In this time-constrained feasibility study the cycle time for an intermediate crystallization step (Compound Y) is reduced by 60% and evidence to support the removal of a difficult seeding step is presented. A simple-to-use image-based method, Relative Backscatter Index (RBI), is used to quickly identify unnecessary hold times and real time microscopy is used to determine how cooling rate influences crystal growth and nucleation. An optimized process is presented that eliminates redundant hold times and utilizes favorable growth kinetics to cool the process at a faster rate while maintaining the same final product crystal size and shape. An unseeded crystallization process is also developed and characterized in order to determine if a difficult seeding step could be removed. While the unseeded process shows promise, it is shown that a series of temperature cycles are needed to achieve the desired final crystal size and shape. Finally, a brief comparison of RBI and turbidity is presented to help benchmark the new technique against a more traditional method.
1 Introduction

Controlling crystal size, shape, and concentration is critical during the development and manufacture of high value chemicals to ensure product crystals have the required attributes and that processing steps downstream from the crystallizers can be optimized and are consistent. Crystallization processes are typically poorly understood, particularly early in the development where complex phenomena such as phase separation, poor impurity rejection, and excessive secondary nucleation can extend development time and cost. Intermediate crystallization steps are particularly prone to poor understanding due to the limited time that is often available for detailed characterization and optimization. As a result intermediate crystallization steps can represent a major component of overall process cycle time during manufacturing, due to unnecessary hold times and difficult filtration and washing steps caused by inconsistent crystal size and shape distributions.

Offline microscopy and inline turbidity measurements are traditional techniques used during crystallization process development to provide useful information to scientists trying to improve crystallization processes. However, offline measurements are often not reliable for delicate crystals that can change during sampling and turbidity signals can be difficult to interpret in systems, such as crystallization, where size, shape and concentration are changing simultaneously.

With these challenges in mind that a simple, probe-based technique has been developed that combines high resolution real time microscopy with an image-based process trend that indicates how crystal size, shape, and concentration change in real time (Figure 1).

ParticleView® V19 allows scientists to directly observe crystals and associated crystallization mechanisms, such as nucleation, growth, phase separation or habit shifts, in process at full process concentrations with no need for sampling. Relative Backscatter Index (RBI) is a simple process trend, provided by ParticleView V19, that uses information from every image collected to indicate how crystallization processes are progressing in real time.

Figure 1: ParticleView V19 combined with EasyMax 402 crystallization workstation
The combination of real time microscopy and RBI in a single probe-based technique allows crystallization processes to be routinely understood using a simple method that is easier to use and understand compared with turbidity and offline microscope analysis. The methods of measurement for ParticleView V19 and RBI are presented in detail in the appendix of this study.

2 Process Understanding for the Intermediate Crystallization Step [Process A]

The standard intermediate crystallization step [Process A] was characterized at a 1 L scale using an OptiMax reactor. 150 g of Compound Y was added to 300 g of solvent and agitation was set at 500 rpm using a pitched blade impeller. The temperature was increased to 58 °C to dissolve the starting material and then decreased to 50 °C before 7.5 g of seed was added. After a hold period the solution was cooled at 0.0625 °C/min until the temperature reached 20 °C. A ParticleView V19 probe was used to provide RBI trending (Figure 2) and real time microscopy (Figure 3).

![Figure 2: Time vs. RBI (green) and Temperature (blue) for Process A](image-url)
In Figure 2 the time vs. RBI trend indicates crystallization progression throughout the 14 hour process. Three distinct phases of crystallization are monitored; seeding at constant temperature (Figure 3a & b), crystallization during cooling phase (Figure 3c), hold period at constant temperature before isolation (Figure 3d). Real time microscopy images provide critical information related to crystal size and shape at key time points as well as the crystallization mechanisms that occur during the process.

During the seeding phase it is clear that the seed "holds", and does not dissolve, with an increase in RBI indicative of seed suspension followed by nucleation and growth. Real time microscopy images during the seed hold indicate that nucleation is prevalent with many small crystals appearing in the presence of the larger seed crystals (Figure 3b). During the cooling phase, RBI increased steadily indicating crystallization progression and real time microscopy showed the growth of elongated platelets over time (Figure 3c). Little evidence of further nucleation was evident during the cooling phase with no fine crystals appearing. During the final hold period, the RBI signal remains constant indicating little change in crystal size and count during that period.

