where is the light I want? (Why wider angles for smaller particles?)

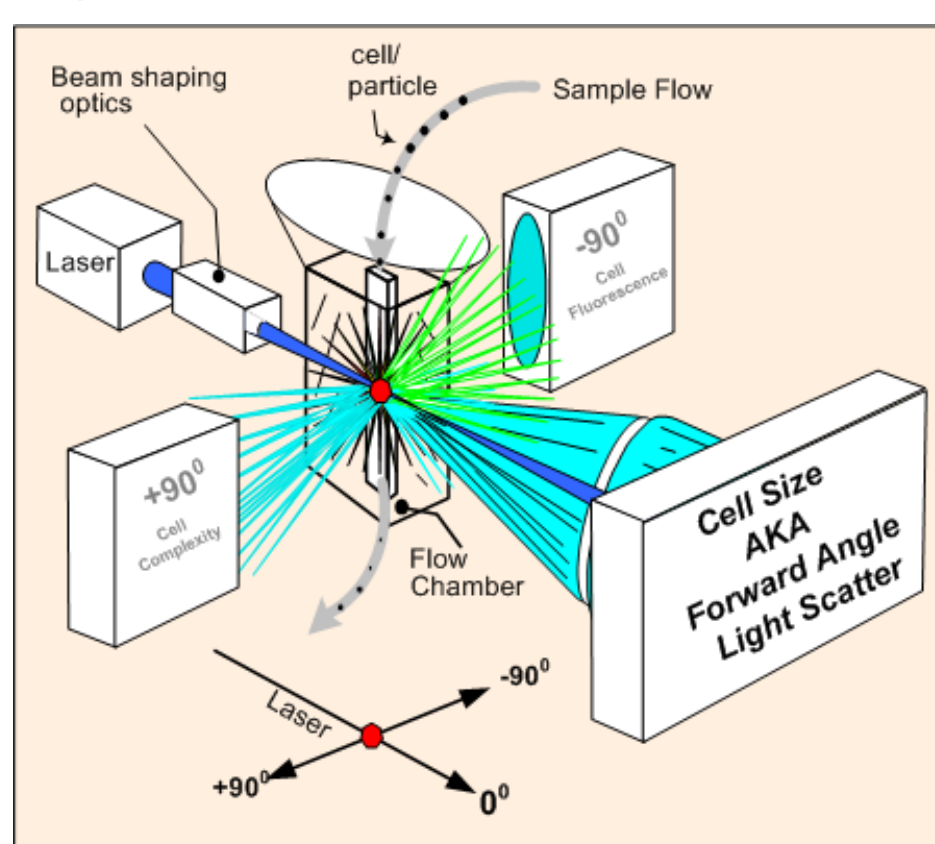
Issue-2: What is Angular range of my system's optical geometry?

Issue-3: What Photo-sensitive detector geometry will I need to collect the small particle generated FALS light?

Issue-4: What is the final FALS detector block diagram and description?

Results of the effort: Actual Histogram Data.

Conclusion: What have we learned?

