Frequently asked questions about Vü

1 **Who said you had to use a CCD camera to capture images of gels and blots?** No one… CCD cameras were first used 30 years ago in the original gel documentation systems. Since then they have been used simply because it has always been done that way and no one thought to think outside of the 'gel box'. The question that should be asked is "why use an expensive CCD camera just to image a small flat object?" After all, in some cases for fluorescence a smart phone is just as good these days. ***TIP:*** **There is a better alternative to a CCD camera system – it's called Vü**

2 **CCD cameras can have mega pixel resolution which means they are more sensitive and can capture low level light which you need for gels and blots**. Wrong….. the number of pixels has nothing to do with the sensitivity. Sensor manufacturers cram more and more pixels onto the sensors which will improve spatial resolution but in doing so the individual pixels have to get smaller which reduces their ability to collect light. Hence smaller pixels are not as sensitive as larger pixels. Most of the gel documentation system manufacturers use the pixel count as a marketing tool claiming their system is better because it has more pixels – do not get pulled into this trap. ***TIP:*** **Beware of the 'pixel trap'**

3 **When is mega pixel resolution good**? In a CCD camera all the light from the gel or blot [which can be anything from an area of 10 x 10cm up to 20 x 20cm or more], is focussed onto one small rectangular sensor which is usually just a few centimetres in size. To increase sensitivity a process called 'binning' is used which groups a cluster of pixels together to make a super pixel which has greater area and hence can capture more light. However, binning does reduce the overall pixel count and hence the spatial resolution is reduced often to the point where you can actually see the pixel clusters on the image. So why is Vü better? Quite simply we have many more pixels to work with. In a 100mm line of sensors there are 7142 elements. If we move this is the X direction across a blot which is 100mm long then the number of pixels we use to capture the image is 7142 x 7142 = 51,008,164 pixels [51megapixels]. We do group pixels to increase sensitivity and so the final image never has this many pixels but it can be anywhere between 1m and 13m depending on the output from the blot. ***TIP:*** **Having 51m pixels as in the Vü rather than just 8.3m in a camera based system is a major advantage.**

4 **So how important is pixel size?** For any chemiluminescence system it is vital. Big pixels mean more ability to capture the light and hence more sensitivity. Ideally you need a system with large area pixels. CCD cameras use a number of different sensors all with different pixel counts and different sizes. The more expensive the camera then typically

the bigger the pixel size. Most of the better CCD based systems will have pixel sizes of the order 6.4um x 6.4um giving a pixel area of 40.96um^2. There are a few that are bigger but many more have smaller pixels. Most gel documentation specifications quote a figure for pixel size – if not they are hiding something. Vü of course is different because of the configuration and type of sensor used. The Vü has a pixel area of 2800um^2. This makes it 70 times more effective at collecting light than a typical gel documentation system. ***TIP:*** **Larger pixel areas are better for chemiluminescence applications**

5 **Is Quantum Efficiency [QE] important?** It certainly is. The QE is a measure of how much light actually gets converted to electrical signal via the sensor and hence how good the image data is going to be. The higher the QE in effect means better sensitivity. In a typical gel documentation system camera there are a range of different sensors that are used. Each of these has a sensor specification which the main sensor manufacturers publish. Some gel documentation companies 'bend' these value to make their systems appear better in specification lists so be aware. The QE varies across the spectrum and so it is wavelength specific. You should always look for the QE which best corresponds to the wavelength of light emitted by your application. As an example, at 428nm the Vü sensor has an impressive QE of 63%. However, go a little further up the spectrum and at 550nm the QE jumps to 83.4%. Both these QE's comfortably exceed those of many of the traditional CCD camera based gel documentation systems meaning the Vü is far more efficient at converting captured light to a measurable signal***. TIP:*** **Higher QE's result in more signal being converted to data.**

