Introduction: Printing with Nexterion® Slide MPX 16

Nexterion® Slide MPX 16 provides distinct subarray wells for multiplexed hybridization experiments. Due to the special slide architecture and the necessary adaptions of the printing process, detailed information to support your experimental microarray design are provided in this section.

The topics covered in this section include:

1) Physical limitations for multiple pin printing configurations due to the interplay between Nexterion® Slide MPX 16, the source plate, and the print head (Figure 1)

2) Determination of printable well area for …

   a) single pin single well printing (Figure 2)
   b) single pin multiple well printing (Figure 3)

3) Determination of useful pin printing configurations for 96 and 384 well source plates (Figure 4)

4) Recommendation of pin printing configurations considering the relative importance of printing time probe density and costs (Table 1)

5) Maximum probe densities per well based on pitch, source plate, and pin configuration (Table 2)

These instructions are intended as a general guideline for contact pin and inkjet printing, however, actual configurations may vary due to software differences and variable print head configurations.

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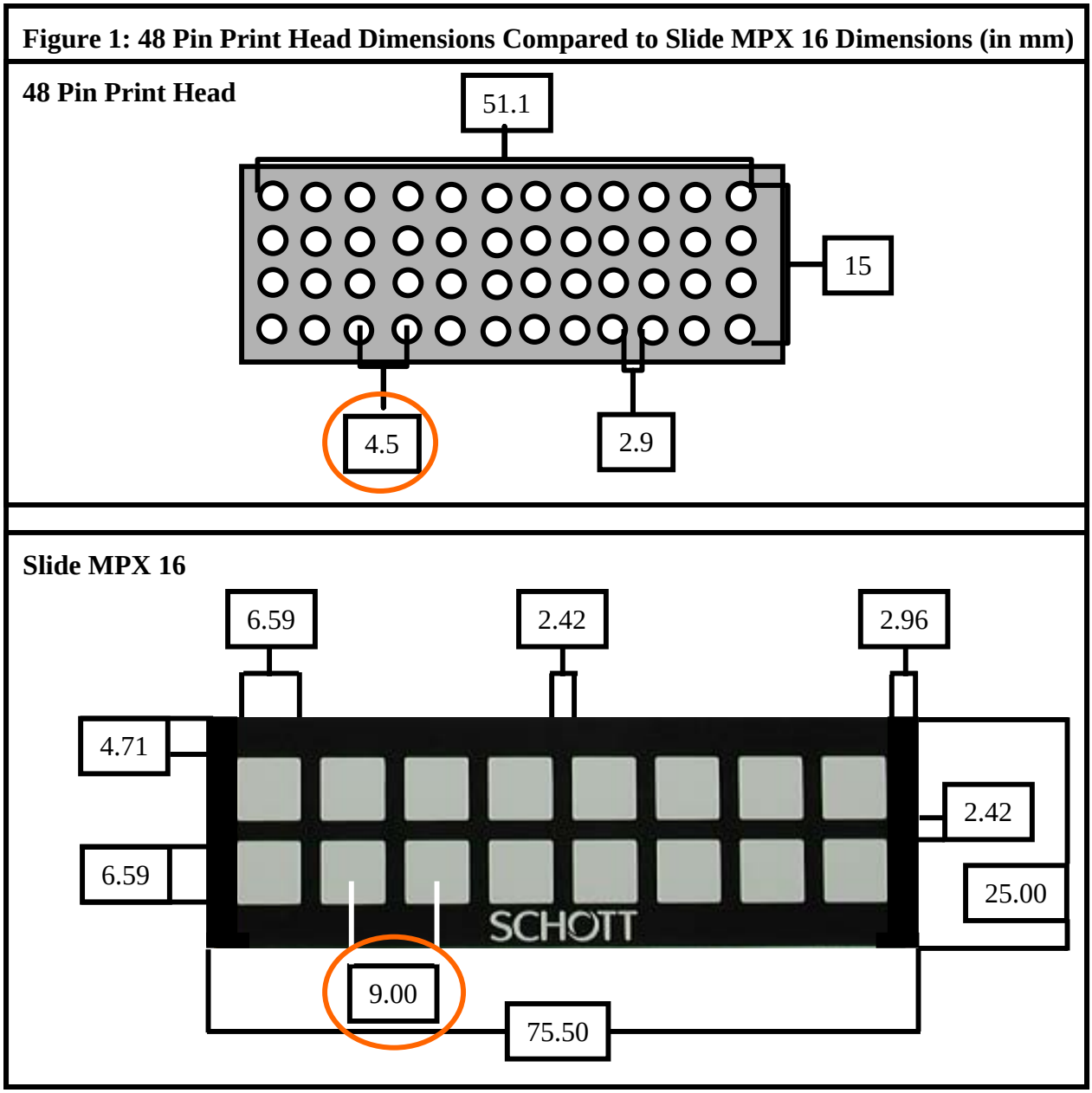
1) Physical Limitations: Nexterion® Slide MPX 16, Source plate and Print Head