**Summary of Process Understanding Obtained for Process A**

Real Time Microscopy: Figure 3
- Seed holds and does not dissolve
- Seed has a variable size and shape
- Nucleation and crystal growth occur during the seed hold
- Crystallization is growth dominated during 8 hour cooling phase
- Crystal shape is elongated platelets
- Approximate aspect ratio at end point 10:1

*Figure 3: Real Time Microscopy Images for Process A during critical process phases; (a) initial seeding; (b) during the seed hold period; (c) at the start of cooling; (d) process endpoint*
3 Designing an Optimized Process [Process B]:

Based on the information obtained from Process A there was a high degree of confidence that process cycle time could be reduced in two areas:

• Reduce seed hold period (to 75 min)
• Cool faster (0.42 °C/min) to take advantage of favorable growth behavior during cooling, observed in Process A

Figure 4 compares Process A (the standard intermediate crystallization step) with Process B (the newly designed process). It is immediately clear that the RBI value for Process B is higher during the seed hold phase (between 0 and 4 hours). Real time microscope images verify a difference in the crystal size and count, and concentration 4 minutes after seeding with more secondary nucleation observed for Process B (Figure 5). Such a difference could result from slight differences in the seeding protocol, inconsistency in the seed itself, or variability in the crystal nucleation kinetics after seeding. In this case, seed was added in the same way, was taken from the same lot, and was anticipated to behave identically – however variability in seed behavior is common and this case warrants further study outside the scope of this work. A proposal to eliminate the seeding step completely will be presented later in this study.

**Figure 4:** Time vs. temperature and RBI for the Process A and Process B
During the cooling phase the RBI increases at a faster rate for Process B, but no sudden spikes in RBI were observed meaning secondary nucleations did not occur at the faster cooling rate. At the end of Process B, the RBI value is slightly higher compared to Process A but the real time microscopy images (Figure 6) comparing both endpoints indicate only minimal differences. A higher RBI value may indicate smaller particles with a higher count and the real time microscope images indicate this may be the case here. This difference likely results from the faster cooling rate which favors nucleation over growth – however the variation in seed performance during the initial stages of the crystallization should also be considered. In order to verify, with a high degree of confidence, the root cause of differences in crystals size and count a method such as ParticleTrack should be used which can provide significantly more detailed information.

Based on this result, and the detailed level of process understanding that has been obtained in a short space of time, it appears highly feasible that simple changes to the crystallization protocol have the potential to reduce the process cycle time by up to 60% (14.4 hours to 5.8 hours) while maintaining a relatively similar crystal size and shape distribution.
4 Building Evidence to Remove the Seeding Step

For intermediate crystallization steps seeding is often undesired, because it adds time and complexity to a process where the crystalline product will likely be dissolved and recrystallized. Also, for this process some variability in the seed performance for two similar experiments has already been observed (Figure 5). Crystal size and shape distribution are important for intermediate crystallization steps when it comes to the speed at which the suspension can be filtered, dried, and transferred to the next step of the process. If crystal size and shape are variable or not optimized, then gains achieved from removing the seeding steps might be lost elsewhere. With this in mind another simple experiment was conducted to assess the feasibility of removing the seeding step completely, while maintaining the same crystal size and shape.

Using evidence obtained from the first two experiments a third process [Process C] was designed that removed the seeding step in favor of a self-nucleating process. Since extensive secondary nucleation was observed at the seeding point for Process A and Process B it was reasonable to assume that an isothermal hold at 50 °C was close to the metastable limit and that primary nucleation would be likely during an extended isothermal hold. Since RBI and real time microscopy provide real time information it was possible to directly observe crystallization behavior as it was happening and essentially design the process in real time. This is shown in Figure 7 where time vs temperature and RBI for Process C is shown.

Figure 7 shows how RBI increases for Process C during the initial hold period indicating nucleation has occurred. Since process cycle time was important for this intermediate crystallization step and it had been shown previously that the crystallization was growth dominated during the cooling phase a fast cooling ramp (0.42 °C/min) was initiated soon after nucleation. This resulted in a very rapid increase in RBI to a level far above the value obtained for Process B. Real time microscopy (Figure 8) shows crystals one hour after nucleation, during this cooling phase, that are very long and thin and clearly exhibiting a dramatically different crystal size and shape distribution.