6 **Why are cameras cooled for chemiluminescence but Vu isn't cooled?** This is very simple. With a CCD camera you need long exposure times to give the sensor long enough time to collect sufficient light to form an image. This of course comes with its own problems. As the exposure gets longer there is more time to collect electronic noise which then shows on the image as a noisy [not smooth] background. To try an overcome this the sensor is cooled to varying degrees using a Peltier system. Cooling on some systems can go as low as -60^{0}C – usually because the camera itself is inherently noisy. Noise levels do reduce the cooler the sensor is made but cooling just adds cost and in some cases can cause condensation on the sensor which is often seen as bubbles. Vu has none of these problems simply because it does not have any extended exposure times. In the Vu the sensor downloads its data in under 1 second as it moves backwards and forwards over the gel or blot. In this minimal time there is little or no noise generated and hence image backgrounds look very even and smooth. ***TIP***: **Avoid cooled systems – pick the Vü**

7 **Why use expensive optical lenses?** Somehow you have to focus the gel or blot onto the small CCD sensor and to do this you need a lens. Whether fixed or zoom lens all the light from the gel or blot has to be channelled through the optical elements of the lens and through the lens opening [aperture]. At each step you are losing more of the light output from the sample. In tests it has been found that as much as 36% of the available light energy gets blocked at some apertures. The optical path of a traditional gel documentation system is therefore not the most efficient way of capturing the low output level from a gel or blot. It is better then to have a system which does not have a traditional lens but which uses something with far greater transmission characteristics. The Vü system has a small single micro-lens element placed directly over the sensor and

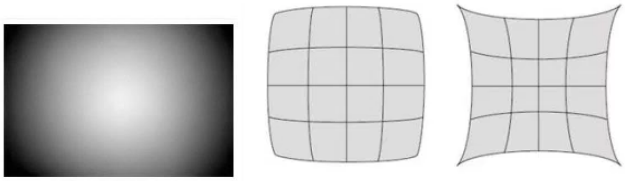
which has superior light transmission qualities compared to a regular optical lens. ***TIP:*** **Zoom lenses and even some fixed lens absorb too much light**

8 **What does f-stop mean?** A traditional photographic lens of the type used on virtually all gel documentation systems if often characterise by its f-stop number. There is a desire to have a lens with a f-stop of 0.95 or better since this means there is a larger aperture allowing more light to pass through [although some light is still blocked by the aperture]. A lens with an aperture of say f1.4 has a smaller aperture and allows even less light through – this can become a real problem when working with low light level samples. An f-stop number as such is just a description of the aperture size and gives no indication as to how much actual light is passed through the lens. Couple that with the light absorbed by each individual element of the lens and you have no idea how much light you have to work with on a traditional gel documentation system. What is the better way of characterising a lens? The answer is to use its 'effective f-stop which is called the T-stop. The T-stop is a fully calibrated value and is a real measure of how much light is transmitted through the lens and aperture and is therefore a much more valuable figure to use when comparing lens systems. The effective f-stop or T-stop for the Vü is 1.01 and this means a transmission of light of 99% [a figure of 1 would mean 100%]. Now compare this with a typical gel documentation system using an f0.95 lens. Using the t-stop value for these types of lens you get a figure of 1.57. Or put another way only 63% of the light is transmitted. Hence, even though the f0.95 lens is considered to be a good choice it still comes nowhere near the transmission qualities of the Vü. ***TIP:*** **Don't use the f-stop to characterise a lens but use the calibrated T-stop**.

9 **Why are traditional Gel Documentation systems so big and heavy?** In a word it's going back to the same old issue – it's always been done this way. Therefore, you need a box capable of housing a CCD camera, lens, filter mechanism and light sources. When you need to take an image of a flat object you firstly need to be a certain distance away to be able to cover the area of interest. You could use a close-up lens but these tend to add additional optical problems. With most of the usual CCD camera and lenses used in a gel documentation system there has to be a distance between the camera/lens assembly and the sample typically anywhere between 20 – 50cm depending on the field of view needed and the focal length of the lens. This results in the need to have a darkroom box which can be large and it can be heavy. In the Vü system there is no camera or any large lens and so the 'head' assembly is placed very close to the gel or blot. The distance between them is 3-5mm. This results in the Vü system being very small in size and taking up minimal footprint although it can still image 10 x 10cm blots and 20 x 20cm gels. ***TIP:*** **Forget the massive darkroom box which takes up valuable bench space and use something small and compact which does the same job – like Vü**.