Figure 1 compares the dimensions for a standard 48 pin print head configuration with those for Nexterion® Slide MPX 16. The potential pin printing configurations are dependent upon the dimensions of Nexterion® Slide MPX 16 and the number of wells in the source plate (i.e., 96 or 384). For instance, a 96 well sourceplate (9.0 mm well-to-well spacing) dictates that only every other pin position could be available for printing while a 384 well sourceplate (4.5 mm well-to-well spacing) potentially allows all pin positions.

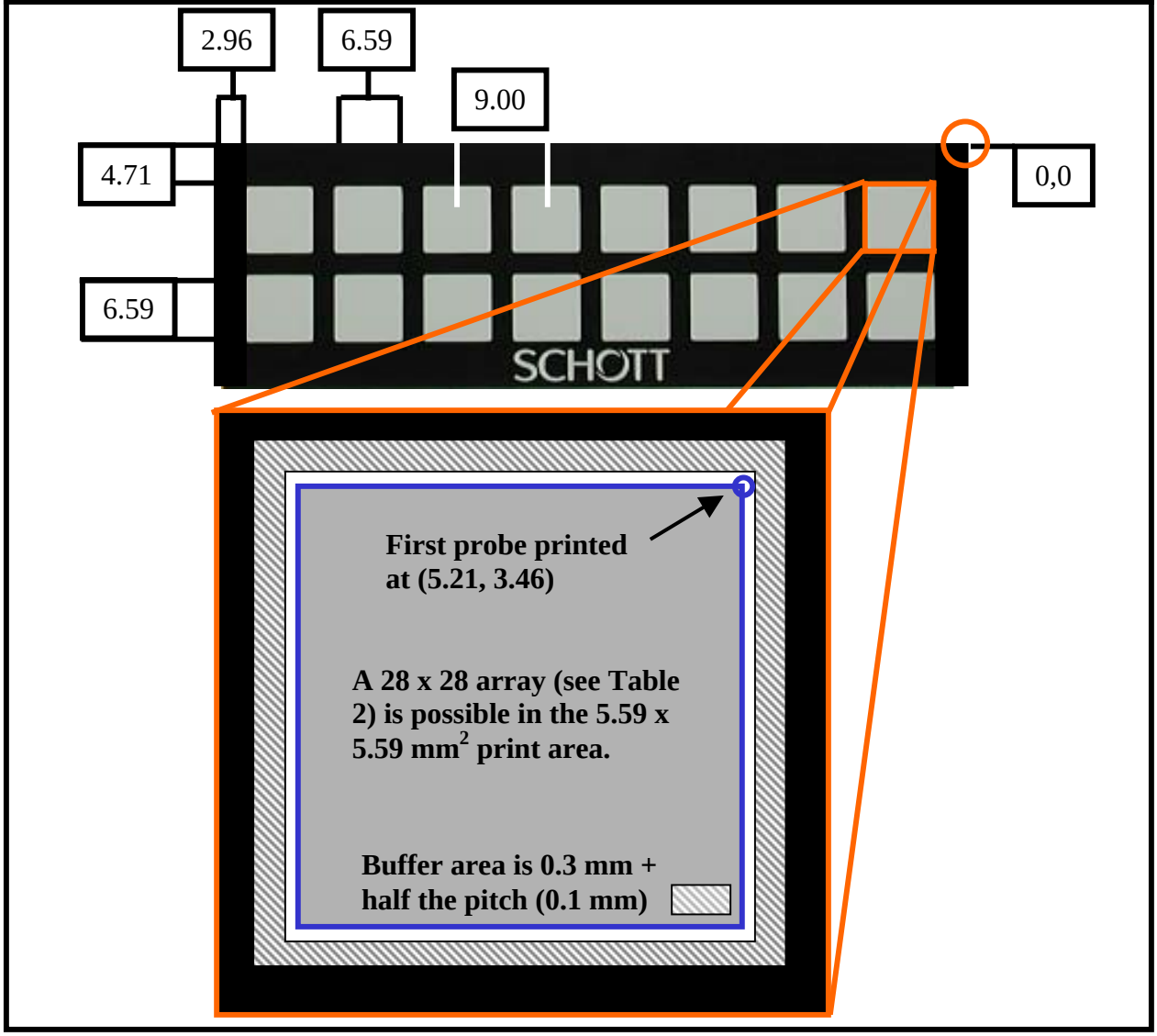
Figure 1: 48 Pin Print Head Dimensions Compared to Slide MPX 16 Dimensions (in mm)