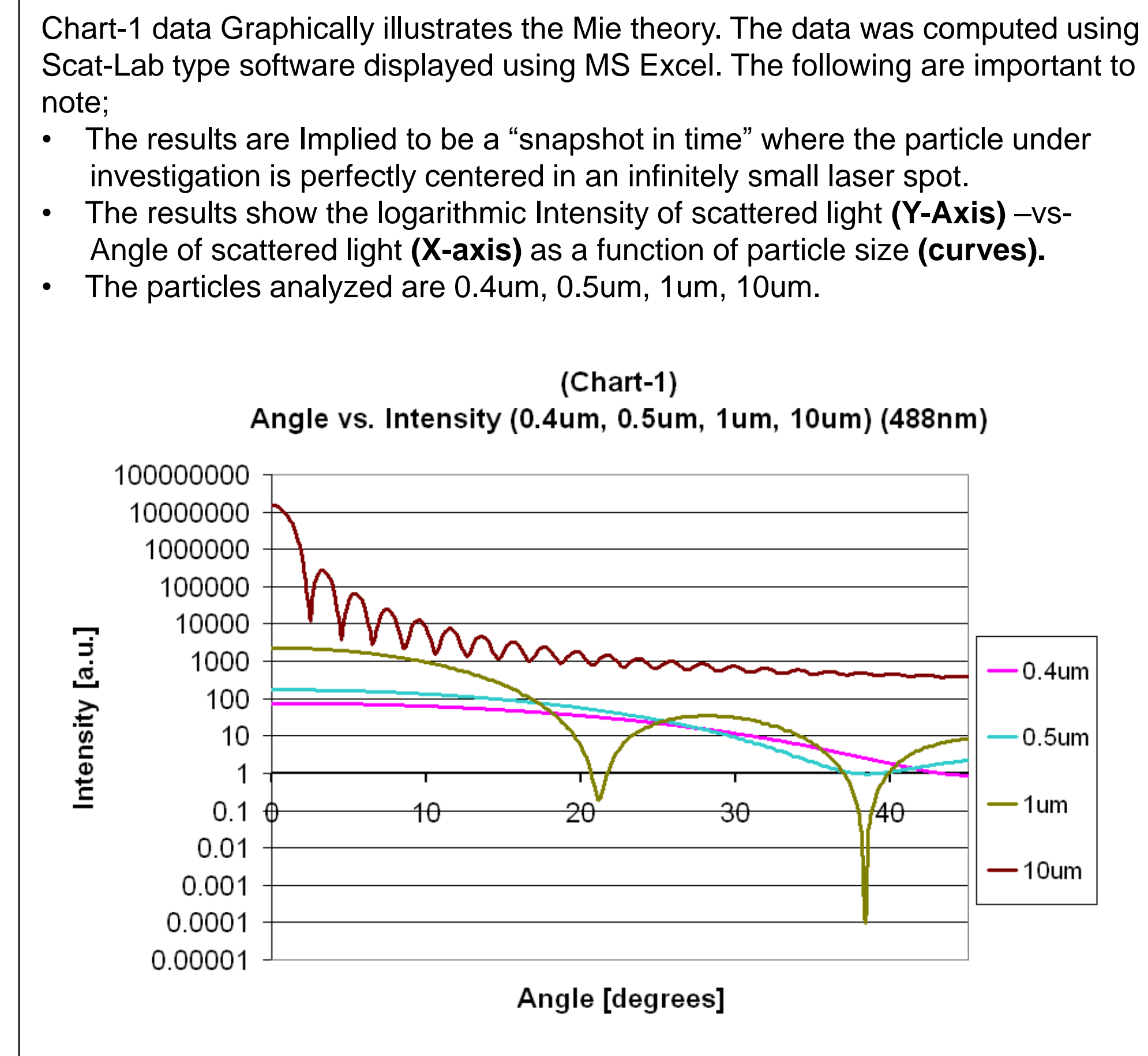
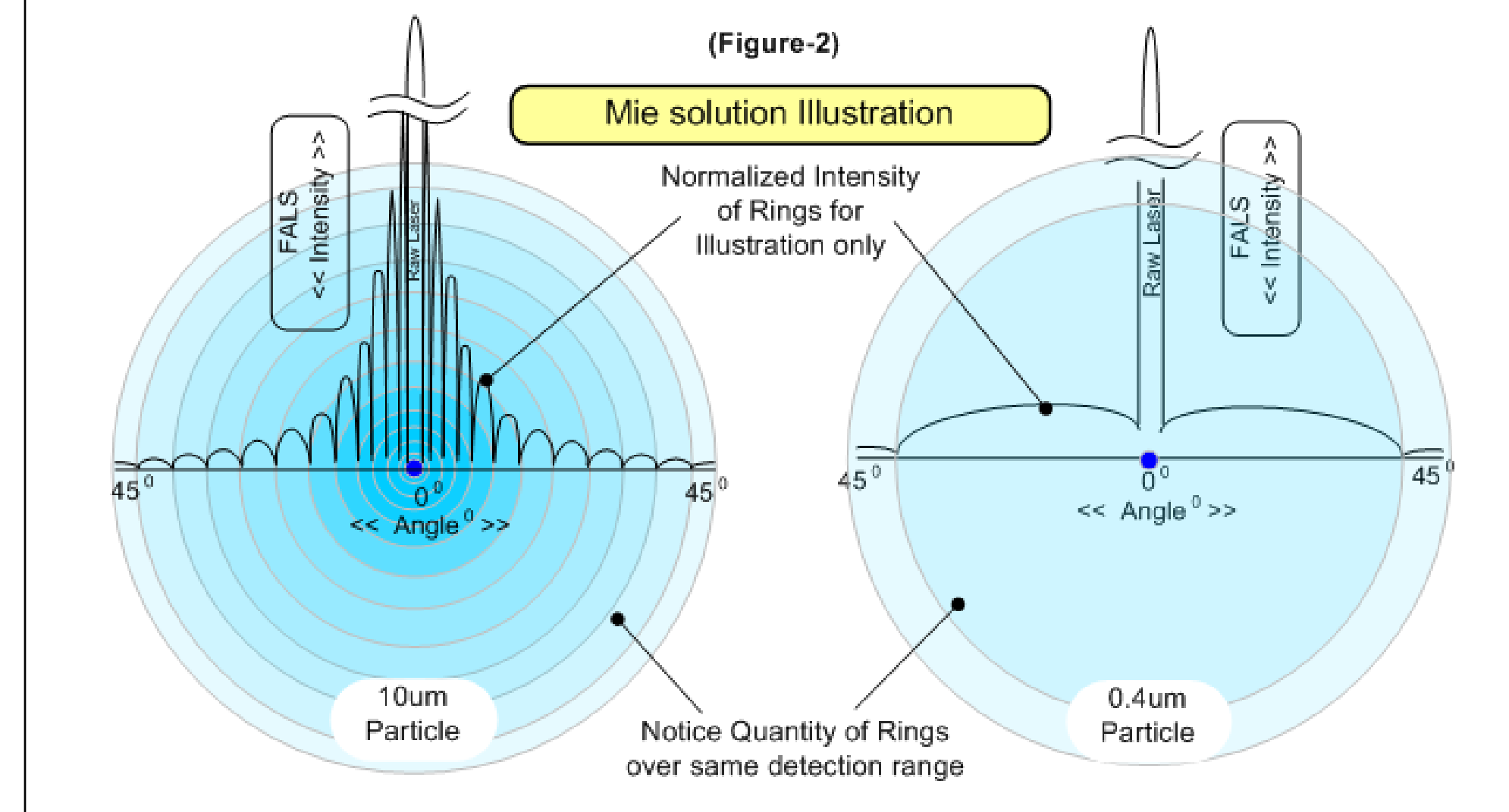