10 **What other imaging problems can you see with a traditional lens?** There are a number of lens issues which can be easily seen in a lot of traditional gel documentation systems. One of the most common is Vignetting which is a darkening of the corners of the image which occurs with wide aperture lenses. So an f0.95 lens could often present this problem. Another is barrel distortion where the image looks as if it has been stretched over a sphere causing the edges to bulge. Wide angle lenses are especially prone to barrel distortion. The opposite of barrel distortion is pincushion distortion. Here it appears that the edges of the image have been tucked towards the centre. This sort of

distortion is most prevalent with longer lenses such as some zoom lens that are used. See these images as examples of Vignetting, barrel distortion and pincushion.



With the Vü system because a traditional photographic lens is not use then these issues are not seen. ***TIP:*** **Avoid systems which have optical faults.**

11 **Why do I have to pay so much for filters?** The cost of filters is of course based on their size and so a 58mm lens needs a 58mm diameter filter – which is large. The way to overcome this is to use smaller filters. However, optically this is not possible with a traditional gel documentation system because you are stuck with the lens that has be used. In the Vü because we are working close to the sample and at sensor level the only filters we use are very small and hence cost a fraction of their large counterparts. However, they still do the same job – they just cost a lot less because their surface area is much smaller. Vü has all the filters you need built into the system. Hence no need for an expensive filter wheel either. ***TIP***: **Forget large filters and filter wheels – they just cost more.**

12 **Why use an elevating table in a gel documentation system?** Motorised stages have been included in some gel documentation systems simple because instead of using a zoom lens a fixed lens is used instead. To bring the gel or blot closer to the camera to reduce the field of view and simulate a zoon function the motorised stage will move up or down. This is vast overkill – why use an industrial scale elevator just to move a small, lightweight gel or blot closer to the camera? Not only does it add to the cost and weight of the system it's yet another moving part [along with the motorised filter wheel] that can go wrong. In the Vü the sensor head is within 3-5mm of the gel or blot and does not need any zoom functions. The whole area is mapped by the head and the relevant parts of the sample are imaged. **TIP: Don't waste money on motorised stages**

13 **Why doesn't Vü have a transilluminator?** The answer is that is does. However, in the Vü there is a single tube transilluminator with a 302nm filter. In a full size transilluminator found in a typical gel documentation system there is often a large filter area [20 x 20cm] in a box with 6-8 UV tubes. Inherently this has problems – a transilluminator will have output variations across its surface either from the UV filter itself or from one or more of the UV tubes. The bottom line is that you are never illuminating the gel with a homogeneous level of UV and so the fluorescence will vary across the gel resulting in doubts as to whether it can ever be quantified accurately. In Vü the UV output comes from one position which moves with the head unit across the gel. This ensures 100% even illumination across all parts of the gel and guarantees total accuracy when carrying out any analysis. The Blue LED illumination within Vü is the same – it also follows the head unit ensuring the same level of illumination is always given to the gel or blot**. TIP: do not use a traditional transilluminators for image capture – they never give even illumination and can lead to inaccuracies.**

14 **Do you really need capture software?** Well in a traditional gel documentation system something needs to control all the components and capture the image. All gel documentation systems have some software which controls the camera setting including exposure and binning levels, the lens aperture and focus position, filter selection and lighting selection. Some attempt to do this automatically while others require a significant amount of user interaction. Even those claiming to be automated require the need to either set up certain protocols or for the user to select application types from a series of menus/buttons. This all requires training and a knowledge of how the system is going to respond before you can consistently produce high quality images. Vü is not like that. With Vü there is no control/capture software. The user simply places the gel or blot on the tray and pushes it in to begin the capture process. Simple ID recognition of the tray being used in the Vü is picked up by the system which then competes the appropriate mapping process. The system has it own advanced intelligent software control system which reads and measures the light output from the gel or blot. From this it can calculate the best capture conditions before producing the ideal image. This technology is called ***eMIT*** (electronic mapping imaging technology) is a patented [applied for] process which replaces the need for any user input or interface. This is without question the way all gel and blots will be captured in the future. **TIP: forget complex image acquisition software and use an automated intelligent system that requires no user input.**