2 a) Printable Well Area: Single Pin Single Well Printing

The Nexterion® Slide MPX 16 format allows for four printing schemes to optimize probe density per well, printing time, and/or probe cost. Single pin single well printing offers the greatest experimental flexibility, allowing the maximum number of probes per well and minimizing replicate probe cost at the expense of increased printing time.
Figure 2 demonstrates the calculation of printable well area and the mechanics of setting up printing when using a 1 x 1 pin configuration, a 384 well sourceplate, and a pitch (spot-to-spot spacing) of 0.2 mm. The printable well area is calculated by setting a well border buffer of 0.3 mm + pitch. This buffer is provided to ensure printing of the arrays within the wells after considering the tolerance variations of the slides, arraying equipment, and superstructures. The calculations provide that for any given pitch, there will be a minimum buffer of 0.3 mm + ½ pitch between the array and the pattern.

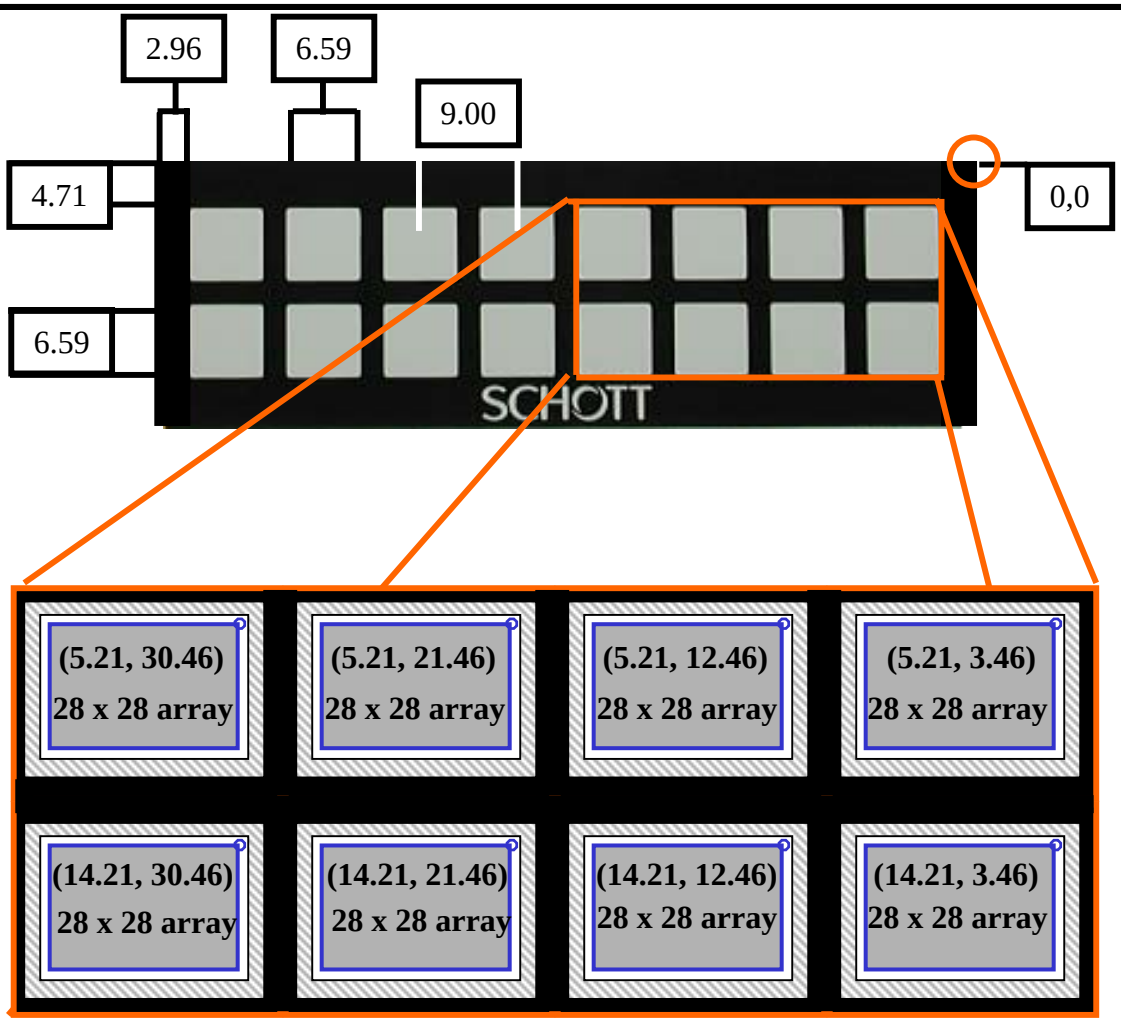
Figure 2: Printing Calculations Based on 384 Source plate & 1 x 1 Pin



2 b) Printable Well Area: Single Pins Multiple Well Printing

Single pins multiple well printing is the most useful multiple pin printing scheme. The scheme allows for full well printing of unique and/or replicate arrays with reduced printing time. The only disadvantage with this scheme is the printing of replicate arrays results in higher probe costs. **Figure 3** demonstrates the calculation of printable well area and the mechanics of setting up well printing when using a 2 x 4 pin configuration, a 96 well source plate, and a pitch (spot-to-spot spacing) of 0.2 mm. The printable well area is calculated by setting a well border buffer of 0.3 mm + pitch. This buffer is provided to ensure printing of the arrays within the wells after considering the tolerance variations of the slides, arraying equipment, and superstructures. The calculations provide that for any given pitch, there will be a minimum buffer of 0.3 mm + ½ pitch between the array and the pattern.