Issue-1: Where is the light? (Why wider angles for smaller particles?)

In the quest for small particle detection, first, we need to determine where the FALS photons should report. Using the Mie theory (Gustav Mie 1908) of small particle light scattering as a Solution to Maxwell's equations, showing the photon trajectories. Mie Solution takes into account both diffraction and diffusion of light around a particle and its medium, making it fitting to explain FALS in a flow Cytometry over a wide range of small particles sizes. The domain of FALS light we will initially discuss is from 0-45° (Figure-1 below).

According to Mie's solution the FALS pattern will be manifested in concentric light rings with decreasing intensity away from the center (center = raw beam) (Figure-2). The number of rings and width of each ring (in the same 45° domain) is dependant on particle diameter and the refractive index of each particle (keeping the excitation wavelength constant).

The Mie solution illustration (Figure-2) shows FALS light rings overlaid with intensity curves (logarithmic intensity response) for 10um particle (many rings with higher intensity) and 0.4um particle (few rings with significantly less intensity).



Issue-2: What is the possible FALS Angular Range of my system's optical geometry?

We have predicted where the Target light is, now, we need to determine the systems FALS Angular range capability using an optical/flowcell system model. See sketch of Optical System model (Figure-3).

Figure-3: FALS Angle is limited by [inv tan ("Y" microns / "X" microns)]

(Top-View Looking Downward)

Using the Flowcell dimensions, and the equation highlighted in Figure-3, the results of our calculations are:
We will collect max 19° from the laser/particle intersection point.
We will collect a minimum of 2° limited by the detector mask.

By collecting 2-groups of angles (Narrow and Wide), we can now treat them differently if we wish. In essence minimizing effects of cell structure (Wide Angle) on larger cell sizes (> 1um), and/or minimize the effects of near-center laser noise (Narrow Angle) from small cell (< 1um) size measurements.

Observe Chart-2 (Below) showing the **Narrow** and **Wide** areas highlighted. (Chart-2)

Angle Isolation vs. Intensity (488nm)

The graph shows Intensity [a.u.] on a logarithmic y-axis (0.00001 to 10,000,000) versus Angle [degrees] on the x-axis (0 to 20). Two regions are highlighted: 'Narrow Angle collection from 2-8°' and 'Wide Angle collection from 9°-19°'. The 0.4um curve (red) is concentrated in the narrow angle region, while the 10um curve (purple) is concentrated in the wide angle region.

Issue-3: What Photo-sensitive detector geometry will I need to collect the small particle generated FALS light?

Since we know our target light angles, and now know our systems FALS Angular range capability, What detector geometry and placement is necessary?

In Figure-4, the Fourier plane is illustrated where a Lens is placed at its Focal distance away from laser / cell intersection point allowing parallel rays to the Photo-detectors.