15 **How does the Vu specification compare with a traditional CCD camera based imaging system?** For a start both use a different technology and so a direct comparison is not really meaningful. The Vu uses no camera or lens and it captures its images in a completely different way. Hence, there is no like-for-like comparison. The following two tables show how a Vu specification looks against a CCD camera based system. **TIP: it is very hard to compare two different specifications for different technologies.**

Typical Chemi system		Vü -C
Camera: 16 bit CCD camera		16bit imaging sensors
1.44-3.2 Megapixel camera		51m pixel sensor resolution
4-5.8 Megapixel image resolution		13.66 Megapixel maximum image resolution
		1 Megapixel typical image resolution for chemi blots
Quantum efficiency >70% at 425nm		QE at 428nm = 64%; QE at 580nm = 83.4%
Lens: f / 0.85-1.2, motor driven		Micro lens with T-stop 1.01
Cooling system		Not required
Dynamic range: 4-4.8 order of magnitude		4.8 orders of magnitude
Exposure time: 0.001 sec- 1hr		0.001 sec - we do not have to do extended exposure and so no maximum time is required
Capture Mode: Both automated and manual		Automatic capture
Cooling down time: 120 sec-5 mins		Not applicable
Application preference: Fluorescence (EtBr, SYBR Green fluorescent gel stains)		Chemiluminescence only
Colorimetry (Coomassie Blue, Silver stain, blots,		
prestained markers) Chemiluminescence		
Fluorescent western blots		
Illumination sources: Trans UV, Epi-White, optional Trans-white light		White LEDs for marker lanes
Sample Stage: motor driven		Push-in drawer
Sample size: 16 / 28 x 22 / 36 cm		10 x 10cm
Emission filters:		
filters for Cy2, Cy3/EtBr, Cy5, blue green filters and filters for commassie blue and ethidium bromide		Not applicable
Operating voltage: 100-240 V		80 - 264v
Operating Temperature: 10-28°C		10- 40C
Optional Item: A desktop computer with suitable operating system compatible		Can be used with external PC or use Cloud transfer of images
with software		
Warranty: 3 years warranty		2 years

Typical Fluorescence system		Vü-F
GEL DOCUMENTATION SYSTEM WITH ANALYSIS SOFTWARE		FLUORESCENCE SYSTEM
General - Easy and simple to use Camera CCD camera Pixel resolution 1.0 to 14.0 Mega Pixel		Advaned sensor technology with resolution 12.7m pixels
Lens type		Micro lens T-stop 1.01
Motorized zoom PC interface Image documentation system suited for fluorescent and non-fluorescent electrophoresis gels		Zoom lens not required
Operation panel		No user interface for operation required
Full function control panel for zoom, focus, Iris and illuminator Increased sensitivity and dynamic range, Dark room Light tight, UV safety shut off, Protecting from UV irradiation with the design of auto shutoff UV lights when the door is open;		Fully automatic control using advanced intelligent embedded software
UV Transilluminator		Not required- uses dedicated 302nm illumination system
Standard UV trans-illuminator;		Not required- uses dedicated 302nm illumination system
Build-in overhead white light, durable LED reflection;		Built in Blue LED's
Slide out tray hold UV trans-illuminator and trans-white converter plates		Slide in/out drawer for application and user ID's
Software		Control software not required
Fully automatic Control through software with latest computer,		Fully automatic control using advanced intelligent embedded software
Gel Imaging software should have the function of database, Image capture, image edit and image analysis.		Analysis software for image edit and image analysis.
Transmitted area		20 x 20cm
UV Transilluminator Transmitted area: not less than 21cm x 26cm		20 x 20cm
Power supply 220/230 V power supply		80-246V

16 **How do the Vu images compare with those from a traditional CCD camera based imaging system?** Of course, this is the real 'test'. It is impractical to compare a Vu image with every traditional gel documentation system on the market – there are too many. Over time our libraries will show more and more comparative images as we gather more data. Ask any current gel documentation system manufacturer to show you comparative images and at best they might be able to show just one or two – and even then how do you know that those images have not been 'doctored'? Our objective is to create a library with as many images as we can which show how much better Vu images are when compared to any other gel documentation system. **TIP: always ask for a demonstration of Vu and see for yourself with your own gels and blots how much better the Vu un-doctored images are.**