Figure 3: Printing Calculations Based on 96 Source plate & 2 x 4 Pin



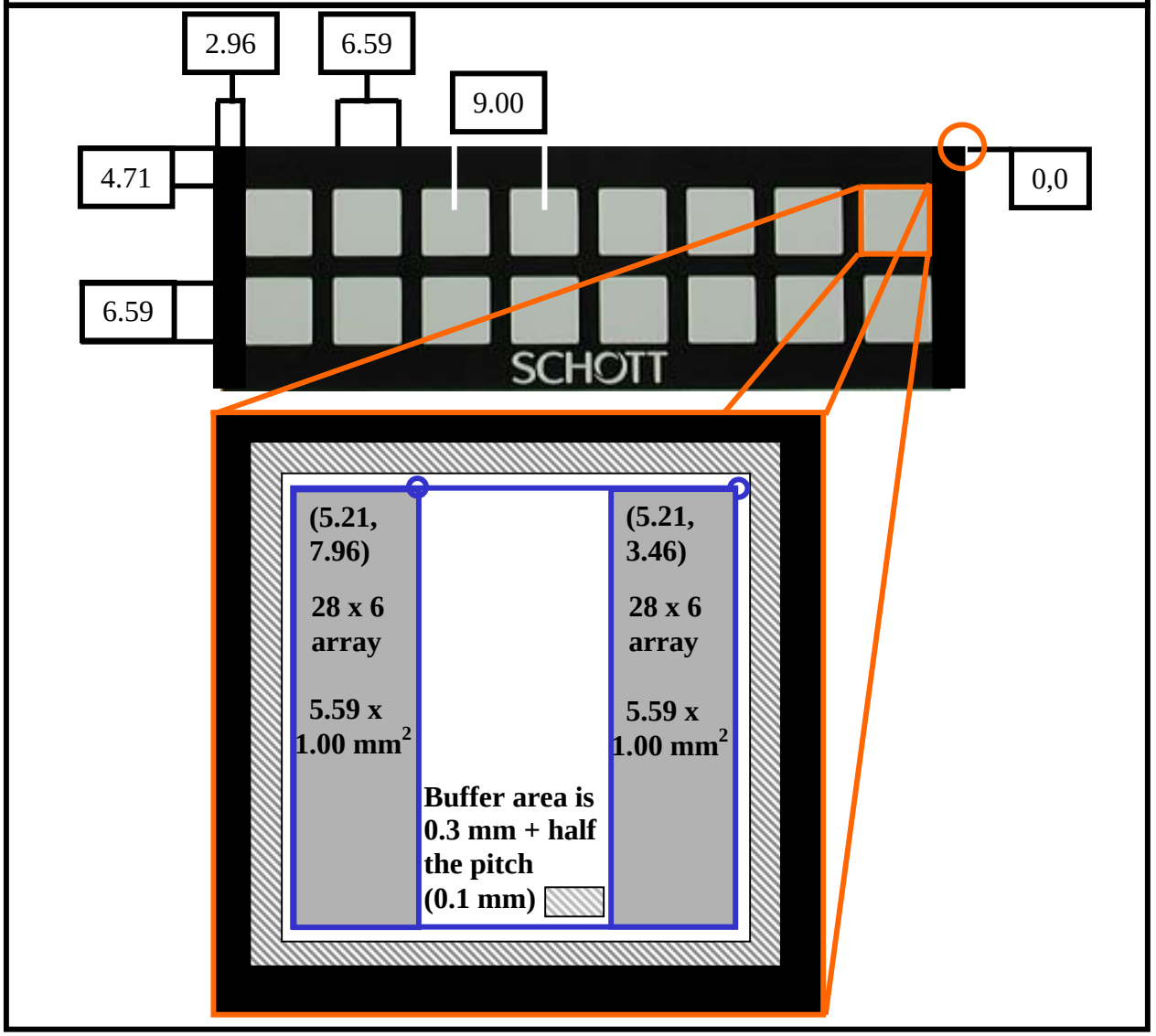
Print area of 5.59 x 5.59 mm^2

Buffer area is 0.3 mm + half the pitch (0.1 mm)

2 c) Printable Well Area: Multiple Pins Single Well Printing

Multiple pin single well printing offers decreased printing time but reduces the achievable probe density, increases replicate probe cost, and results in discontinuous intra-well arrays. **Figure 4** demonstrates the calculation of printable well area and the mechanics of setting up well printing when using a 1 x 2 pin configuration, a 384 well source plate, and a pitch (spot-to-spot spacing) of 0.2 mm. A cosmetic consequence of multiple pin printing is discontinuous arrays, as shown by the two arraying regions in Figure 4. The printable well area is calculated by setting a well border buffer of 0.3 mm + pitch. This buffer is provided to ensure printing of the arrays within the wells after considering the tolerance variations of the slides, arraying equipment, and superstructures. The calculations provide that for any given pitch, there will be a minimum buffer of 0.3 mm + ½ pitch between the array and the pattern. For intra-well printing, due to the pin spacing of 4.5 mm required by a 384 well source plate, all of the well area cannot be printed.