The Lens "F" and Lens proximity to the intersection point determines optimum detector size.

Figure-4: Photo-Detector Geometry

The diagram shows a laser beam passing through a cell/laser intersection point. A lens with focal length F is placed at a distance F from the intersection point. The light rays are focused onto a photo-sensitive detector. The detector is divided into 'Wide Angle' and 'Narrow' regions.

Issue-4: What is the FALS detector block diagram and description?

Three Gallios FALS detector modes exceed your variety of needs and are found on the Parameters menu of Gallios User Interface software (Figure-5, 6, 7).

Narrow Detector selection only (2° - 9°) mode.
Narrow detector at **Low** sensitivity.
Best Large Particle (<40um) resolution.
Usually followed by Low Step Gain control.

Wide (9° - 19°) + Narrow (2° - 9°) mode.
Narrow & Wide detectors at **Low** sensitivity.
Best non-specific size performance blend (1 - 20um).
Step Gain control dependant on Cell size.

Wide-High (9° - 19°) + Narrow (2° - 9°) mode.
Narrow detector at **Low** sensitivity.
Wide detector at **Very-High** sensitivity.
Provides **Best micron-micron** particle resolution.
Step Gain control dependant on Cell size.

Results of the Effort.

Histograms were collected on a Beckman Coulter Gallios with 488nm Laser at 222mw. (* Total Gain FALS & SS = (((HV / 1000) x 3) + 1) x Gain)

These are the equations for **%Population Overlap₁** (P-Ovlp) as a function of **Fisher Distance₁** (FD) (Results are in Histograms):

$$FD = \frac{(ABS(P2-P1))}{((P1CV/100)*P1) + ((P2CV / 100)*P2)}$$

P1 = Population-1 (X-Mean), P1CV = Population-1 (X-CV)
P2 = Population-2 (X-Mean), P2CV = Population-2 (X-CV)

$$\% \text{ Population Overlap} = (1 - \text{ERF} (FD / \text{SQRT}(2)))$$

FD = Fisher Distance
ERF = MS Excel function (Divide ERF Result / 100 for %)

Water with Total FALS Gain = 620*

Two histograms are shown. The left one is a scatter plot of FALS vs. SS. The right one is a histogram of FALS intensity. The histogram shows a peak at approximately 19 degrees.

Results (Continued)

0.4um with Total FALS Gain = 620*

Two histograms are shown. The left one is a scatter plot of FALS vs. SS. The right one is a histogram of FALS intensity. The histogram shows a peak at approximately 19 degrees. Statistics: FD = 4.19, P-Ovlp = 0.003%.

0.4um + 0.5um with Total FALS Gain = 620*

Two histograms are shown. The left one is a scatter plot of FALS vs. SS. The right one is a histogram of FALS intensity. The histogram shows a peak at approximately 19 degrees. Statistics: P2 to P1 % Ovlp = 0.003%, P3 to P2 % Ovlp = 0.0015%, FD = 4.19, FD = 4.33.

Water with Total FALS Gain = 1550*

Two histograms are shown. The left one is a scatter plot of FALS vs. SS. The right one is a histogram of FALS intensity. The histogram shows a peak at approximately 19 degrees.

0.4um with Total FALS Gain = 1550*

Two histograms are shown. The left one is a scatter plot of FALS vs. SS. The right one is a histogram of FALS intensity. The histogram shows a peak at approximately 19 degrees. Statistics: FD = 4.78, P-Ovlp = 0.00018%.

Conclusion: What have we learned?

- All particles generate light scatter over 360° as light rays hit their surface. Particle size and excitation wavelength determine the number and intensity of light rings generated. (Figure-1, Chart-1, 2).
- When focusing on a certain particle range, Keys to developing the optimal FALS detector are;
 - 1) It is important to collect the correct range of light angles as signal.
 - 2) It is equally as important to **exclude** the range of light angles considered as noise!
 - a) Wanted signals are plagued by improper exclusion of light noise.
 - b) Proper detector geometry, placement, and elimination of unwanted light deliver best detector efficiency (signal/noise), and are most capable of higher fidelity at increased post detector gains.
- The worst case % Population Overlap is 0.003% (0.4um at Gain=620).

Citations:
1 John Steven Riley, "Statistical analysis and optimal classification of blood cell populations using Gaussian distributions" (January 1, 2003). *ETD Collection for Florida International University*. Paper AA13085820. <http://digitalcommons.fiu.edu/dissertations/AA13085820>

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