Since this process was being designed in real time, a temperature cycling program was implemented to determine if cycling offered an opportunity to adjust the crystal size and shape by dissolving thin crystals and growing longer elongated platelets – that were apparent during Process B. With each successive temperature cycle, the RBI dropped until it reached a level similar to that observed for Process B. Comparing real time microscopy images at the end of Process B and Process C (Figure 9) it is shown that crystal size and shape is much closer than would have been expected if temperature cycling had not been implemented (it was also noted that the final temperature cycle did not have a significant impact on RBI and did not result in significant changes in crystal size observed with real time microscopy).
Information obtained from Process C indicates that there is scope for removing the seeding step from this intermediate crystallization step, however further feasibility work needs to be done in order to confirm this is a viable option. Temperature cycling at the end of an intermediate crystallization step may be difficult to implement reliably in manufacturing and adds complexity that may not be warranted. However, since this crystallization exhibits good growth behavior it may be possible to modify the initial stages of Process C to build a seed bed that can then be cooled quickly and efficiently to generate larger crystals. An extended hold period at 50 °C – just after the nucleation should be investigated. This approach will be evaluated in a follow up study.

**Figure 7:** Time vs. RBI and Temperature for Process B and C

**Figure 8:** Real time microscope image 1 hour after spontaneous nucleation event (Process C)
5 Benchmarking RBI against Turbidity

Since RBI is a new process monitoring technique, a simple study was conducted to benchmark performance in a model system. Serial additions of wollastonite particles were made to 300 g of water in an agitated system and RBI and turbidity was recorded for each addition. A METTLER TOLEDO InPro 8200 Turbidity Sensor with Trb 8300 meter was used to measure turbidity and ParticleView V19 was used to measure RBI. Figure 10 compares RBI and turbidity from 0 w/w% up to 35 w/w% and shows that turbidity begins to saturate above 10 w/w% solids. The RBI signal continues to increase as solids concentration increase up to 35 w/w%, the point where mixing and solids suspension was no longer reliable in the system. This simple result indicates that RBI has the potential to monitor a wider range of processes with a higher degree of sensitivity and shows promise for crystallization processes where solids concentrations are often high, up to 40 w/w%. It should also be noted that RBI is derived from a continuous stream of high resolution microscope images that provide comprehensive process understanding and can help interpret changes in the RBI signal easily.

![Figure 9: Comparing real time microscope images at process endpoint Process B and C](image1)

![Figure 10: Comparing RBI and turbidity for increasing solids concentration of wollastonite in water](image2)
6 Summary

Cycle time reduction for intermediate crystallization steps offers the opportunity to improve the performance of processes that may have received relatively little attention during the development phase. In this study it has been shown that during development itself, or during a process optimization project for an existing process, it is possible to apply a new real time image-based technique to more completely understand and characterize difficult crystallization processes. Images are straightforward to interpret and provide an unparalleled level of process understanding with little or no ambiguity. Utilizing these images to provide a process trend indicates how particle size, shape, and concentration are changing and allows scientists to choose suitable process parameters to achieve the desired crystal size and shape. This combination of tools in a small and easy to use probe-based instrument offers the opportunity to develop better, more scalable, crystallization processes, in less time at a lower total cost.

7 Appendix

ParticleView Method of Measurement:

Figure A: Schematic illustrating principle of operation of ParticleView V19 and capture of high resolution image of known brightness
RBI is an image-based trend that indicates how particle size, shape, and concentration is changing in real time.

**Figure B:** Method of measurement for RBI

Reflectivity of Particle System = \[
\frac{\text{Image Brightness}}{\text{Intensity of Light Output}} \times \frac{\text{Particle Size}}{\text{Particle Shape}} \frac{\text{Solids Concentration}}{\text{Refractive Index}} \frac{\text{Particle Brightness}}{\text{Constant for most systems}}
\]

Relative Backscatter is a function of:
- Particle Size
- Particle Shape
- Solids Concentration
- Refractive Index
- Particle Brightness

**Figure C:** ParticleView V19 with Real Time Microscopy and Relative Backscatter Index (RBI) describes nucleation and dissolution as temperature changes for a crystallization process
White Paper: How Real-Time Microscopy Simplifies Crystallization and Precipitation

Further examples of how real-time microscopy simplifies crystallization and precipitation: This white paper discusses how GlaxoSmithKline, Merck, Sintef, and University College Dublin (UCD) use in-line microscopy to understand crystallization processes – faster than any other method. Scientists can now directly observe key crystallization mechanisms including:

- Polymorphism
- Crystal Growth
- Secondary Nucleation
- Seed Aggregation
- Oiling Out

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