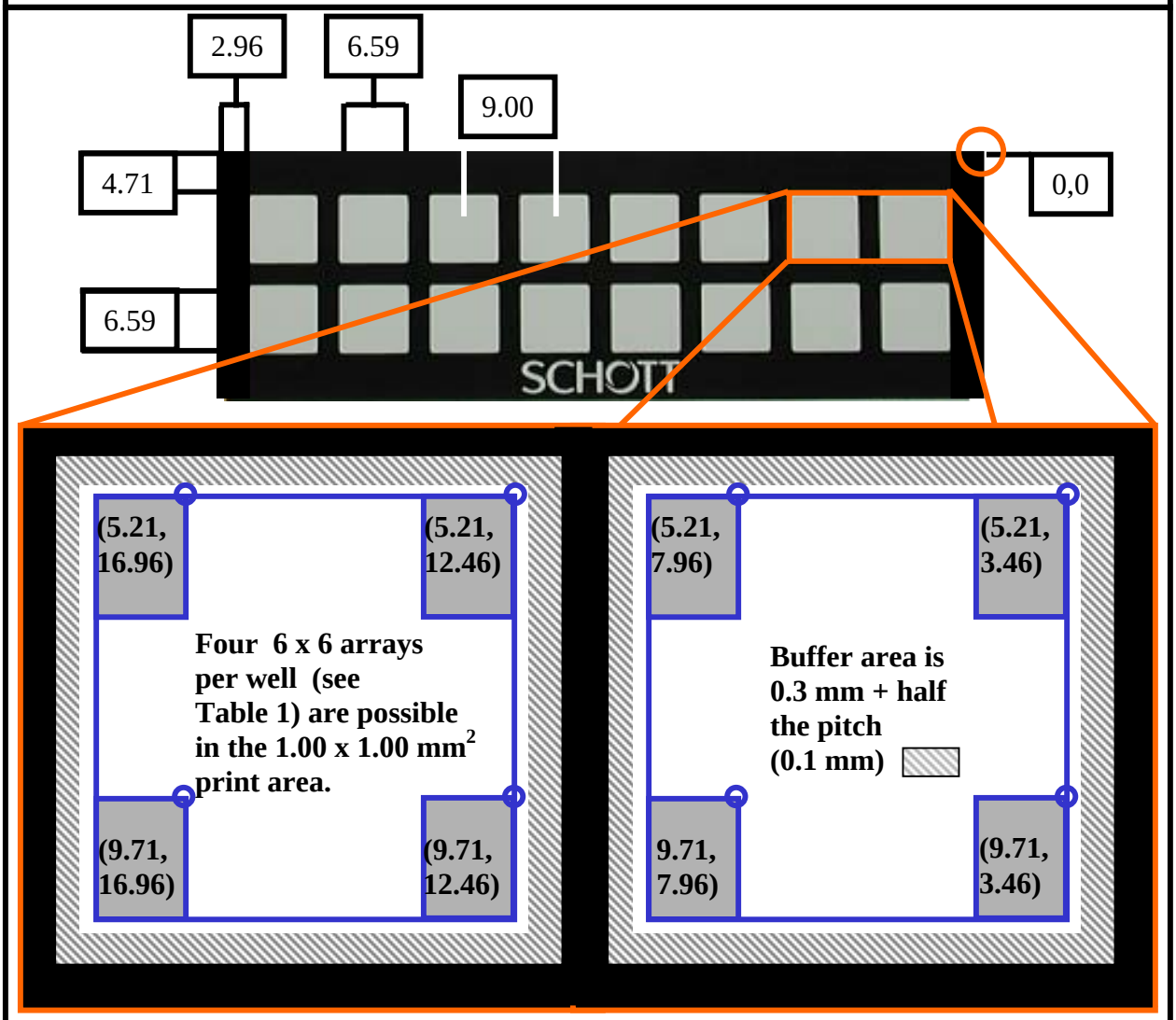
Figure 4: Printing Calculations Based on 384 Source plate & 1 x 2 Pin



2 d) Printable Well Area: Multiple Pins Multiple Well Printing

Multiple pin multiple well printing reduces printing time at the expense of probe density, higher replicate probe cost, and discontinuous intra-well arrays.
Figure 5 demonstrates the calculation of printable well area and the mechanics of setting up to print an array in well 1 using a 4 x 4 pin configuration (4 pins per well), a 384 well source plate, and a pitch (spot-to-spot spacing) of 0.2 mm. The printable well area is calculated by setting a well border buffer of 0.3 mm + pitch. This buffer is provided to ensure printing of the arrays within the wells even after considering the tolerance variations of the slides, arraying equipment, and superstructures. The calculations provide that for any given pitch, there will be a minimum buffer of 0.3 mm + ½ pitch between the array and the pattern. For intra-well printing, due to the pin spacing of 4.5 mm required by a 384 well source plate, all of the well area cannot be printed.

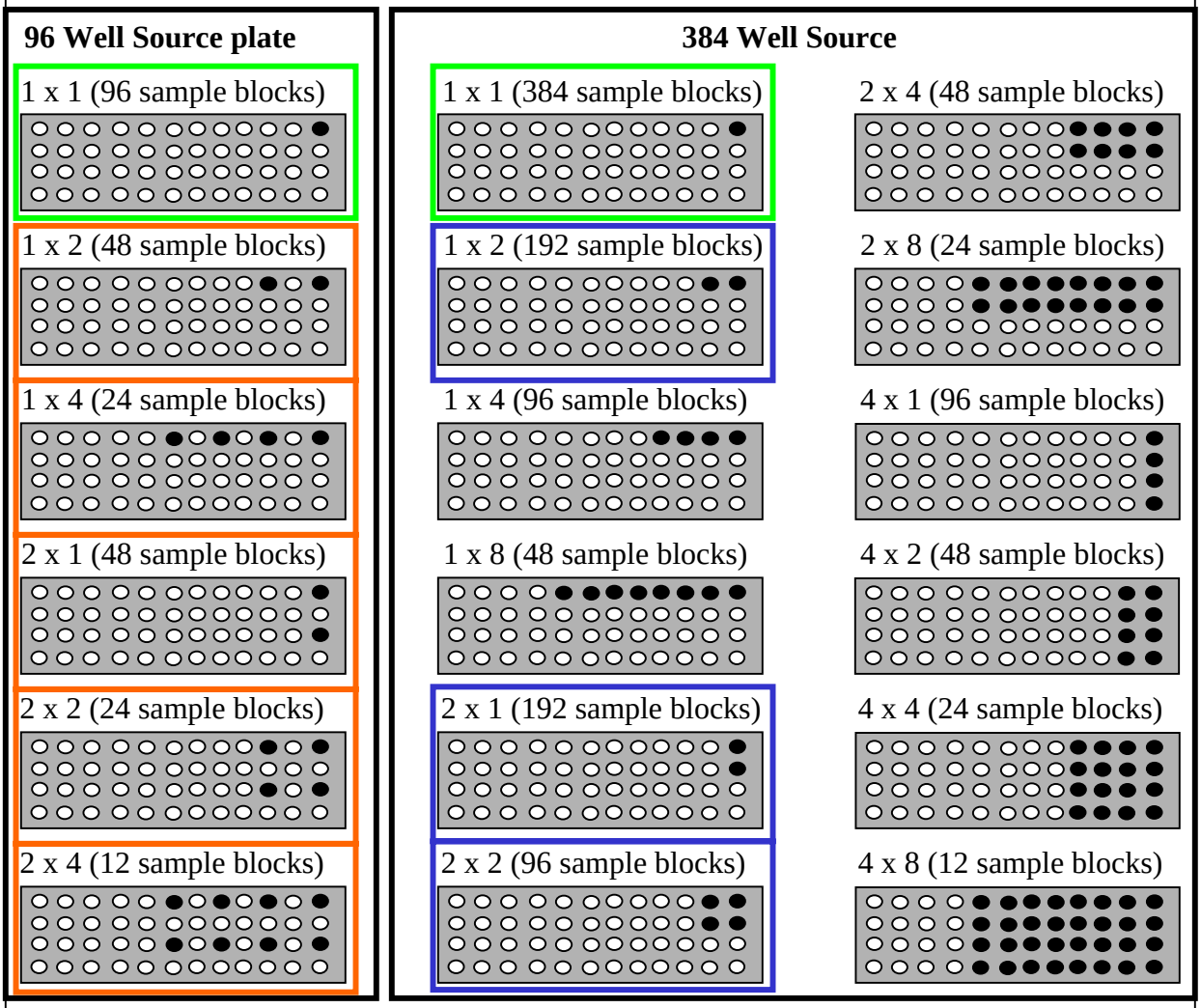
Figure 5: Printing Calculations Based on 384 Source plate & 4 x 4 Pin



3) Useful Pin Printing Configurations

Figure 6 demonstrates the useful pin configurations for the printing of probes based on either a 96 well or 384 well source plate. “Sample blocks” designates the groupings of probes within the source plate (each probe in the sample block being unique or replicate) which allow full utilization of the source plate. The pin configurations are deemed “useful” if they enable (1) the printing of all Nexterion® Slide MPX 16 wells in a single print run; and (2) the use of all wells in the source plate.

Figure 6: Useful Pin Printing Configurations Using a 48 Pin Print-Head



Filled circles represent pin positions. **Configurations inside green boxes are for single pin printing**. **Configurations inside red boxes are for single pins multiple well printing**. **Configurations inside blue boxes are for multiple pins printing in single wells**. Unboxed configurations are for multiple pins printing in multiple wells.

4) Recommendation of Pin Printing Configurations

Table 1 compares the probe density, printing time, and replicate probe cost as a function of the printing schemes. The final experimental design will dictate which printing scheme is best suited for a particular application.

Table 1: Printing Considerations as a Function of Pin Configuration			
	# Probes per Well	Printing Time	Probe Cost - Replicates
Single Pin Single Well	3	1	3
Single Pins Multiple Wells	3	2	1
Multiple Pins Single Well	2	2	2
Multiple Pins Multiple Wells	2	3	1

Note: 3 = best, 2 = medium, 1 = worst

5) Maximum Probe Densities per Well

Table 2 displays the maximum number of probes per well based on theoretical calculations using pitch and type of source plate. The color-coding in the table indicates, for each pin configuration, the number of pins used for intra-well printing. Please note that the results in Table 2 are based on theoretical calculations. However, they have been experimentally validated in part using a GeneMachines Omnigrid Accent (contact printer) and Packard Bioscience Biochip Arrayer (piezoelectric printer).

Maximum Probe Densities for Nexterion Slide MPX 16							
Pitch (microns)	96 Well Sourceplate	384 Well Sourceplate					
Pin Configurations	*(1 x 1-2-4), (2 x 1-2-4)*	*(1 x 1)*	*(1 x 2-4-8)*	*(2 x 1)*	*(2 x 2-4-8)*	*(4 x 1)*	*(4 x 2-4-8)*
100	3364	3364	1508	1508	676	1508	676
150	1444	1444	608	608	256	608	256
200	784	784	336	336	144	336	144
250	484	484	220	220	100	220	100
300	324	324	108	108	36	108	36
350	256	256	96	96	36	96	36
400	169	169	52	52	16	52	16
450	144	144	48	48	16	48	16
500	100	100	20	20	4	20	4

1 Pin / MPX well
2 Pins / MPX well
4 Pins / MPX well

Security buffer is 300 microns + pitch from well borders.